UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

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VOL 33, 2
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2017

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2017

Biotechnology in Animal Husbandry 33 (2), p 135-259, 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636

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Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164;
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Biotechnology in Animal Husbandry is covered by Agricultural Information Services (AGRIS) -Bibliographic coverage of abstracts; Electronic Journal Access Project by Colorado Altiance Research Libraries -Colorado, Denver; USA; Matica Srpska Library -Referal Center; National Library of Serbia; University Library "Svetozar Markovic", Belgrade, Serbia; EBSCO, USA; DOAJ and European Libraries
According to CEON bibliometrical analysis citation in SCI index 212, in ISI 9, impact factor (2 and 5) of journal in 2012: 0,667 and 0,467, - M51 category

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

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

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

Biotechnology in Animal Husbandry 33 (2), p 135-149 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.082'64
https://doi.org/10.2298/BAH1702135S

FERTILITY OF BOARS – WHAT IS IMPORTANT TO KNOW

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Review paper

Abstract: The most important part in reproductive management is the control of boar fertility. A common division of fertility traits is on the: *in vitro* (sperm traits) and *in vivo* (return rate, farrowing rate and litter size traits) fertility. In many studies were found differences between breed in the both groups of fertility traits. Variability of sperm traits of boars during the reproductive exploitation is influenced by various genetic (boar, breed) and paragenetic factors/effects (age, season, intensity of use). Good libido is desirable characteristics in boars, but the knowlegde of the correlation of libido and boar fertility traits are limited. Also, there is no standardised procedure or methods for the estimation of libido of the boars. The permanent ranking of boars according to the reproductive efficiency should be performing. Good reproductive management implies the timely identification of boars with the low fertility (or close to the average).

Key words: boar, artificial insemination, sperm traits, libido, reproductive efficiency

Introduction

A large number of descendants produced by boar at an annual level requires a permanent control of its fertility and timely culling of the animals whose performance is below the population or herds average. The application of artificial insemination requires fast and accurate estimation of boar breeding value on the basis of libido, sperm evaluation, success of insemination and the litter size. The boar fertility traits are represented by *in vitro* (sperm traits) and *in vivo* (reproductive efficiency and litter size) fertility. In selected pig populations the boars remain in reproduction from 6 to 8 months on average. In a review of the

results realised by a number of artificial insemination centres, *Robinson and Buhr (2005)* state the following most frequent reasons for replacement of boars: genetic reasons (20-45%), sperm quality (10-30%), libido (1-21%), physical health (13-60%) and other reasons (10-20%).

The research results obtained by *Feitsma (2009)* showed that variability of litter size was from 4-7% caused by sperm components, 10% by sow genotype, 10% by herds and 17% by parity. In pig populations where continuos selection is being conducted there is a tendency to use the boars of good production performances in the best way possible what is reflected in obtaining as more doses per ejaculate of optimal fertilising capacity as possible *(Savić et al., 2013b).*

The size of litter varies among the boars, when insemination doses contain the same number of spermatozoa, while an increase of the number of spermatozoa at insemination has a positive effect on the number of live-born piglets particularly in the interval of $1\text{-}3x10^9$ spermatozoa *(Flowers, 2002)*. Farrowing rate and litter size are important traits of the economy of pig breeding industry. A litter size is a low heritable trait and variability of this trait depends in a highest degree on the environmental factors. Number of piglets in litter depends on a sow's genetic potential, but it is thought that boars determine constitution and vitality of fruits and piglets influencing the number of prenatal, perinatal and postnatal losses *(Petrović, 1990)*. According to the research of *Ruiz-Sánchez et al. (2006)* there are some differences between the boars regarding the percentage of determined pregnancies on the 30^{th} day after insemination (73-98%), farrowing rate (71-98%) and total number of born piglets (8.8-12.0 piglets). The boars are being selected mostly on the traits having a primary economic importance such as the rate of liveweight gain or the age at certain body mass, bacon thickness and productivity of their daughters, report *Robinson and Buhr (2005)*.

The control of boars productivity is an important segment of reproductive management so the objective of this research is the effect of evaluation of the most important factors on boar fertility. In addition, this research will partly answer the question whether sexual drive (libido) can have some influence on fertility in boars.

Sperm traits

Evaluation of sperm traits is a standard procedure in the application of artificial insemination. Various factors like boar, breed, age, season, intensity of use have been evidenced to show effect on these traits.

An important step in improving a pig population is a production and exploitation of boar sperm of high genetic potential, good productive performances and high fertilising capacity. Sperm fertilising capacity depends on a larger number of traits. The most important quantitative and qualitative traits of ejaculate are: volume of ejaculate, density or concentration of sperm, motility, percent of

abnormal spermatozoa, total number of spermatozoa and number of functional spermatozoa, vitality of spermatozoa and number of doses produced per ejaculate.

A precise determination of concentration, volume and percent of live spermatozoa is very important for the evalution of maximal dilution of sperm which can be used either for artificial insemination or for a number of sows which can be inseminated *(Kanokwan, 2011).* Sperm concentration, volume of ejaculate and motility of spermatozoa are the most important factors which determine the number and fertility of doses produced per one jump *(Savić et al., 2013b).* The choice of extender in the process of preparation of doses for insemination can have an effect on fertility. The results of the research of *Berg et al. (2014)* show differences in the farrowing rate and total number of live piglets at birth of 2.5% and 0.6 piglets which occurred as a result of application of different types of extenders.

Effect of breed

An average manifestation and variability of the boar sperm traits depend on a breed. The research by *Borg et al. (1993)* shows breed differences in body mass, size of testicles, number of spermatozoa per ejaculate and volume of ejaculate. In their research, *Wierzbicki et al. (2010)* determined the differences in all ejaculate traits among 10 examined boar genotypes.

Table 1 shows parameters of quantitative and qualitative traits of boar ejaculate per breed in accordance with the results obtained in different studies. The lowest volume of ejaculate (VOL) and the highest sperm concentration (CON) were determined in Duroc breed boars while the highest volume was determined in LWxP cross-bred boars.

Table 1. Ejaculate traits per breed

Traits	*Wolf and Smital (2009b)*						*Wolf and Smital (2009a)*		*Wolf (2010)*	
	Pure breed			Cross-breed			Pure breed		Pure breed	
	D	LW^s	P	DxLW	DxP	LWxP	LW	L	L	LW
VOL	200	270	275	236	241	282	276	273	276	275
CON	491	401	453	431	445	407	430	422	418	428
MO	73.6	76.6	76.8	71.6	74.2	76.6	76.0	75.6	75.8	76.2
AB	10.8	11.2	11.8	13.1	10.8	10.8	11.4	11.2	11.2	11.5
NT	93.7	101.3	118.7	95.1	102.1	107.4	112	107	108	111
NF	61.5	69.3	80.3	59.1	67.5	73.8	75.5	72.6	72.9	75.2

D- Duroc, LW- Large White (^{s}-sire line), P- Pietrain, L- Landrace, LW- Large White, VOL- Volume of ejaculate *(ml)*, CON- Sperm concentration *(x10³ spermatozoa/mm³)*, MO- motility *(%)*, AB- percentage of abnormal spermatozoa *(%)*, NT- total number of spermatozoa *(x10 ^{9}spermatozoa)*, NF- number of functional spermatozoa *(x10^9 spermatozoa)*

Pietrain boars had a large volume of ejaculate, high concentration of sperm, the largest percent of motility of spermatozoa (MO) with the largest number of functional spermatozoa (NF=80.3x10^9) what enables obtaining a larger number of fertilising doses for insemination from one ejaculate. Percent of motile spermatozoa is in all genotypes more or less similar and ranges in the interval from 71.6 to 76.8%. Cross-breds boars DxLW had the worst parameters of the sperm traits with the lowest motility, highest percent of abnormal spermatozoa and smallest number of functional spermatozoa determined.

In the research of *Smital (2009)* significant differences existed in all examined sperm traits with maximum differences between analysed breeds (Czech meaty pig, Duroc, Hampshire, Landrace, Great White, Czech Great White, Pietrain and their different cross-breds) being: 95 ml (VOL), 109 x10^3/mm^3 (CON), 9% (MO), 1.6% (AB), 24x10^9 (NT) and 19x10^9 (corrected number of spermatozoa).

The research of *Stanić et al. (2003)* showed differences in sperm traits existing between boar breeds and cross-breds so that the highest VOL (303 ml) and lowest CON (181x10^6 per ml) were determined in Hampshire boars. The lowest VOL was determined in Pietrain boar (177 ml), while the highest CON in Duroc (218x10^6 per ml). The VOL of ejaculate in Duroc was lower than average in all examined breeds and cross-breds (191 vs. 262 ml). In the research by these authors an average progressive motility of spermatozoa was 78%, the lowest being in Duroc boar (75%), and the highest in Pietrain (83%).

Values of VOL determined in the research of *Savić (2014)* for Landrace and Large White fertile breeds were 236.30 ml and 239.57 ml, while in extremely meaty breed Duroc, value of VOL was 218.09 ml. In the same research the motility of spermatozoa (in native state and after dilution; subjective evaluation) was examined and the Duroc boars were superior in relation to fertile breeds. Differences existed also in the number of doses produced per ejaculate (NPD) because the smallest number of doses for artificial insemination (9.56 doses) was obtained from the ejaculates produced by Duroc. Superiority of swine fertile breeds in relation to Duroc regarding VOL was determined also in other studies (*Wolf, 2009; Savić et al., 2013a).*

In the research of *Knecht et al. (2014)* the highest volume of ejaculate was determined in Polish Great White breed (258.6 ml), but also the smallest number of insemination doses (22.44) due to the lowest concentration (345.1x10^6/ml) and total number of live spermatozoa (68.8x10^9) in ejaculate. The results of this research indicate, a negative relationship between the ejaculate volume and sperm concentration.

Many studies indicate differences in ejaculate traits between the breeds, but in some studies the effect of boar genotype on the ejaculate characteristics was not determined. Thus in the research of *Šernienė et al. (2002)* the effect of breed on the sperm traits (motility, total number of pathological spermatozoa, percent of vital

spermatozoa, percent of spermatozoa with anomalies of the tail and head) was not determined .

Differences in sperm traits phenotypic values can be a consequence of the purpose meant for the breeds in a breeding programme. Actually, inferiority of exceptionally meaty Duroc breed in the volume of ejaculate and number of doses produced per ejaculate in relation to fertile breeds might be a consequence of selection directed towards high meat production. Some studies indicate possible disorder of the functions of accessory sexual glands when genotypes with exceptionally small volume of ejaculate are in question. Taking into account a complexity of reproductive mechanism this can most likely be the question of a number of genetic and hormonal effects.

Effect of ejaculation frequency

The intensity of exploitation or a frequency of sperm collection affects boar quantitative and qualitative parameters *(Wolf and Smital, 2009a; 2009b)*. According to the reports of a number of researchers an optimal pause between two jumps for boars in exploitation is 3-5 days, however for young boars the pause between two jumps should be larger (minimum 7 days). Number of spermatozoa in ejaculate is gradually decreasing when boar is used more than once weekly in spite of slight increase of the production of sperm with ejaculation frequency *(Rothschild and Ruvinsky, 2011*). The research conducted by *Savić et al. (2015)* showed a significant effect of interval between two jumps on a volume of ejaculate, total number of spermatozoa and total number of doses per ejaculate. An acceptable level of volume of ejaculate occurred after a 3-day sexual pause, spermatozoa reserves replenished after 5-7 days, while full recovery took about 10-11 days (*Smital,2009)*.

Interval between two successful collections of ejaculates has a great effect on semen concentration *(Wolf and Smital, 2009a).* Prolonging this interval from 2 to 6, that is, 10 days, semen concentration increases for approximately $100x10^3$, i.e., $150x10^3$ spermatozoa per mm^3. As regards the volume of ejaculate, the effect of interval between the two semen collections is considerably weaker and slight increase when interval was prolonged from 2 to 7 days was perceived. Intervals longer than 12 days result botn in a decrease of the percent of motile spermatozoa and certain increase of the percent of abnormal spermatozoa in ejaculate.

By prolonging the interval between sperm collections the motility shows a tendency to decrease while a percent of abnormal spermatozoa shows a tendency to grow but the changes are relatively small *(Wolf and Smital, 2009b).*The results obtained in the studies of the same authors show that phenotypic values of the traits of total count and count of functional spermatozoa may increase when the interval between two semen collections is being prolonged to 10 days and when longer intervals are in question the values of these traits are slightly reduced.

Interval between two collections (≤7 days) showed better effect on sperm production implying that collecting the boar sperm be performed at least once a week *(Savić et al., 2016)*. The intervals between two collections lasting 7 days or less resulted in higher volume of ejaculate (by 42.64 ml), higher count of spermatozoa in ejaculate (by $7.68x10^9$) and higher number of doses per collection (by 2.64) compared to the intervals lasting longer than 7 days. When the intervals exceed 7 days, a productivity of boar per collection decreases at an annual level as well what can be negatively reflected on boar performance increasing the cost of production (less number of doses per collection, less number of collections at an annual level and necessity to raise greater number of boars).

In practical pig production some boars are more often exploited for ejaculate collection because of the ease of manipulation or shorter preparation time for a jump but at the same time we ignore the intensity of exploitation and make shorter pauses between jumps. In this way the animals are not only extremely exhausted but also obtain a weaker fertilising capacity ejaculates. For this reason it is necessary to take care of frequency of boar semen collection in order to obtain the sperm of optimal fertilising capacity.

Libido

Libido is important feature in boars but there is no standardised procedure for the assessment of sexual behaviour of boars used in artificial insemination. Also, the knowlegde of the effect of libido on boar fertility are limited.

Swine reproductive ability depends on the environment they live in. Pigs are social animals therefore for their normal sexual development a contact with other individuals of the same species in the group is necessary. Social environment can affect the age of puberty maturing, manifestation of sexual instinct, behaviour during mating, manifestation of oestrus and alike.

By reviewing the literature there could not be found any standardized procedure for the evaluation of sexual behaviour of boars used for artificial insemination and insight into the effect that sexual behaviour can have on boar reproductive performances is much more inferior than insight into a physiological mechanism of sperm production *(Levis and Reicks, 2005)*. Libido manifestation is under the effect not only of genetic and hormonal factors, but also of paragenetic effects (social environment, season, housing, boar training), so that changes in libido can indirectly indicate possible technological neglects (bad microclimate, fattening condition, stress exposure, badly skilled workers who manipulate the animals).

Social environment has a significant effect on boar sexual ability taking into account that modern testing of male breeding material means housing of a boar in individual or group boxes for purpose of controlling its productive parameters. According to different studies *(Petrović et al., 1994; Levis et al., 1997; Knox, 2003)*, boars raised separately either from female of male animals, mature later

sexually, show weaker sexual drive and lower volume of ejaculate. Such animals can even be asocial, showing aggressivness towards individuals of the same breed. For the purpose of improving reproductive ability and socialisation it seems to be necessary to provide the presence of female animals in test stations for testing the male breeding material. By this approach it is possible to expect that boars raised in that way will have better libido, higher volume of ejaculate, and indirectly, higher conception rate and larger size of litter during their reproductive life. *Petrović et al. (1994)* in a review paper reports an unfavourable effect of isolation of boar on his sexual behaviour, occurence of later puberty and decrease of ejaculate volume. Keeping the sexually matured boars in the group had a favourable effect on their libido and boars who were in contact with female breeding animals had better sperm parameters. Raising the boars individually (for purpose of measuring liveweight gain, conversion, daily consumption) can have a negative effect on age at puberty, sexual behaviour and firmness of legs and toes *(Levis et al., 1997).* Besides this, it is also necessary to take into account the behaviour of people towards animals, where different veterinary or zootechnical interventions must be performed in such a way so as not to endanger the welfare of animals and by gaining confidence, particulary towards people who are in direct contact with the boars.

According to different studies, the libido evaluation is performed on the basis of duration of preparation time for a jump, duration of erection, period from the entrance into the room with a dummy sow to the beginning of ejaculation, duration of ejaculation or total time elapsed from the entrance into the room for sperm collection to the end of ejaculation *(Okere et al., 2005; Szostak and Sarzyńska, 2011; Oberlender et al., 2012).* The libido evaluation only on the basis of duration of ejaculation is not sufficient so it is also essential to take into account a pre-jump period. When the evaluation of libido is performed on the basis of total duration of period from the entrance into the room with dummy sow to the end of ejaculation (total manipulative time) the period of preparation of boar is not being separated from the duration of ejaculation. Manifestation of sexual instinct in boar is more complex and requires different defining of libido, and taking into account its importance it is quite certain that in the future it will be a subject of numerous studies.

It was assumed that longer duration of ejaculation and shorter preparatory time are indicators of a good libido (*Savić and Petrović, 2015a)*. Preparing to collect is a non-productive period (NP) within the total manipulative time, which is calculated from when a boar enters the room with the dummy sow until the end of ejaculation. The productive period (PP) is defined as the time during which the boar ejaculates. Libido may be defined as the ratio of productive to non-productive period, based on this formula: I = PP/NP and obtained numerical value representing the libido index (I). When it came to libido, L boars had better sex drive. That is, the PP to NP ratio was higher ($p<0.05$) by 0.10 and 0.12 index

points, compared with Large White and Duroc boars, respectively. The main reason for pronounced libido in Landrace boars is the result of the shorter duration of preparing for the collection. Duroc boars were inferior to the fertile breeds (Large White and Ladrcae) with the the weakest libido (1.73).

Phenotype relationship of the traits of sexual activity indicates the possibility of simultaneous improvement of these traits (*Savić and Petrović, 2015b*). A weak relationship (r<0.2000) exists between libido and intensity of ejaculation, but values of coefficients are positive what makes possible simultaneous improvement of both traits. By comparing relationship of libido with VOL and intensity of ejaculation (the flow of ejaculate per time) among the breeds, the values of correlation coefficients are the least within Large White breed.

The one study with libido effect on boar fertility, found that the boars expressing the highest level of sexual behavior were characterized by the greatest number of ejaculate, the best quality of ejaculate (the higher percentage of spermatozoa with progressive motility, the small percentage of defect spermatozoa) and the high number of insemination doses from one ejaculate *(Szostak et al., 2015)*.

In the research of *Szostak and Sarzyńska (2011),* the value of preparation time before the jump was higher in L boars (4.19 min) compared to LW boars (3.10 min). Total time (preparation time+ejaculation) was shorter in LW boar (10.36 min) compared to L boar (12.62 min) and the best libido was determined in hybrid (6.47 min) and Duroc (7.05 min) boars. More distinct sexual drive is probably a consequence of a higher level of testosterone in blood, and the research by *Williams (2009)* indicates close relationship of the level of testosterone and sexual behaviour and libido. This can be confirmed by castrated male animals with a low level of hormones that do not show sexual interest.

Differences in libido among studies, when the same breeds are in question, seems to be the consequence of differences in genetic structure of studied populations, technology of keeping, but also of the way of defining and evalution of boar libido. Taking into account that boars are primarily selected on the traits which have an economic importance (liveweight gain, meatiness, fertility) it is also necessary to take presence of libido into consideration as one of more important criteria at choosing male breeding animals.

Reproductive efficiency of boars

Regardless the mode of mating (natural or artificial) a mating can be either successful or unsuccessful. A successful mating is the one in which a physiological duration of pregnancy in a breeding female results in farrowing. Unsuccessful serving can result in a repeated occurrence of oestrus or pregnancy may end in a miscarriage. When we talk about a reproductive efficiency of herd we primarily think both of a realised return rate and farrowing rate. Taking into account a wide application of artificial insemination and number of breeding females that are

inseminated by the sperm of one boar during a determined period an importance to understand parameter values of these traits is for that reason even greater and represents an unavoidable segment in the control of boar productivity. Control of productivity and ranking of boars based on the farrowing rate should be continuously carried out *(Savić et al., 2015).*

Oestrus can be both regular and irregular. If we presumed that an average duration of oestrous cycle is from 18 to 24 days, a regular oestrus would be in the period from 18 to 24 or 36 to 48 days post service. A repeated occurrence of oestrus in breeding females in the period ≤17, 25-35 and ≥49 days is an irregular oestrus.

In the research of *Didion et al. (2009)* involving 18 boars analysed, the percent of farrowing varied in the interval from 38.9-82.7%. *Holm et al. (2005)* confirmed that the value of oestrus in gilts was 14% and in the first farrowing sows 18%.

There are different studies on the traits of boar sperm and fertility in *in field* conditions. *Flowers (2003)* in his review paper presented the results of a several studies which indicate the differences in farrowing rate (from 65.8 to 92.5%) depending on the way of insemination, spermatozoa contained in dose, volume of doses and number of doses per sow.The results of *Tsakmakidis et al. (2010)* showed that the farrowing rate, in relation to differences in boar sperm, varied from 59.3 to 88.9%. For this reason, recommendation of some researchers *(Sutkevičienė and Žilinskas, 2004)* implies that maintaining the conception rate at a constant level means that ejaculates with lower motility of spermatozoa should be used so as to have larger number of spermatozoa in insemination doses. The success of insemination can depend upon a quality of ejaculate what is confirmed also by the research of *McPherson et al. (2014)* about the relationship of acrosomal defects of spermatozoa with a repeated occurrence of oestrus and their research implicate that the estimation of morphological characteristics of spermatozoa (particularly acrosomal region) can be used for predicting a repeated occurrence of oestrus. The research of *Park (2013)* in which a stimulative linear regression effect of overall motility of spermatozoa on the farrowing rate was established reports in favour of relationship between the ejaculate traits and success of insemination. The research of *Savić (2014)* implicates that fertilising capacity of sperm *in field* conditions depends primarily on spermatozoa motility.

In the research of *Savić (2014)* the average value of return rate in breeding females mated with boars of L, LW and D breeds was 11.70% and varied from 4.82% to 28.04%. The farrowing rate was on average 81.40% with variations from 63.55% to 90.00%. An average index of mating (100/81.40) indicates that 1.23 matings are necessary for one farrowing. The largest number of breeding females in which oestrus reappeared was mated with the boars of LW breed (12.79%). The lowest value of return rate was confirmed in the boars of Duroc breed (10.79%). Difference in return rate in breeding females between the boars of LW and D

breeds with which they mated was 2%. Breeding females that mated with LW boars also had lowest farrowing rate of 79.66% with highest varying interval of 22.60%, while breeding females mated with D boars, and compared to them, had farrowing rate higher for about 3%.The average value of return rate in breeding females mated with L breed boars was 11.55% and varied in the interval of 4.82 to 25.60%. Difference among the boars with the lowest and highest farrowing rate was 21.20%.

Bringing the sows into a contact with boars has a favourable effect on parameters of reproductive efficiency. In the research of *Umesiobi (2010)* different ways of exposing sows to the boars were applied (no contact, contact over the fence and physical contact) where the farrowing rates were 50.4, 62.9 and 88.3%, respectively, while depending on the frequency of ejaculation (interval between two jumps, 24 h and 92 h), the conception rates were 76.8% and 93.5% and farrowing rates were 56.8% and 85.5%. Contrary to previously mentioned, different frequency of exposing the sows after weaning (once, twice and three times daily) to the presence of boars showed no differences in the farrowing rate *(Knox et al., 2002;* 75%; 87% and 83%*)*.

Boar direct effect explains 5.3% of total variability of farrowing rate *(Broekhuijse et al., 2012b)*. The highest share in boar direct effect involve an individual (29%) and breed (22%), age of boar participates with 0.3%, progressive motility of sperm with 9%, and about 40% variability of the farrowing rate cannot be explained, according to the results of these authors. The research of *Broekhuijse et al. (2012a)* showed that only 5.9% of total variations of farrowing rate was caused by boar, while a variability of the sperm traits was by 21% explained by the effect of a boar genetic line, 11% by laboratory technique and 7% by the centre for artificial insemination. *Park (2013)* reported that variability of the farrowing rate was by 3.33% explained by the effect of boar, 1.22% by the effect of breeding female and 0.57% and 0.17% by the effect of year and season (month) of mating. If these rates were applied on a large population of animals a boar significant effect on reproductive parameters would be clearly seen.

The ranking of boars according to the value of return and farrowing rate during the reproductive exploitation is important part in the reproductive managment.

Conclusions

A large number of descendants per boar at an annual level clearly shows the need to control boar productivity. It is necessary to develop testing methodology by which their reproductive potential could be estimated in the most objective way possible and as earlier as possible. Reproductive parameters vary among the breeds and intensity of utilisation. Differences in return and farrowing rates between the boars can be larger than 20% what indicates the importance of

the research on fertility in boars. Early identification of hypoprolific boars (or close to the average) is needed. That is why the improving of reproductive traits means improving the traits of ejaculate, libido, reproductive efficiency and litter size traits. Therefore it is important to observe genetic structure of pig populations, to implement continuous control of productivity, to respect the mating plans and to exploit the boars in an optimal way.

Plodnost nerasta - šta je važno znati

Radomir Savić, Raquel Ausejo Marcos, Milica Petrović, Dragan Radojković, Čedomir Radović, Marija Gogić

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Ključne reči: nerast, veštačko osemenjavanje, osobine sperme, libido, reproduktivna efikasnost

Acknowledgment

This review research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia, project TR 31081.

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Received 5 May 2017; accepted for publication 20 June 2017

Biotechnology in Animal Husbandry 33 (2), p 151-159 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.082.453.64
https://doi.org/10.2298/BAH1702151Z

DEPENDENCIES BETWEEN SOME TRAITS OF SPERM PRODUCTION OF BOARS AT DIFFERENT AGE

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Original scientific paper

Abstract: The aim of the study was to establish correlation and regression dependencies between some traits of sperm production of terminal boars (Large White x Pietrain). The analyzed material consisted of 347 ejaculates, received in the period from 2011 to 2014. The boars were divided in three groups according to the age the ejaculate was obtained at (up to 12 months, from 12 to 24 months, and above 36 months). A moderate and negative correlation was established (r_p=-0.34, $p<0.001$) between the volume of the ejaculate and the concentration of the sperms in the second age group. With the youngest boars we established poor and positive correlation (r_p=0.29, $p<0.05$) between the volume of the semen and the motility of the sperm. The correlations between the traits concentration and motility are poor in the third (r_p=0.18, $p<0.05$), and moderate in the second age group (r_p=0.49, $p<0.001$). Between the motility from one side and the agglutinated and dead sperms from the other, they are within the range of r_p =-0.46 to r_p=-0.87, $p<0.001$. The correlations between the agglutinated and dead sperms are positive, from moderate to high and reliable ($p<0.001$) in all three age classes. The regression trend between the traits of sperm production are almost the same in all three age groups with the exception of volume of the ejaculate to the concentration of sperms.

Key words: boars, semen traits, correlations, regression, age class

Introduction

Artificial insemination (AI) has large economic importance in pig-breeding and in the past twenty years there has been a significant increase in its use. The results of AI depends mostly on quantitative and qualitative properties of the sperm *(Savić et al., 2015)*. According to *Ciereszko et al. (2000)* cross-bred boars are frequently used for reproduction. Numerous authors have determined that

ejaculates, received from cross-bred boars have better quantitative and qualitative semen traits in comparison with the pure-bred animals – *(Kondracki et al., 2003; Wysokińska and Kondracki, 2004) on Szostak and Przykaza (2016).* On the other hand, the establishment of the correlation and regression regularities appear as a reliable factor for the effective performance of the selection of farm animals *(Gerzilov, 2004).*

All of the above gave us reason to establish the correlation and regression dependencies between some of the sperm production traits of terminal boars (Large White x Pietrain), grouped in three age classes.

Materials and Methods

The study includes a total of 347 ejaculates, obtained in the period from January 2011 to May 2014, from 11 breeding boars (Large White x Pietrain), bred in a pig farm located in the region around the town of Plovdiv. The animals were divided in three groups according to the age the semen was obtained at (up to 12 months, from 12 to 24 months, and above 36 months).

The ejaculates were obtained by the double glove method, collected in a graduated semen-collection cup, covered with sterile gauze. Immediately after the collecting and filtering, the material was assessed for quantitative and qualitative traits, including:

- volume of the ejaculate (cm^3),

- concentration of the sperms ($x10^6$ sperm/cm^3), measured in a sperm densitometer

- motility (%), determined by a routine method, under microscope with standard magnification *(Nikolov et al., 2012).*

- Agglutinated and dead sperms (%) - determined by a routine method under light microscope, with magnification x400 *(Nikolov et al., 2012).*

Linear regression and Pearson's coefficient of correlation was used for bivariate correlation analysis, and were performed with SPSS software product version 19.

Results and Discussion

The average values of the studied traits of sperm production at the different age classes are shown in Table 1.

Table 1. Seminal characteristics of terminal boars

Traits	1-st age class		2-nd age class		3-th age class	
	n	LS±SD	n	LS±SD	n	LS±SD
Volume, (cm^3)	43	237.67±71.9	172	330.47±107.8	132	301.8±90.6
Concentration, ($x10^6$ sperm/cm^3),	43	468.84±82.9	171	393.86±109.8	131	436.6±73.6
Motility, (%)	43	74.88±8.34	169	73.05±8.4	132	71.06±3.4
Agglutinated spermatozoa, (%)	37	7.03±6.06	133	6.82±3.5	91	6.81±2.7
Dead spermatozoa, (%)	37	6.62±3.1	134	9.22±6.7	91	9.23±2.9

LS Mean; SD- Standard deviation

According to *Savić et al. (2013)* the volume and progressive motility of sperm are significant characteristics that determine the reproductive ability of semen. Based on a previous publication of ours *(Zapryanova and Hristev, unpublished data)*, whose aim was to make an analysis of the influence of the year, season and age when collecting semen from hybrid imported boars on the volume and concentration of the ejaculate, and the motility of the sperms, we determined that the age of the boar when collecting the ejaculate has a significant effect on the volume and concentration of the semen ($p<0.001$), and on the motility of the sperms ($p<0.05$). Boars below the age of 12 months have the smallest semen volume and the highest sperms motility. The breeding boars from the third age group are characterized with the lowest motility (Table 1). The largest percentage of abnormal sperms are found in the semen of the youngest boars, which is with the highest concentration as well. In the other two age classes the percentage of agglutinated sperms is practically the same. The results of our study are in unison with the information established by *Kondracki et al., (2013)*, who also report the highest presence of abnormal sperms in the semen with highest concentration. The group of up to 18 months of age have the smallest fraction of dead sperms, while the second and the third have almost identical values in this trait.

Table 2. Phenotypic correlations (r_p) between some indices of semen

Traits	Concentration	Motility	Agglutinated	Dead
Volume				
I age class	-0.06	0.29*	-0.08	-0.13
II age class	-0.34***	0.06	-0.03	-0.06
III age class	-0.03	0.06	0.01	-0.08
Concentration				
I age class		0.13	0.08	-0.13
II age class		0.49***	0.01	-0.26**
III age class		0.18*	-0.03	-0.04
Motility				
I age class			-0.65***	-0.77***
II age class			-0.56***	-0.77***
III age class			-0.46***	-0.64***
Agglutinated				
I age class				0.27
II age class				0.51***
III age class				0.41***

The correlations between the volume of the ejaculate and the sperms concentration are poor and unproven in age classes I and III, and moderate (r_p=-0.34, $p<0.001$) in the breeding boars from 12 to 24 months old (Table 2). For different grade phenotype correlations between the volume of the semen and the concentration of sperms of boars is also reported by *Buranawit and Imboonta (2016) and Wolf (2009)*, in whose experiments the values of the correlation coefficient is within the range of r_p=-0.20 to r_p=-0.6. In their own study of Duroc x Pietrain boars and their reciprocal crosses, *Szostak and Przykaz (2016)* establish strong correlations between the volume and the concentration of the sperms in the

semen depending of the season of collection of the ejaculate. The correlations between the volume and the motility are also poor, but positive and reliable ($p<0.05$) and they are only in the first group. The correlations between the motility and the concentration of the sperms in the second and third age class are poor to moderate r_p =0.49 ($p<0.001$) and r_p=0.18 ($p<0.05$) respectively. Between the traits of ejaculate volume and sperms concentration from one side, and the percentage of dead sperms from the other, there is a poor negative correlation, and only the connection between the sperms concentration and the percentage of dead sperms of boars below the age of two years is statistically proven ($p<0.001$).

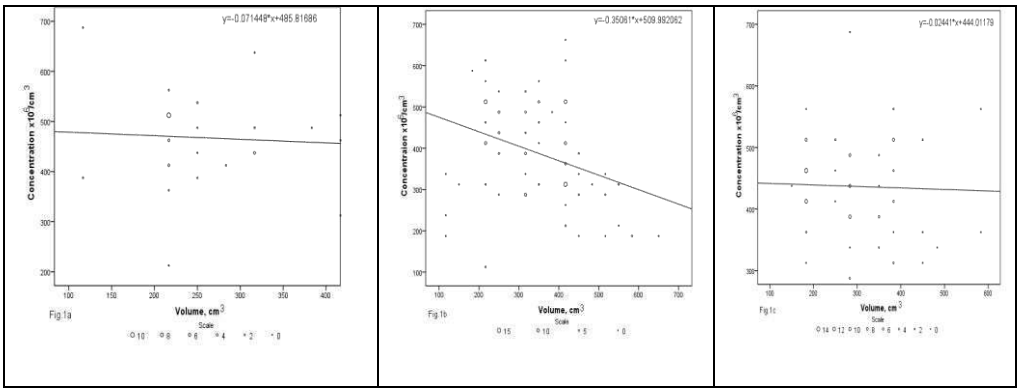

Figure 1. Regression between sperm volume and sperm concentration
1a-1-st age class, 1b- 2-nd age class, 1c- 3-th age class

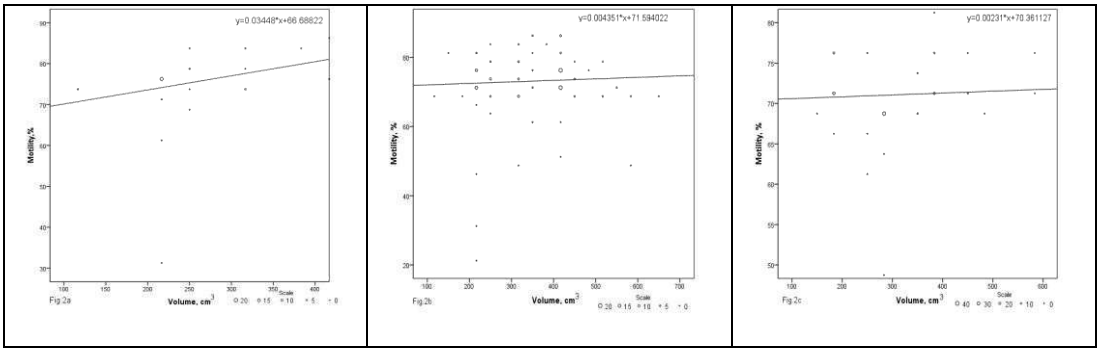

Figure 2 a, b, c. Regression between sperm volume and sperm motility
2a-1-st age class, 2b- 2-nd age class, 2c- 3-th age class

We have a moderate to strong correlation between the sperms motility and the percentage of agglutinated and dead sperms in all three age classes ($p<0.001$). The correlation between dead and agglutinated sperms is reliable ($p<0.001$) and positive in the second (r_p=0.51) and third (r_p=0.41) age class. Similar results are achieved with other animal species *(Gerzilov, 2004)*. The author establishes a

coefficient of phenotype correlation between the motility of the semen and the percentage of dead sperms (r_p=-0.430÷-0.440), during trials with Muscovy drakes in first and second reproductive season.

The regression dependencies between some of the studies traits of sperm production of boars are presented on figures 1-5. From figure 1a, c it is clear that the trend to a decrease of the volume of the ejaculate and the sperms concentration of the first and third age class is almost the same and quite slanting, while the regression line is a lot steeper for the boars from 12 to 24 months old (Fig. 1b)

With the exception of the first age group, where we have a reliable increase of the sperms motility in a larger volume of ejaculate (Fig.2a), the inclination of the other two groups is insignificant (Fig. 2b, c). In the conditions of our study we report increase in the sperms motility with increase of their concentration. This is especially noticeable with breeding boars before the age of 2 years (Fig. 3b), while in the youngest boars the positive dependency is the most poorly expressed (Fig. 3a).

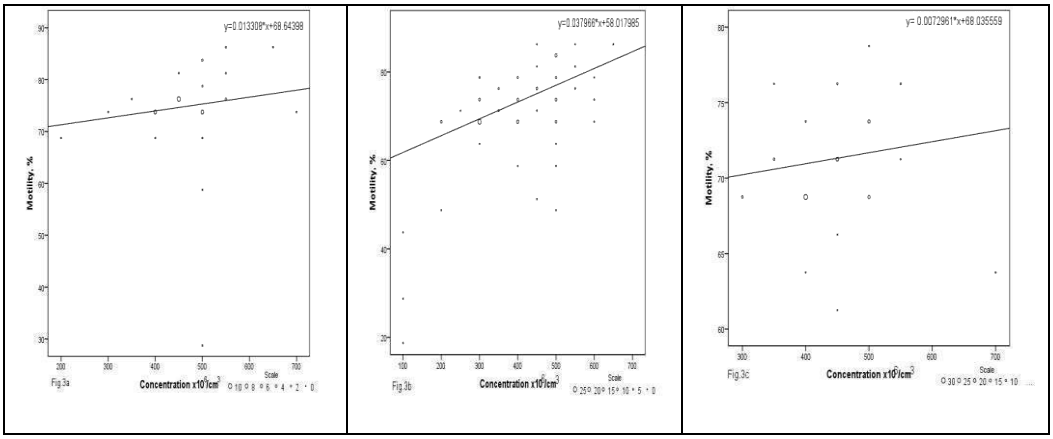

Figure 3 a, b, c. Regression between sperm concentration and sperm motility
3a-1-st age class, 3b- 2-nd age class, 3c- 3-th age class

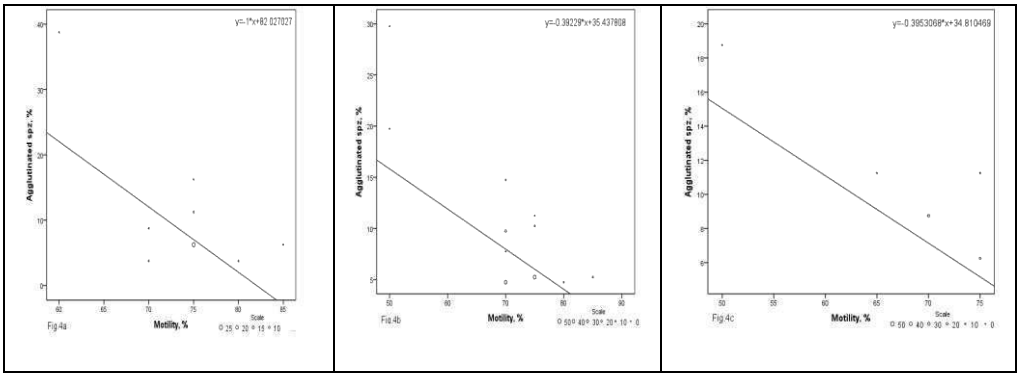

Figure 4 a, b, c. Regression between sperm motility and agglutinated spermatozoa
4a-1-st age class, 4b- 2-nd age class, 4c- 3-th age class

For the rest of the studied traits of sperm production – percentage agglutinated (Fig. 4a, b, c) and dead (Fig. 5a, b, c) sperms to the sperms motility trait, the regression trend is almost identical in all three age groups.

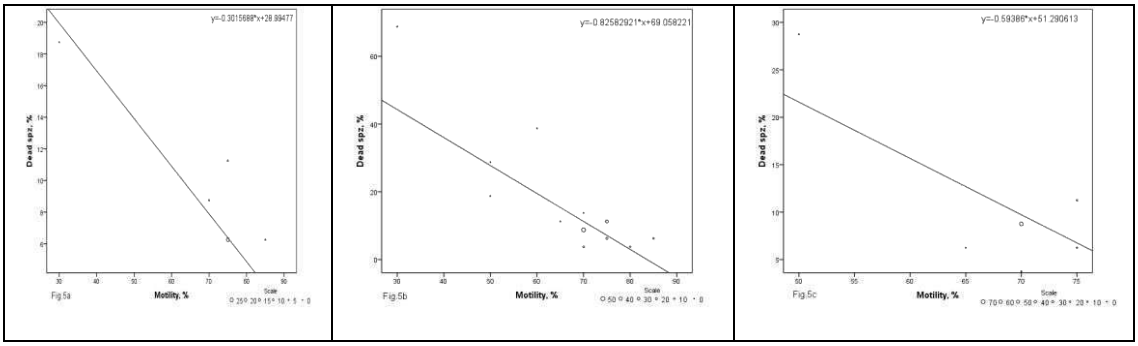

Figure 5 a, b, c. Regression between sperm motility and dead spermatozoa
5a-1-st age class, 5b- 2-nd age class, 5c- 3-th age class

Conclusion

The correlation between the volume of the ejaculate and the concentration of the sperms is negative and proven to be moderate for boars from the second age class (r_p=-0.34, $p<0.001$). Between the volume of the ejaculate and the motility of the sperms we have poor, positive and reliable correlation for the youngest breeding boars. The correlation links between the concentration and motility of the sperms are positive by trend, poor to moderate by grade for the second and third age class. Between the sperm motility from one side, and the agglutinated and dead

sperms from the other, we established reliable ($p<0.001$), moderate to strong and negative correlations in all three age classes.

The regression trend for the volume of the ejaculate to the concentration of the sperms is more clearly expressed for breeding boars up to the age of 2 years. The variation direction for the traits of volume and concentration towards the sperms motility as well as of the motility to the percentage of agglutinated and dead sperms is almost the same. There is an exception in the group from 18 to 24 months old regarding the traits of semen volume from one side towards the concentration and motility of the sperms from the other, where the regression dependencies are expressed more clearly.

Proizvodnja spermatozoida nerastova različitog uzrasta

Ivelina Zapranova

Rezime

Cilj istraživanja je bio da se utvrdi korelacija i regresione zavisnosti između nekih osobina u proizvodnji semena terminalnih nerastova (jorkšir x pijetren). Analizirani materijal se sastojao od 347 ejakulata, primljenih u periodu od 2011. do 2014. godine. Nerastovi su bili podeljeni u tri grupe, prema uzrastu nerastova u trenutku uzimanja ejakulata (do 12 meseci, od 12 do 24 meseci, iznad 36 meseci). Umerena i negativna korelacija ustanovljena je (r_p=-0.34, $p<0.001$) između zapremine ejakulata i koncentracije sperme u drugoj starosnoj grupi. Kod najmlađih nerastova ustanovili smo slabu i pozitivnu korelaciju (r_p=0.29, $p<0.05$) između zapremine semena i pokretljivosti sperme. Korelacije između osobina koncentracija i pokretljivosti su slabe u trećoj (r_p=0.18, $p<0.05$), odnosno umerene u drugoj starosnoj grupi (r_p=0.49, $p<0.001$). Između motiliteta, sa jedne strane, i aglutiniranih i mrtvih spermatozoida, sa druge, oni su u rasponu od r_p =-0.46 do r_p=-0.87, $p<0.001$. Korelacije između aglutiniranih i mrtvih spermatozoida su pozitivne, od umerenih do visokih i pouzdanih ($p<0,001$) u sve tri starosne grupe. Regresioni trend između osobina proizvodnje sperme je skoro isti u sve tri starosne grupe, sa izuzetkom zapremine ejakulata u odnosu na koncentraciju spermatozoida.

Ključne reči: nerastovi, osobine semena, korelacije, regresija, starosna klasa

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Received 23 November 2016; accepted for publication 23 January 2017

Biotechnology in Animal Husbandry 33 (2), p 161-169 , 2017
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.5'64
https://doi.org/10.2298/BAH1702161B

INFLUENCE OF LITTER SIZE ON GROWTH AND STRUCTURE OF *M. SEMITENDINOSUS* IN NEWBORN PIGLETS AND SLAUGHTER PIGS

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Original scientific paper

Abstract: Modern meat production has to meet the requirements of profitability, while meeting the demands of consumers in terms of meat quality at the same time. Huge scientific work has been done in order to find balance between quantity and quality of meet. Most authors agree that piglets of lower birth weight have less muscle fibers within muscle, grow slower, compensating the muscle growth with increase of muscle fiber diameter and accumulating carcass fat. In recent years, selection in pig production has been directed towards increase of piglet number per litter. Since the inverse relation of litter size and birth weight has been well documented, the purpose of this work was to investigate the possible effects of litter size as a factor on pig growth and *m. semitendinosus* characteristics. Except the statistically significant difference (p=0,05) in number of primary fibers among piglets from small litter (15053) and large litter (11347), litter size did not influence birth weight, or other observed morphological and histological characteristics of the muscle significantly. Similarly, results of this research show that litter size as a factor did not affect final weight, morphological characteristics or fiber type distribution within the muscle in slaughter pigs.

Key words: litter size, muscle structure, muscle fibers, growth, pigs

Introduction

Quantity and quality of pig meat are largely depending on structure and growth of skeletal muscles (*Picard et al., 2002*). In adult animals, skeletal muscles are built of elongated muscle cells – muscle fibers. Based on speed of contraction

and type of metabolism, muscle fibers can be classified as: slow twitch oxidative (STO), fast twitch oxidative (FTO) and fast twitch glycolitic (FTG) (*Lefaucheur and Gerrard, 2000)*. During myogenesis, primary fibers occur first, followed by the development of huge number of secondary fibers and some tertiary fibers. After birth in pigs, primary fibers transform into STO fibers, while secondary and tertiary fibers will transform into fibers of fast contractile type (*Brameld et al., 2008*). After birth, the number of muscle fibers increases only during first weeks in pigs (*Rehfeldt et al., 2000; Rehfeldt et al., 2008; Berard et al., 2011*). In adult pigs, muscle weight increases based on hypertrophy – enlargement of girth and length of a muscle fiber (*Te Paas et al., 2004*). Therefore, the final weight of the muscle is determined by the number of muscle fibers given at birth, and growth of fibers during production.

Different factors are influencing growth and development of muscle fibers (*Vitorović and Adamović, 2012*). During last years, selection in pig production was directed towards increasing the number of piglets per litter, which led to a big variation of body weight and general decrease of piglets birth weight and higher number of piglets born dead in larger litters (*Quiniou et al., 2002; Sorensen et al, 2000*). A possible explanation could be a weaker supply of nutrients to fetuses in large litters (*Perre and Etienne, 2000*), or much stronger competition for nutrients *in utero* (*Quiniou et al., 2002*), leading to decrease of fetus weight and piglet birth weight with increase of litter size. Large number of authors agrees that piglets of higher birth weight have a higher total number of muscle fibers (*Wigmore and Stickland, 1983; Dwyer and Stickland, 1991; Rehfeldt et al., 2001; Nissen et al., 2004; Rehfeldt and Kuhn, 2006*). At the same time, newer research (*Rehfeldt and Kuhn, 2006; Gondret et al., 2006; Berard et al., 2008*) have shown that piglets of lower birth weight have slower growth, ending with larger diameter muscle fibers (*Gondret et al., 2006*). Such animals have higher percent of carcass fat, higher percent of undesirable giant fibers, and poor meat quality (*Rehfeldt and Kuhn, 2006). Berard et al. (2008)* suggest that litter size affects growth, carcass characteristics and meat quality only indirectly, and that this impact is realized through an inverse correlation with birth weight. On the other hand, *Beaulieu et al. (2010)* have found litter size influencing piglet birth weight, and somewhat slower growth of smaller litter mates, but the authors did not determine any significant influence of litter size on carcass composition and meat quality.

The aim of this paper was to examine the possible effects of litter size on growth, morphological and histological characteristics of *m. semitendinosus* muscle in piglets at birth and animals at the end of fattening period.

Material and methods

Animals and feeding

The research was conducted at the experimental station of the Leibnitz Institute for Farm Animal Biology - FBN Dummerstorf, Rostock, Germany. Pigs of German Landrace breed were used for the investigation. All procedures including use and treatment of animals were in accordance with the guidelines set by the Animal Care Committee of the State Mecklenburg-Vorpommern, Germany, based on the German Law of Animal Protection. Eight multiparous sows were bred to the same German Landrace boar. Sow pregnancy was confirmed at day 28 of gestation by ultrasound. The sows were housed individually, under controlled environmental conditions (temperature 19°C, relative humidity 60-80 %). All animals had free access to water, and were manually fed twice daily with standard soy based concentrate (Denkavit, Trede&Pein GmbH&Co. KG, Itzehohe, Germany). During the experiment two sows had to be excluded, due to bone desease and premature farrowing. To induce farrowing, on day 114 of pregnancy all sows were injected intramuscularly with 1 ml of a synthetic prostaglandin (cloprostenol, 75 mg/ml: AniMedica West, Chemische Produkte GmbH, Senden, Germany). After birth, the body weight of piglets was recorded, as well as the litter size of each sow. Mediane value for number of piglets per litter was 14, and therefore litters were classified as small (<14 piglets) or large (>14 piglets). From each litter two male piglets with body weight closest to the average for that litter were sacrificed for further analysis by injection of 1 ml mixture of Ursotamin (Ursotamin, Serumwerk Bernburg AG, Germany) and Combelen (Combelen, Bayer AG, Leverkusen, Germany) in proportion 1:1. The remaining male piglets were castrated at day 5 after birth, and all piglets were weaned at day 28 of age. During the whole growing-finishing period the offspring was fed ad libitum, with standard commercial starter, grower and finisher feed mixtures. The growing period lasted until 180 days of age, and average market weight of slaughter pigs at the end of fattening was 108,35 kg.

Muscle histology and histochemistry

For histological and histochemical analysis, right side *m. semitendinosus* was used in piglets, and left side *m. semitendinosus* in slaughter pigs. In newborn piglets, muscle cross sectional area (MCSA) was estimated from the circumference of the muscle mid belly. Pieces of the mid belly from the neonatal muscle were mounted on cork-chucks and snap frozen in isopentane cooled in liquid nitrogen. Whole muscle serial transverse sections of 10 and 16 μm were cut at -20°C in a cryostat (Reichert-Jung, Leica, Nussloch, Germany). Muscle tissue sections of 10 μm were stained with eosin (Romeis, 1989) and used for determination of the total

muscle fiber number per cross section, and sections of 16 μm were stained for myosin ATPase after acid preincubation at pH 4.2 (Guth and Samaha, 1970), and used for determination of primary and secondary fibers. Since in the pig muscle the central slow fiber in each cluster developed as primary fiber, the number of primary fibers corresponds to number of dark, central fibers of the largest diameter in the cluster. The number of secondary fibers was calculated by difference.

In adult pigs, two samples were taken from *m. semitendinosus* of each individual: one sample was taken from the deep dark portion of the mid belly, and the second sample was taken from the superficial bright portion of the mid belly. Pieces of the muscle were mounted on cork-chucks and snap frozen in liquid nitrogen. Serial sections were cut at 10 μm in a cryostat, and stained for cytoplasm and nuclei by hematoxylin/eosin (Romeis, 1989), or exposed to a combined reaction for NADH-tetrazolium reductase (NADH-TR) (Novikoff et all, 1961) and acid preincubated ATPase at pH 4,2 (Guth and Samaha, 1970), which enables to classify STO, FTO and FTG muscle fibers. Further image analysis of sections of adult pig muscle was done by AMBA software (AMBA, IBSB, Berlin, Germany). On hematoxylin/eosin stained sections the number of fibers and cross sectional area (FCSA) of individual fibers were determined first, and immediately afterwards the sections stained for fiber types were analyzed. Average values for investigated parameters calculated from both regions of the muscle were taken for further analysis. TFN was calculated by multiplying the number of fibers/unit area with FCSA.

Statistical analysis

Data were subjected to analysis of variance using the GLM and mixed classification models of SAS, tested with Students T-test (SAS System for Windows Release 8e, SAS Institute Inc., Cary, NC 27513, USA).

Results and discussion

Piglets

Besides the birth weight, most important morphological characteristics (weight, length, girth and cross section) and histological characteristics of *m. semitendinosus* (total fiber number, number of primary and secondary fibers, percent of primary fibers and secondary:primary fibers ratio) were analysed in this experiment. The results are shown in the Table 1.

Table 1. Influence of litter size on birth weight, morphological characteristics and histological structure of *m. semitendinosus* in piglets, LSM±SE

Parameter	Small litter	Large litter	P value
Number of animals, n	8	4	
Birth weight, kg	1.32±0.06	1.13±0.08	0.106
Morphological characteristics			
- weight of *m. semitendinosus*, g	2.98±0.20	2.54±0.25	0.218
- length of *m. semitendinosus*, cm	4.79±0.21	4.68±0.25	0.734
- girth of *m. semitendinosus*, cm	3.78±0.16	3.53±0.19	0.357
- cross section of *m. semitendinosus*, cm^2	1.14±0.09	1.00±0.11	0.382
Histological characteristics			
- total fiber number	379248±23716	325343±29046	0.201
- number of primary fibers	15053±964.55	11347±1181.33	0.051
- number of secondary fibers	364196±22876	313996±28018	0.201
- primary fibers, %	3.97±0.18	3.56±0.22	0.197
- secondary:primary ratio	24.33±1.25	27.47±1.53	0.164

Body weight of newborn piglets was higher in animals from small litters (1,32 kg compared to 1,13 kg), but no statistically significant difference was determined. P value for this parameter (p=0.106) could be interpreted as a tendency. Similar situation was registered for all morphological characteristics of the muscle. Numerically higher values were found for weight, length, girth and cross section of the muscle in small litter piglets, but the differences were not statistically significant.

The influence of litter size on histological structure of the muscle was monitored through total fiber number, number of different types of fibers, percentage of primary fibers and secondary:primary fibers ratio. Although all characteristics were higher in small litter piglets, only for the number of primary fibers statistical significance (p=0.05) was determined. Significantly higher number of primary fibers was found in piglets from small litters (15053) compared to piglets from large litters (11347).

Slaughter animals

In animals at the end of fattening period, numerous muscle characteristics besides the final weight have been analyzed, and the results are summarized in Table 2.

Table 2. Influence of litter size on final weight, morphological characteristics and histological structure of *m. semitendinosus* in pigs at the end of fattening period, LSM±SE

Parameter	Small litter	Large litter	P value
Number of animals, n	n=40	n=22	
Body weight, kg	108.16±1.96	103.28±2.57	0.18
Morphological characteristics	n=23	n=8	
- weight of *m. semitendinosus*, g	477.80±21.37	465.03±36.03	0.78
- length of *m. semitendinosus*, cm	23.65±0.47	23.34±0.83	0.76
- girth of *m. semitendinosus*, cm	21.52±0.40	21.27±0.68	0.77
- cross section of *m. semitendinosus*, cm^2	37.00±1.31	36.04±2.21	0.73
Histological characteristics	n=22	n=7	
- total fiber number	953127±42791	937878±72637	0.87
- STO fiber area	4518.94±373.13	4451.11±632.22	0.93
- FTO fiber area	4137.42±245.74	4001.44±417.30	0.79
- FTG fiber area	3673.07±138.26	3679.65±246.95	0.98
- Average fiber area	3972.92±173.66	3928.01±301.27	0.90
- STO fibers, %	18.82±0.88	22.09±1.57	0.15
- FTO fibers, %	30.81±1.45	26.20±2.49	0.19
- FTG fibers, %	49.66±1.44	50.31±2.50	0.83

Although all characteristics were numerically higher in animals from small liters, no statistically significant differences were determined neither for slaughter weight nor for morphological characteristics of *m. semitendinosus* between animals from small and large litters. Accordingly, analysis of live weight gain (data not shown) showed no statistically significant impact of litter size. Also, litter size did not affect total fiber number and area of different fiber types. Histological structure of *m. semitendinosus* was different in slaughter animals from small and large litters, but the differences were again not statistically significant. The percent of STO fibers was higher in large litter animals, while percent of FTO fibers was higher in animals from small litters compared to animals from large litters (31% and 26% respectively). The percent of FTG fibers was eqal in both groups, about 50%.

The data on litter size influence on muscle morphological and histological characteristics are very scarce in literature, especially for piglets. Our results have shown no statistically significant impact of litter size on muscle characteristics in pigs. However, since all investigated muscle characteristics had higher numerical values in small litter piglets compared to large litter animals, and having in mind that due to complexity of this research only two average weight male piglets per litter were used for analysis, further investigations would be required. Results monitored in slaughter pigs in this experiment are in accordance with findings of *Beaulieu et al. (2010),* showing no significant affects of litter size on *m. semitendinosus* characteristics.

Conclusion

Pig meat production has been directed towards the increase of number of piglets per litter in the past decades. In recent years, increased need of consumers for meat of better quality focused the attention of researchers on examination of possible influence of piglet birth weight on muscle structure. Previous studies have shown that lower birth weight piglets are growing slower, by increasing muscle fiber diameter (hypertrophy) and accumulation of carcass fat. Some authors reported that litter size is affecting muscle characteristics indirectly, through an inverse correlation with birth weight. Although values for all investigated parameters in this research were numerically higher in piglets from small litters, statistically significant differences among piglets from small and large litters were determined neither for birth or final body weight, nor for *m. semitendinosus* morphological and histological characteristics, with exception of number of primary fibers in piglets. However, data on the influence of litter size on the growth and muscle properties are rare in the literature. Therefore, further studies would be necessary to examine the possible impact of litter size either as independent factor or in combination with other factors that are influencing pig meat production.

Uticaj veličine legla na porast i strukturu *m. semitendinosus*-a kod novorođene prasadi i tovljenika

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Rezime

Savremena proizvodnja mesa usmerena je na profitabilnost sa jedne strane uz istovremeno odgovaranje zahtevima potrošača u pogledu kvaliteta mesa sa druge. Isrcpna naučna istraživanja obavljaju se u cilju pronalaženja balansa između količine i kvaliteta mesa. Najveći broj autora slaže se da prasad manje porođajne mase ima manji broj mišićnih vlakana u skeletnim mišićima, sporije raste, kompenzujući mišićni porast povećanjem prečnika mišićnih vlakana i deponovanjem veće količine masti u trupu. Poslednjih godina selekcija u svinjarstvu bila je usmeravana u pravcu povećanja broja prasadi u leglu. Obzirom da je utvrđena inverzna korelacija između veličine legla i mase prasadi na rođenju, cilj ovog rada bio je da se prouče mogući uticaji veličine legla kao faktora na porast svinja i karakteristike *m. semitendinosus*-a. Osim utvrđene statistički značajne razlike (p=0,05) u broju primarnih vlakana kod prasadi iz malog legla (15053) u odnosu na prasad iz velikog legla (11347), analiza nije pokazala uticaj veličine legla na masu prasadi pri rođenju i druge posmatrane morfološke i

histološke osobine mišića. Slično tome, ni kod tovljenika nije utvrđen uticaj veličine legla kao faktora na klaničnu masu, morfološke karakteristike ispitivanog mišića ili na zastupljenost pojedinih tipova mišićnih vlakana u mišiću.

Ključne reči: veličina legla, struktura mišića, mišićna vlakna, porast, svinje

Acknowledgement

The work was supported by the project TR 31081 of the Ministry of Education, Science and Technological Development, Republic of Serbia.

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Received 19 April 2017; accepted for publication 3 June 2017

Biotechnology in Animal Husbandry 33 (2), p 151-159 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.082.453.64
https://doi.org/10.2298/BAH1702151Z

DEPENDENCIES BETWEEN SOME TRAITS OF SPERM PRODUCTION OF BOARS AT DIFFERENT AGE

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Abstract: The aim of the study was to establish correlation and regression dependencies between some traits of sperm production of terminal boars (Large White x Pietrain). The analyzed material consisted of 347 ejaculates, received in the period from 2011 to 2014. The boars were divided in three groups according to the age the ejaculate was obtained at (up to 12 months, from 12 to 24 months, and above 36 months). A moderate and negative correlation was established (r_p=-0.34, $p<0.001$) between the volume of the ejaculate and the concentration of the sperms in the second age group. With the youngest boars we established poor and positive correlation (r_p=0.29, $p<0.05$) between the volume of the semen and the motility of the sperm. The correlations between the traits concentration and motility are poor in the third (r_p=0.18, $p<0.05$), and moderate in the second age group (r_p=0.49, $p<0.001$). Between the motility from one side and the agglutinated and dead sperms from the other, they are within the range of r_p =-0.46 to r_p=-0.87, $p<0.001$. The correlations between the agglutinated and dead sperms are positive, from moderate to high and reliable ($p<0.001$) in all three age classes. The regression trend between the traits of sperm production are almost the same in all three age groups with the exception of volume of the ejaculate to the concentration of sperms.

Key words: boars, semen traits, correlations, regression, age class

Introduction

Artificial insemination (AI) has large economic importance in pig-breeding and in the past twenty years there has been a significant increase in its use. The results of AI depends mostly on quantitative and qualitative properties of the sperm *(Savić et al., 2015)*. According to *Ciereszko et al. (2000)* cross-bred boars are frequently used for reproduction. Numerous authors have determined that

ejaculates, received from cross-bred boars have better quantitative and qualitative semen traits in comparison with the pure-bred animals – *(Kondracki et al., 2003; Wysokińska and Kondracki, 2004) on Szostak and Przykaza (2016).* On the other hand, the establishment of the correlation and regression regularities appear as a reliable factor for the effective performance of the selection of farm animals *(Gerzilov, 2004).*

All of the above gave us reason to establish the correlation and regression dependencies between some of the sperm production traits of terminal boars (Large White x Pietrain), grouped in three age classes.

Materials and Methods

The study includes a total of 347 ejaculates, obtained in the period from January 2011 to May 2014, from 11 breeding boars (Large White x Pietrain), bred in a pig farm located in the region around the town of Plovdiv. The animals were divided in three groups according to the age the semen was obtained at (up to 12 months, from 12 to 24 months, and above 36 months).

The ejaculates were obtained by the double glove method, collected in a graduated semen-collection cup, covered with sterile gauze. Immediately after the collecting and filtering, the material was assessed for quantitative and qualitative traits, including:

- volume of the ejaculate (cm^3),

- concentration of the sperms ($x10^6$ sperm/cm^3), measured in a sperm densitometer

- motility (%), determined by a routine method, under microscope with standard magnification *(Nikolov et al., 2012).*

- Agglutinated and dead sperms (%) - determined by a routine method under light microscope, with magnification x400 *(Nikolov et al., 2012).*

Linear regression and Pearson's coefficient of correlation was used for bivariate correlation analysis, and were performed with SPSS software product version 19.

Results and Discussion

The average values of the studied traits of sperm production at the different age classes are shown in Table 1.

Table 1. Seminal characteristics of terminal boars

Traits	1-st age class		2-nd age class		3-th age class	
	n	LS±SD	n	LS±SD	n	LS±SD
Volume, (cm^3)	43	237.67±71.9	172	330.47±107.8	132	301.8±90.6
Concentration, ($x10^6$ sperm/cm^3),	43	468.84±82.9	171	393.86±109.8	131	436.6±73.6
Motility, (%)	43	74.88±8.34	169	73.05±8.4	132	71.06±3.4
Agglutinated spermatozoa, (%)	37	7.03±6.06	133	6.82±3.5	91	6.81±2.7
Dead spermatozoa, (%)	37	6.62±3.1	134	9.22±6.7	91	9.23±2.9

LS Mean; SD- Standard deviation

According to *Savić et al. (2013)* the volume and progressive motility of sperm are significant characteristics that determine the reproductive ability of semen. Based on a previous publication of ours *(Zapryanova and Hristev, unpublished data)*, whose aim was to make an analysis of the influence of the year, season and age when collecting semen from hybrid imported boars on the volume and concentration of the ejaculate, and the motility of the sperms, we determined that the age of the boar when collecting the ejaculate has a significant effect on the volume and concentration of the semen ($p<0.001$), and on the motility of the sperms ($p<0.05$). Boars below the age of 12 months have the smallest semen volume and the highest sperms motility. The breeding boars from the third age group are characterized with the lowest motility (Table 1). The largest percentage of abnormal sperms are found in the semen of the youngest boars, which is with the highest concentration as well. In the other two age classes the percentage of agglutinated sperms is practically the same. The results of our study are in unison with the information established by *Kondracki et al., (2013)*, who also report the highest presence of abnormal sperms in the semen with highest concentration. The group of up to 18 months of age have the smallest fraction of dead sperms, while the second and the third have almost identical values in this trait.

Table 2. Phenotypic correlations (r_p) between some indices of semen

Traits	Concentration	Motility	Agglutinated	Dead
Volume				
I age class	-0.06	0.29*	-0.08	-0.13
II age class	-0.34***	0.06	-0.03	-0.06
III age class	-0.03	0.06	0.01	-0.08
Concentration				
I age class		0.13	0.08	-0.13
II age class		0.49***	0.01	-0.26**
III age class		0.18*	-0.03	-0.04
Motility				
I age class			-0.65***	-0.77***
II age class			-0.56***	-0.77***
III age class			-0.46***	-0.64***
Agglutinated				
I age class				0.27
II age class				0.51***
III age class				0.41***

The correlations between the volume of the ejaculate and the sperms concentration are poor and unproven in age classes I and III, and moderate (r_p=-0.34, $p<0.001$) in the breeding boars from 12 to 24 months old (Table 2). For different grade phenotype correlations between the volume of the semen and the concentration of sperms of boars is also reported by *Buranawit and Imboonta (2016) and Wolf (2009)*, in whose experiments the values of the correlation coefficient is within the range of r_p=-0.20 to r_p=-0.6. In their own study of Duroc x Pietrain boars and their reciprocal crosses, *Szostak and Przykaz (2016)* establish strong correlations between the volume and the concentration of the sperms in the

semen depending of the season of collection of the ejaculate. The correlations between the volume and the motility are also poor, but positive and reliable ($p<0.05$) and they are only in the first group. The correlations between the motility and the concentration of the sperms in the second and third age class are poor to moderate r_p =0.49 ($p<0.001$) and r_p=0.18 ($p<0.05$) respectively. Between the traits of ejaculate volume and sperms concentration from one side, and the percentage of dead sperms from the other, there is a poor negative correlation, and only the connection between the sperms concentration and the percentage of dead sperms of boars below the age of two years is statistically proven ($p<0.001$).

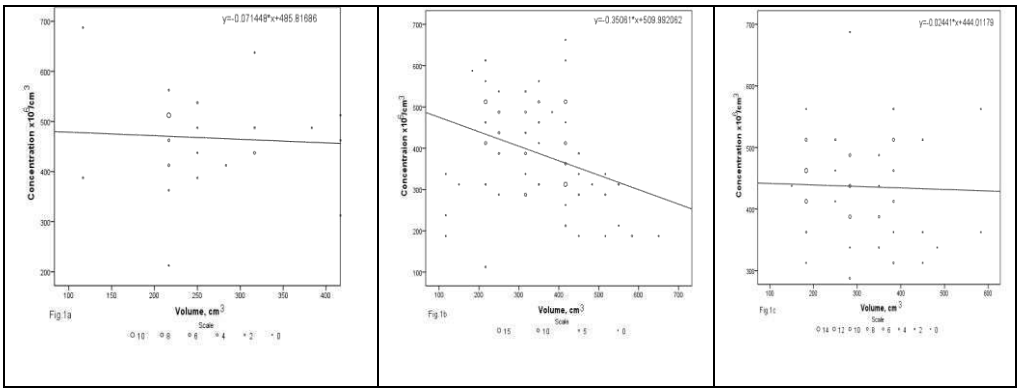

Figure 1. Regression between sperm volume and sperm concentration
1a-1-st age class, 1b- 2-nd age class, 1c- 3-th age class

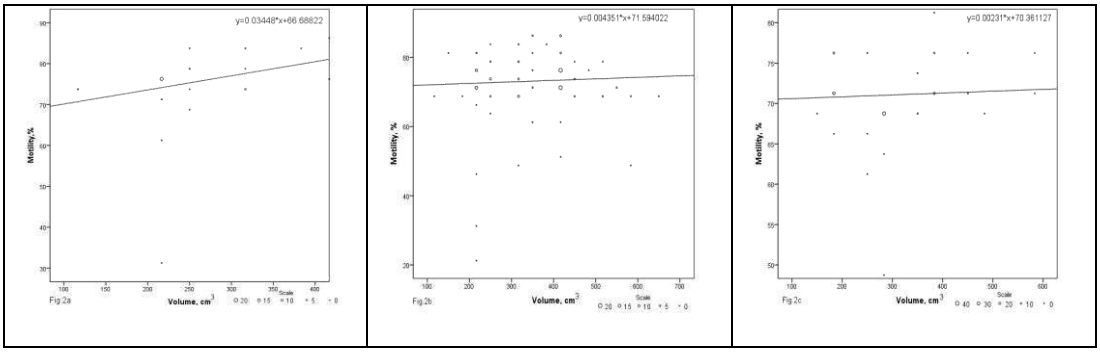

Figure 2 a, b, c. Regression between sperm volume and sperm motility
2a-1-st age class, 2b- 2-nd age class, 2c- 3-th age class

We have a moderate to strong correlation between the sperms motility and the percentage of agglutinated and dead sperms in all three age classes ($p<0.001$). The correlation between dead and agglutinated sperms is reliable ($p<0.001$) and positive in the second (r_p=0.51) and third (r_p=0.41) age class. Similar results are achieved with other animal species *(Gerzilov, 2004)*. The author establishes a

coefficient of phenotype correlation between the motility of the semen and the percentage of dead sperms (r_p=-0.430÷-0.440), during trials with Muscovy drakes in first and second reproductive season.

The regression dependencies between some of the studies traits of sperm production of boars are presented on figures 1-5. From figure 1a, c it is clear that the trend to a decrease of the volume of the ejaculate and the sperms concentration of the first and third age class is almost the same and quite slanting, while the regression line is a lot steeper for the boars from 12 to 24 months old (Fig. 1b)

With the exception of the first age group, where we have a reliable increase of the sperms motility in a larger volume of ejaculate (Fig.2a), the inclination of the other two groups is insignificant (Fig. 2b, c). In the conditions of our study we report increase in the sperms motility with increase of their concentration. This is especially noticeable with breeding boars before the age of 2 years (Fig. 3b), while in the youngest boars the positive dependency is the most poorly expressed (Fig. 3a).

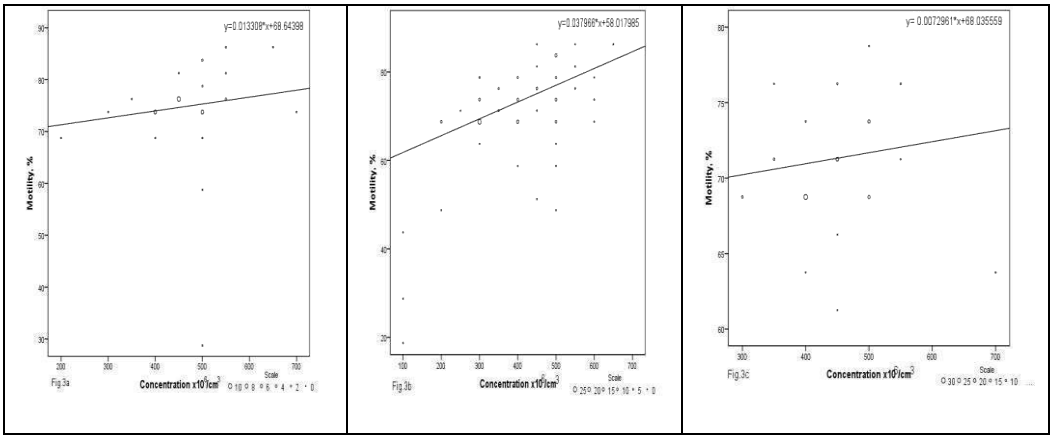

Figure 3 a, b, c. Regression between sperm concentration and sperm motility
3a-1-st age class, 3b- 2-nd age class, 3c- 3-th age class

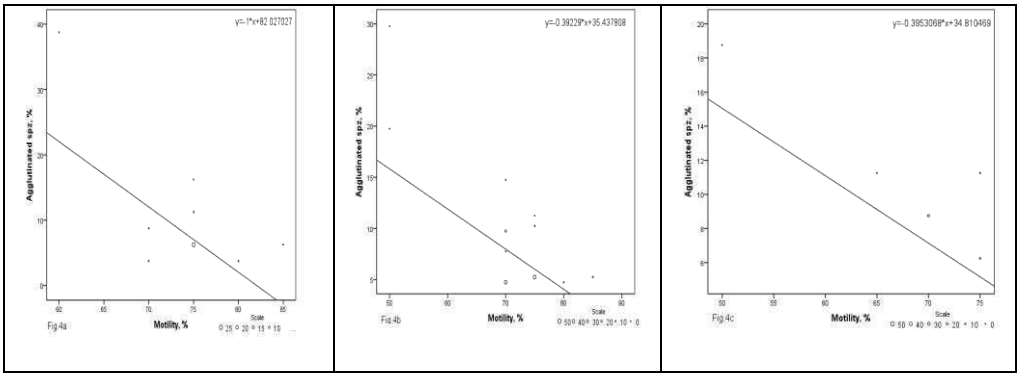

Figure 4 a, b, c. Regression between sperm motility and agglutinated spermatozoa
4a-1-st age class, 4b- 2-nd age class, 4c- 3-th age class

For the rest of the studied traits of sperm production – percentage agglutinated (Fig. 4a, b, c) and dead (Fig. 5a, b, c) sperms to the sperms motility trait, the regression trend is almost identical in all three age groups.

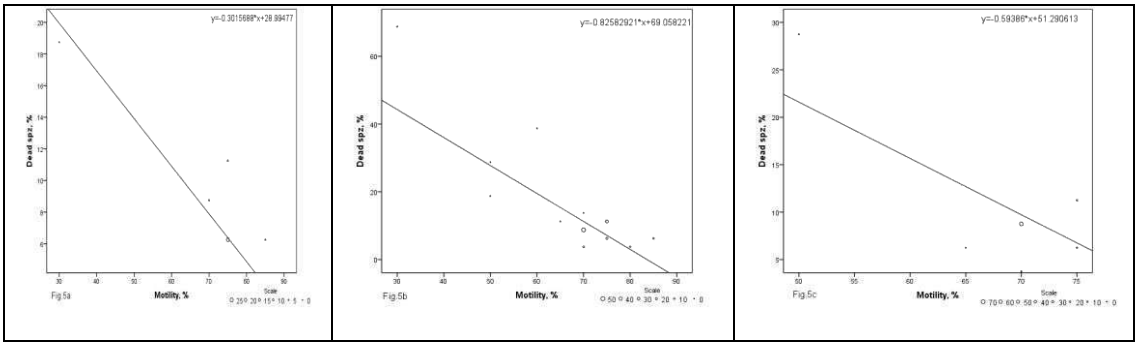

Figure 5 a, b, c. Regression between sperm motility and dead spermatozoa
5a-1-st age class, 5b- 2-nd age class, 5c- 3-th age class

Conclusion

The correlation between the volume of the ejaculate and the concentration of the sperms is negative and proven to be moderate for boars from the second age class (r_p=-0.34, $p<0.001$). Between the volume of the ejaculate and the motility of the sperms we have poor, positive and reliable correlation for the youngest breeding boars. The correlation links between the concentration and motility of the sperms are positive by trend, poor to moderate by grade for the second and third age class. Between the sperm motility from one side, and the agglutinated and dead

sperms from the other, we established reliable ($p<0.001$), moderate to strong and negative correlations in all three age classes.

The regression trend for the volume of the ejaculate to the concentration of the sperms is more clearly expressed for breeding boars up to the age of 2 years. The variation direction for the traits of volume and concentration towards the sperms motility as well as of the motility to the percentage of agglutinated and dead sperms is almost the same. There is an exception in the group from 18 to 24 months old regarding the traits of semen volume from one side towards the concentration and motility of the sperms from the other, where the regression dependencies are expressed more clearly.

Proizvodnja spermatozoida nerastova različitog uzrasta

Ivelina Zapranova

Rezime

Cilj istraživanja je bio da se utvrdi korelacija i regresione zavisnosti između nekih osobina u proizvodnji semena terminalnih nerastova (jorkšir x pijetren). Analizirani materijal se sastojao od 347 ejakulata, primljenih u periodu od 2011. do 2014. godine. Nerastovi su bili podeljeni u tri grupe, prema uzrastu nerastova u trenutku uzimanja ejakulata (do 12 meseci, od 12 do 24 meseci, iznad 36 meseci). Umerena i negativna korelacija ustanovljena je (r_p=-0.34, $p<0.001$) između zapremine ejakulata i koncentracije sperme u drugoj starosnoj grupi. Kod najmlađih nerastova ustanovili smo slabu i pozitivnu korelaciju (r_p=0.29, $p<0.05$) između zapremine semena i pokretljivosti sperme. Korelacije između osobina koncentracija i pokretljivosti su slabe u trećoj (r_p=0.18, $p<0.05$), odnosno umerene u drugoj starosnoj grupi (r_p=0.49, $p<0.001$). Između motiliteta, sa jedne strane, i aglutiniranih i mrtvih spermatozoida, sa druge, oni su u rasponu od r_p =-0.46 do r_p=-0.87, $p<0.001$. Korelacije između aglutiniranih i mrtvih spermatozoida su pozitivne, od umerenih do visokih i pouzdanih ($p<0,001$) u sve tri starosne grupe. Regresioni trend između osobina proizvodnje sperme je skoro isti u sve tri starosne grupe, sa izuzetkom zapremine ejakulata u odnosu na koncentraciju spermatozoida.

Ključne reči: nerastovi, osobine semena, korelacije, regresija, starosna klasa

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Received 23 November 2016; accepted for publication 23 January 2017

Biotechnology in Animal Husbandry 33 (2), p 171-180 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 575
https://doi.org/10.2298/BAH1702171A

GENETIC DIVERSITY OF LACTOFERRIN GENE IN-SILICO ON SELECTED MAMMALIAN SPECIES

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Original scientific paper

Abstract: A total of 17 lactoferrin gene sequences belonging to 6 species: cattle (3), buffalo (3), sheep (3), goat (3), horse (2) and camel (3), were retrieved from Genbank (www.ncbi.nlm.nih.gov). Sequences alignment, translation and comparison were done with ClustalW of the MEGA 6.0. The present study therefore aimed at examining the genetic diversity of Lf gene *in-silico* on selected mammalian species. The Dxy inferred using p-distance revealed a maximum value of 0.50 between buffalo, sheep and goat whereas a minimum value of 0.01 was realized between sheep and goat. The maximum Dxy value of 0.15 were observed between horse and camel whereas no minimum value was recorded during the investigation. The Neighbour Joining tree from the phylogenetic analysis showed trans-species evolution however, UPGMA tree topology was species-wise. This genetic tree obtained advance some form of proximity and differentiation in Lactoferrin gene sequences within and among the mammalian species studied which provide basis for selection of livestock in terms of genetic relationship.

Keywords: Diversity, In-silico, Lactoferrin, Mammalian, Sequence, Phylogenetic

Introduction

Lactoferrin (LF) is a single-chain, iron-binding glycoprotein of 80 kDa that belongs to the serum transferrin gene family called the red protein. LF is present in milk but also in other exocrine secretions such as tears, semen, saliva, and cervical mucus (*Wakabayashi et al., 2006*). The protein is synthesized by granulocytes and mammary epithelial cells in response to infections such as mastitis (*Kaminski et al., 2006*). Lactoferrin is able to sequester 2 molecules of iron making them unavailable to pathogenic organisms. In addition to this bacteriostatic activity, LF is endowed with antifungal and bactericidal effects (*Wakabayashi et al., 2006*).

Indeed, LF can interact with the lipid A of LPS contained in bacterial membrane. Lactoferrin can affect the destabilization of gram-negative bacterial membranes by preventing LPS from interacting with the main actors of LPS signaling such as CD14 (*Baveye et al., 1999*). Further, LF modulates the inflammatory process, immune system response, and cell growth. This multifunctional protein plays a key role in the health of mammary gland. Thus, it could be considered as a potential candidate gene in dairy mastitis resistance selection (*Seyfert et al., 1996; Wojdak-Maksymiec et al., 2006).*

Protein molecule of lactoferrin contains two lobes, both built of two globular domains (*Moore et al. 1997)*. There is a galore of lactoferrin biological functions and among them a special attention is being paid to its antibacterial (*Małaczewska and Rotkiewicz, 2007; Gonzalez-Chavez et al., 2009*), antiviral (*Rotkiewicz, 2007; Gonzalez-Chavez et al., 2009*), antitumor (*Małaczewska and Rotkiewicz, 2007; Gonzalez-Chavez et al., 2009*) and immunomodulatory properties (*Wakabayashi et al., 2006; Małaczewska and Rotkiewicz, 2007; Gonzalez-Chavez et al. 2009*).

A direct antimicrobial activity of lactoferrin affecting the bacterial cell wall, occurs due to two antimicrobial peptides of an N-terminal part of amino acid chain of this protein, called lactoferricin and lactoferrampin. These peptides are released from native protein by pepsin-mediated digestion (*Kraan et al., 2004; Exposito and Recio, 2006*). Lactoferrampin derived from bovine lactoferrin is bactericidal, where as this human peptide is probably inactive under normal conditions (*Haney et al., 2009*).

In human milk, there is 1 to 5 mg of lactoferrin /ml (*Teng, 2002*), contrary to bovine milk, where this protein concentration reaches maximum level of 0.1 mg/ml (*Schanbacher et al., 1993; Molenaar et al.,1996*). A dramatic increase in lactoferrin has been noticed in colostrum, mammary gland secretion during involution (*Schanbacher et al., 1993*) and in milk obtained from females suffering from a mammary gland inflammation (*Hagiwara et al., 2003; Malinowski et al. 2008*). Milk from quarters, in which *mastitis* pathogens are observed, contains more lactoferrin than that obtainable from uninfected ones, and protein concentration is to some extent pathogen-specific (*Chaneton et al., 2008*).

A lactoferrin gene has developed during evolutional mutations in a transferrin gene. There is 60-65% identity of nucleotide sequences between these two genes (*Baker and Baker, 2005*).The present study therefore aimed at examining the genetic diversity of Lf gene *in-silico* on selected mammalian species.

Materials and methods

Sequence sources

A total of seventeen (17) Lf sequences from six species: Cattle (3), Buffalo (3), Sheep (3), Goat (3), Horse (2) and Camel (3) were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The GenBank accession number of these cattle, buffalo, sheep, goat, horse and camel, sequences were: AH010864.2, AB046664.1, L19981.1 (Cattle), EU669579.1, KC415279.1, HG515533.1 (Buffalo), AF091651.1, NM_001009769.1, LQ223516.1 (Sheep), DI012102.1, GQ149766.1, FM8875929.1 (Goat), AJ010930.1, NM_001163974.1 (Horse), KF915308.1, NM_001303567.1, AJ131674.1 (Camel).

Sequence alignment, translation and comparison

Sequence alignments, translations and comparisons were done using ClustalW as described by (*Larkin et al.,* 2007).

Phylogenetic analysis

Neighbor-Joining trees were constructed each using P-distance model and pair wise deletion gap/missing data treatment. The construction was on the basis of genetic distances, depicting phylogenetic relationships among the lactoferrin nucleotide sequences of the investigated species. The reliability of the trees was calculated by bootstrap confidence values (*Felsenstein, 1985*), with 1000 bootstrap iterations using MEGA 6.0 software (*Tamura et al., 2013*).

UPGMA tree construction

Unweighted pair group method using arithmetic average (UPGMA) trees for each gene was constructed with consensus sequences (a sequence from each species based on similarity was selected for the UPGMA); using same model as that of the tree. All sequences were trimmed to equal length corresponding to same region before generating the tree.

Results

Table 1. **Lactoferrin sequence variation within and among selected mammalian species**

Species	Number of sequences	Sequence length variation (bp)
Cattle	3	1784, 2127, 2357
Buffalo	3	281, 822, 1505
Sheep	3	299, 468, 2127
Goat	3	2326, 2327, 2356
Horse	2	2231, 2270
Camel	3	2250, 2304, 2337

bp means base pair

The length of the Lf gene varied from 281- 2357 within and across species. Cattle and Sheep have similar coding region of 2127 base pair as presented in table 1.

Table 2. Evolutionary divergence between species (Dxy) per site

	Cattle	Buffalo	Sheep	Goat	Horse	Camel
Cattle		0.01	0.00	0.00	0.01	0.01
Buffalo	0.49		0.01	0.01	0.01	0.01
Sheep	0.06	0.50		0.00	0.01	0.01
Goat	0.05	0.50	0.01		0.01	0.01
Horse	0.21	0.54	0.20	0.20		0.01
Camel	0.18	0.54	0.17	0.17	0.15	

Standard error estimate is presented at the upper diagonal while average genetic distances between species is presented at the lower diagonal.

Estimates of Evolutionary Divergence (Dxy) between Sequences is presented in table 2. The number of base differences per site from between sequences are shown. The analysis involved 6 nucleotide sequences.

Estimated distance matrix for lactoferrin gene between consensus sequences of 6 mammalian species selected is as shown. In ruminants, a maximum Dxy of 0.50 between buffalo, sheep and goat and minimum Dxy value of 0.01 was realized between sheep and goat. Amongst non-ruminants (pseudo- ruminant), maximum Dxy value of 0.15 was seen between horse and camel respectively. Generally a maximum value of 0.54 Dxy was realized between buffalo, horse and camel respectively.

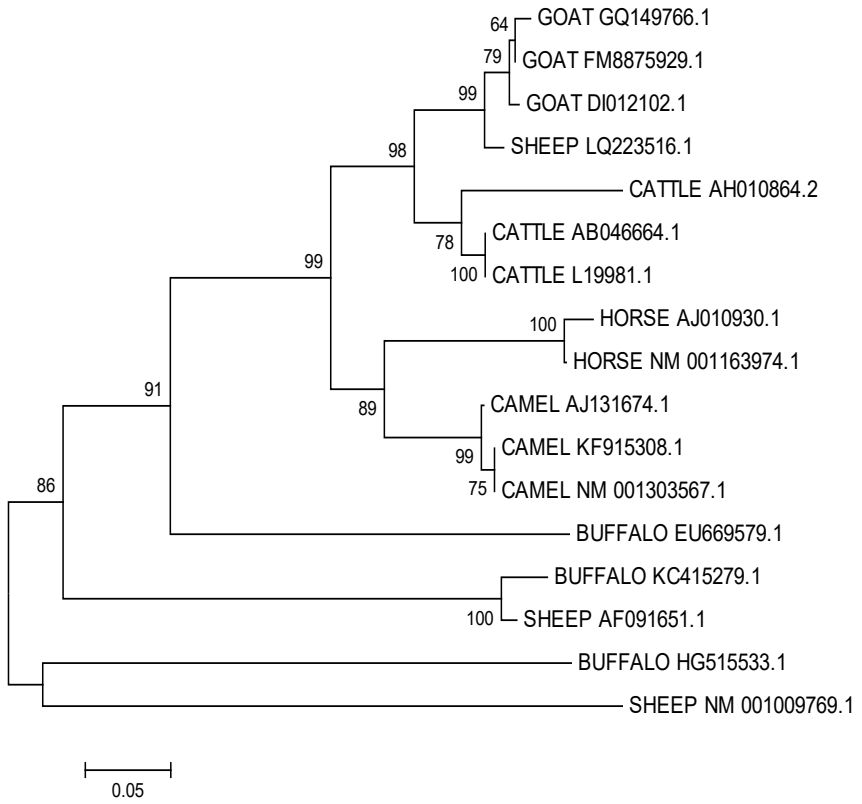

Fig 1.0 Phylogenetic tree construction from the p-distance option of neighbour joining tree.

Neigbhour Joining tree, showed that the sequence of ruminants and non-ruminants were separated from each other due to their respective genetic distance (Dxy) value. The larger the Dxy value the more the distance across the mammalian species selected.

This variation of the genetic distance across species could clearly be explained based on the UPGMA tree deduced from the consensus sequence of the neighbour Joining tree.

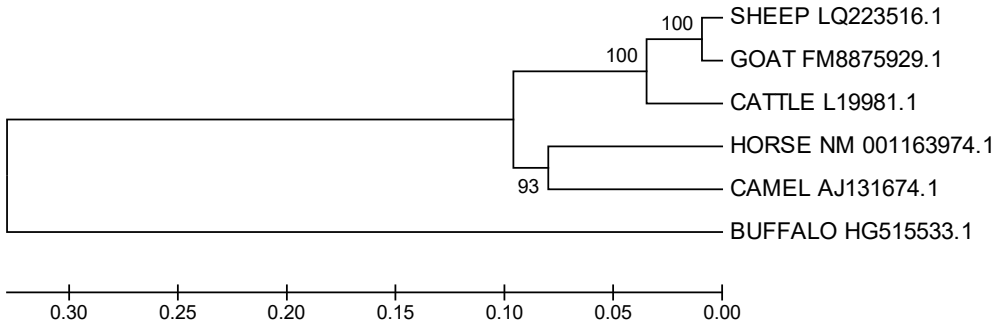

Fig 2. Consensus sequence of the UPGMA tree derived from the neighbour joining tree.

Evolutionary history inferred using UPGMA, revealed that all the goat sequences (FM8875929.1) were closer to sheep (LQ223516.1) sequences compared to those of cattle sequence (L19981.1) (Figure 2). Horse sequences (NM001163974.1) were also closer to those of camel sequence (AJ131674.1). Also this current result revealed that the sequence of buffalo (HG515533.1) tends to be closer to sequence of camel and horse respectively than the ruminant especially the cattle, this variation could be explained based on their genetic distance value from each other from evolution.

Discussion

Dxy is the index of DNA divergence between or among the sequences. The larger the Dxy is, the smaller the genetic distance is (*Kang et al., 2008*).

Lactoferrin is an iron-binding glycoprotein and is considered a major part of the non-specific disease resistance complex in the mammary gland (*Hiss et al., 2008*). The length variation of the LF gene within and among species might result from evolution and differentiation. Many length variations caused by insertions and deletions resulting in amino acid variation within species have been found by comparison with known sequences. This kind of mutation may be related to antibacterial activity or other functions, and needs to be investigated further (*Kang et al., 2008*).

The neighbour-joining tree clearly revealed that clustering was largely species-wise. The presence of numerous alleles at a particular Lf locus is evidence of the long-term evolutionary persistence of the locus (*Yakubu et al., 2013*). This is suggested by the fact that the alleles in one species are often more closely related to the alleles in closely related species than to the other alleles in the same species. The species wise clustering might be due to species specific residues (*Takahashi and Nei, 2000*) and such patterns of the sequences may be explained by gene conversion and balancing selection.

This study may also provide a useful marker for selection of highly expressing animals. By identifying more efficient lactoferrin promoters, a potential exists to identify better sires faster, thereby accelerating the improvement of livestock through breeding (*Daly et al., 2006*).

The genetic relationships of the Lf gene in sheep and goat showed by the phylogenetic tree were in accordance with the speciation of these species in the evolution history as reported in similar work by (*Yakubu et al., 2014*). The same is applicable to the association between horse and camel. The close relationship between horse and camel was also in accordance with the results of *Yakubu et al. (2014),* Yang *et al.* (2004) and Tang *et al.* (2006), which showed that the comparability of cDNA sequences was highest between the pig and the camel by alignment of the full-length sequences of gene caBD21 cDNA of camel, pig, cattle, and sheep. The analysis of molecular evolution could help us to understand Lf antibacterial mechanism from the view of evolution pressure.

Conclusion

There was a great genetic variation in the aligned sequences of Lf gene within and across species whereas the genetic tree obtained advance some form of proximity and differentiation in Lactoferrin gene sequences within and among the mammalian species studied.

Genetička različitost gena laktoferina in-silico na izabranim vrstama sisara

Faith Elijah Akumbugu, Owoeye Ayoadele Olusegun

Rezime

Ukupno 17 laktoferin genetskih sekvenci koje su pripadale 6 vrsta: goveda (3), bivoli (3), ovce (3), koze (3), konji (2) i kamile (3), su preuzete iz banke gena (www.ncbi.nlm.nih.gov). Ređanje sekvenci, translacija i upoređivanje sekvenci je urađeno korišćenjem ClustalW - MEGA 6.0. Stoga, ovo istraživanje je usmereno na ispitivanje genetičke raznovrsnosti Lf gena *in-silico* na odabranim vrstama sisara. Dxy zaključen korišćenjem p-distance pokazao je maksimalnu vrednost od 0,50 kod bivola, ovaca i koza, dok je između ovaca i koza ostvarena minimalna vrednost od 0,01. Maksimalna Dxy vrednost 0,15 je primećena između konja i kamile, dok tokom istraživanja nije zabeležena minimalna vrijednost. Međutim, filogenetsko stablo u okviru filogenetske analize je pokazalo evoluciju između vrsta međutim, UPGMA topologija stabla bila je specifična za vrstu. Ovo genetsko stablo unapređuje neki oblik blizine i diferencijacije u sekvencama gena lactoferina unutar i između proučavanih vrsta sisara koje pružaju osnovu za selekciju stoke u smislu genetskog odnosa.

Ključne reči: Različitost, in-silico, lactoferin, sisar, sekvence, filogenetski

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Received 27 February 2017; accepted for publication 5 May 2017

Biotechnology in Animal Husbandry 33 (2), p 181-191 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 619:616'62
https://doi.org/10.2298/BAH1702181C

DISTRIBUTION OF DOMINANT FOLLICLES IN POSTPARTUM DAIRY COWS

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Original scientific paper

Abstract: Clinical and subclinical disorders and diseases cause reproductive failures and decline in milk production. Etiology of disorders is mainly because of pathological effect of microorganisms, lapses in nutrition and lodging, as well as in management. After partrition, body is under stress and milk yield is highest, which favors appearance of metabolic and infective diseases. Status of puerperium, number of lactation, body condition score and season of parturition, have highest effect to cyclic ovarian activity. Regular development of dominant follicles, ovulation, formation of *corpus luteum* and luteolysis is necessary for establishment of regular cyclic ovarian activity, which leads to better fertility. Experiment had included 50 cows during first 52 days of lactation. Cows were separated in two main groups, those with normal puerperium - NP (n=32) and abnormal puerperium - AP (n=18). Examinations have been performed during period of 6 to 52 days postpartum. Ovarian dominant follicles have been observed using diagnostic ultrasound linear scanner. The highest number of dominant follicles are present during first two examinations, then their number declines and later in last two examinations rise again. Decrease in number of dominant follicles in both groups is most expressed in period of 14-30 days. During first examination, left ovaries have more dominant follicles, compared with right ovaries, while during later examinations, it is changed in favor of right ovaries. Increased number of vital dominant follicles from period 38-45 days postpartum and absence of abnormal uterine content in lumen in same period postpartum is sign of upcoming fertile estrus.

Keywords: cattle, ovaries, dominant follicles, puerperium

Introduction

Cattle industry and especially dairy cattle breeding is important and often primary goal in many farms. This industry is directed for increase in fertility, milk yield, meat quantity and many other products. Clinical and subclinical manifested disorders and diseases cause reproductive failures and decline in milk production. Etiology of disorders is mainly because of pathological effect of microorganisms, lapses in nutrition and lodging, as well as in management. After partrition, body is under stress and milk yield is highest, which favors appearance of metabolic and infective diseases. Management lapses in period of puerperium, may have long lasting negative effects for uterine involution and cyclic ovarian activity, silent estrus is more expressed and open days period becomes longer.

Absence of conception, leads to decreased milk yield, because successful conception is required for development of mammary parenchim and better milk production (*Senger, 2005*). Metabolical excretion of estradiol and progesterone is higher in lactating then in non-lactating cows and is related with blood flow through liver. This process enable that more steroide hormones become metabolised and excreted from body, while low circulated levels of estradiole and progesterone have effect to decreased intensity and lenght of estrus. Follicular activity postpartum should appear 5-10 days postpartum with development of antral follicles (*Leslie, 1983; Sheldon et al., 2002*), which is proof of new follicular phase.

Follicles continue to grow, while one becomes pronouncedly dominant and ready to ovulate after LH wave, while some cows are not observed in estrus before 40 days postpartum (*Mutevelić et al., 2003*). Following number of dominant follicles, it is observed that estral cycle is characterised with growth of 2 or 3 dominant follicle, which is called follicular waves (*Savio et al., 1988*) and this dominant follicles, supress growth of other follicles. During puerperium, before normal cyclic ovarian activity is restored, dominant follicles regress and becomes atretic (*Mutevelić et al., 2003*). Status of puerperium, number of lactation, body condition score and season of parturition, have highest effect to cyclic ovarian activity (*Williams et al., 1995*). Cows with normal puerperium, have earlier ovulation than cows with puerperal disorders (*Williams et al., 1995*). Teatfeeding of calf, levels of IGF-I and insulin, also have important role in development of dominant follicles (*Kawashima et al., 2007; Montiel et al., 2005*).

Follicular activity is supressed in period 14-28 days postpartum, but later it becomes less and less expressed (*Sheldon et al., 2000*). Size of dominant follicle is different among younger and older cows, but also in lactating and non-lactating cows. Regular development of dominant follicles, ovulation, formation of corpus luteum and luteolysis is necessary for establishment of regular cyclic ovarian activity, which leads to better fertility (*Kasimanickam et al., 2004*).

The purpose of this research was to using ultrasonography, observe appearance and distribution of dominant follicles in left and right ovaries, among cows with normal and abnormal puerperium status.

Material and methods

Research has been conducted at dairy farm of Holstein-Friesian cows during autumn and winter season in 2009/2010. Experiment had included 50 black Holstein cows during first 52 days of lactation. All cows were kept in same nutritive and lodging conditions, in tie-stall, without ability to move around. All anamnestic data, have been taken from farm protocols. Cows were separated in two main groups, those with normal puerperium - NP (n=32) and abnormal puerperium - AP (n=18). Cows with disorders of any kind like retained placenta, metritis, purulent vaginal discharge and similar, were placed in group of abnormal puerperium.

Examinations of ovaries, have been performed in period of 6 to 52 days postpartum, during next time phases: 6-13 days, 14-21 days, 22-29 days, 30-37 days, 38-45 days and 46-52 days, when experiment had ended. Ovarian dominant follicles have been observed using diagnostic ultrasound linear scanner SHIMADZU SHIMASONIC SDL-32 with 3,5 MHz linear probe.

Obtained data are graphically represented using software package Microsoft Office Excell 2010.

Results

In cows with normal and abnormal puerperium, all dominant follicles were calculated and graphically represented in next 5 charts.

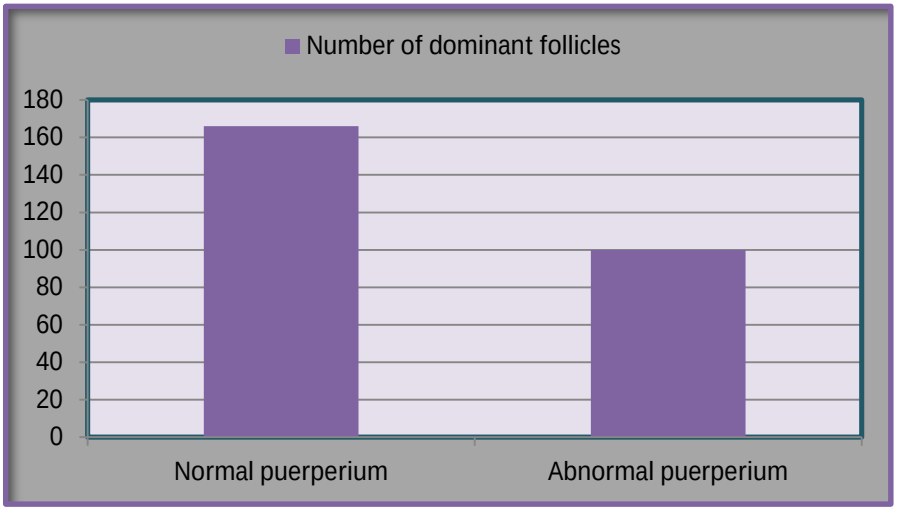

Chart 1. In total 266 dominant follicles were counted, 166 in NP and 100 in AP group.

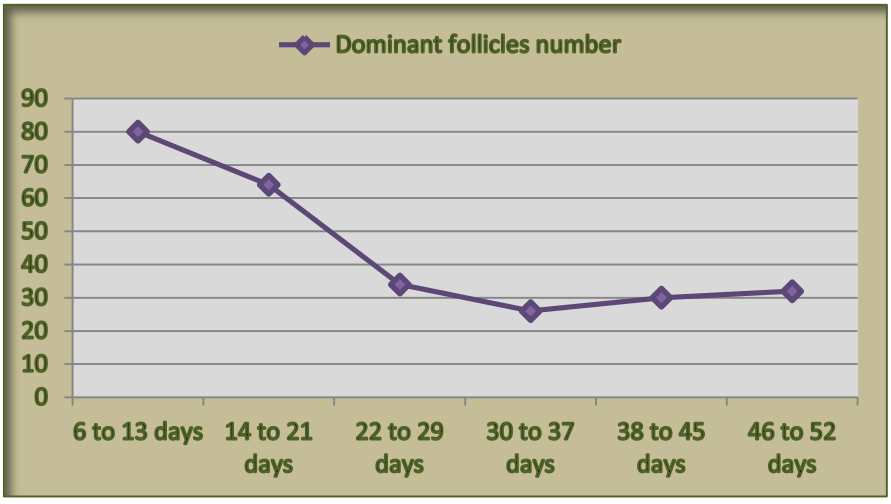

Chart 2. Total number of dominant follicles in left and right ovary in NP and AP group during observation 6-52 days of postpartum period.

In chart 2. can be seen that highest number of dominant follicles are present during first two examinations, then their number declines and later in last two examinations rise again, which may be seen in charts 3 and 4 also.

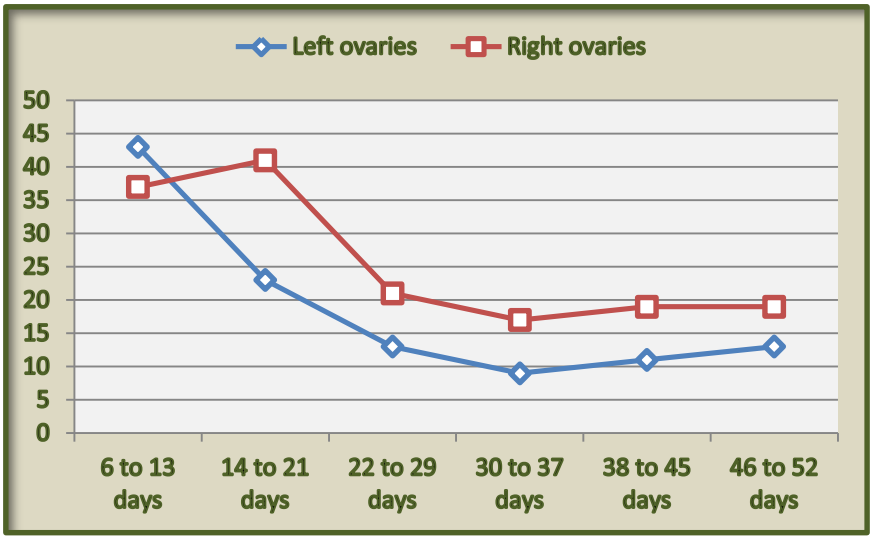

Chart 3. Distribution of dominant follicles in left and right ovaries in NP and AP group observed during 6-52 days of postpartum period.

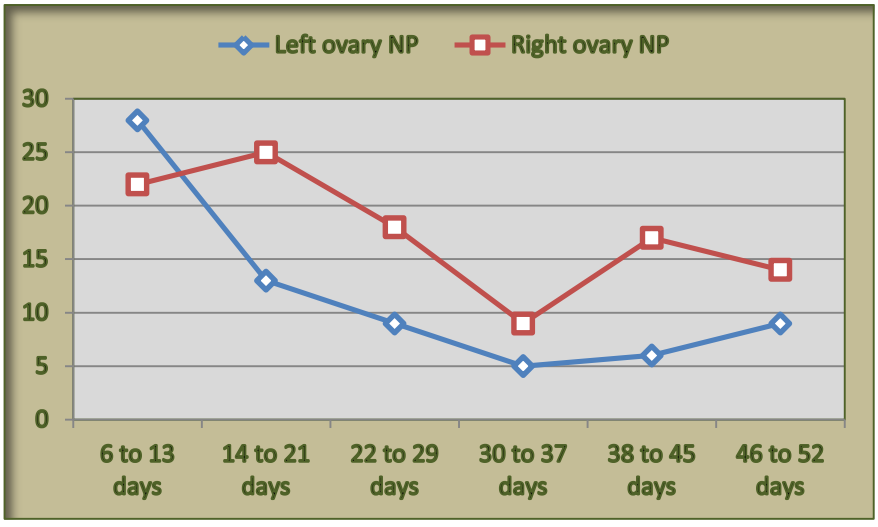

Chart 4. Distribution of dominant follicles in left and right ovaries in NP group during 6-52 days of postpartum period.

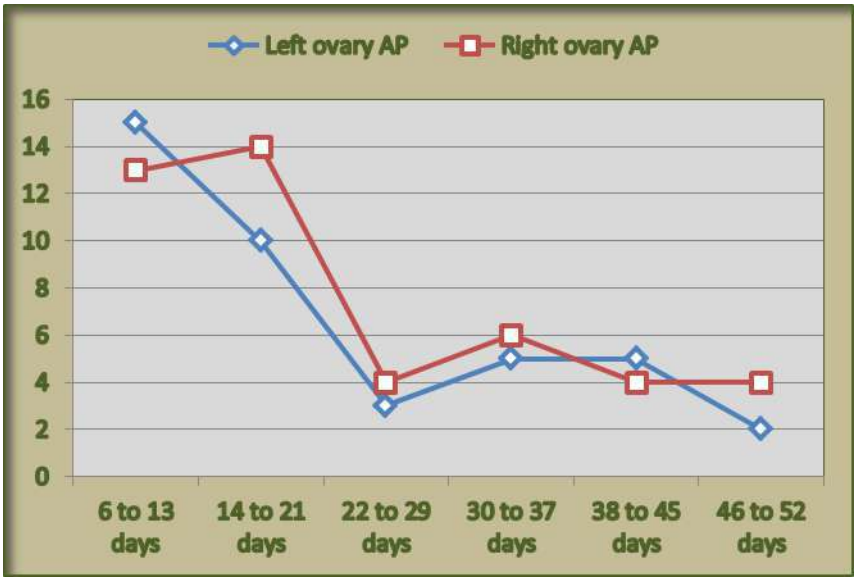

Chart 5. Distribution of dominant follicles in left and right ovaries in AP group during 6-52 days of postpartum period.

In charts 4 and 5, it can be seen that during first examination, left ovaries have more dominant follicles, compared with right ovaries, while during later examinations, it is changed .

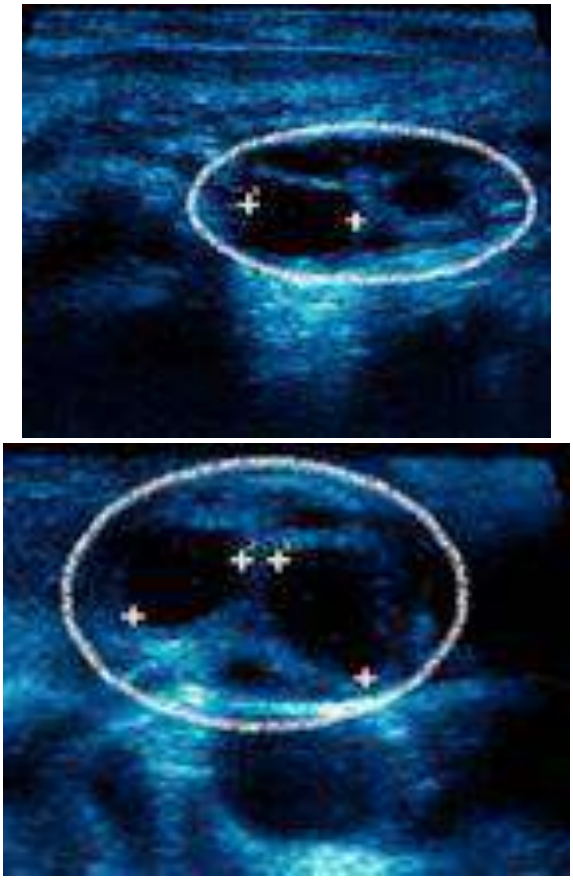

Figure 1 and 2. Appearance of one (left) and two (right) dominant follicle in ovary.

Discussion

Lapses in dairy cows management followed with intensive explotation during puerperium, may lead to irregularities in uterine involution and ovarian cyclicity, which decrease reproductive performances during lactation (*Földi et al., 2006*). About 50% of all cows have irregular ovarian function after parturition (*Bisinotto et al., 2010*). When uterine problems are absent or resolved quickly, pattern of dominant follicle development is changed and becomes more dominant in ovary contralateral of previously pregnant uterine horn (*LeBlanc et al., 2002*). Irregular ovary function is in most cases caused by retention of placenta, *metritis*, delayed uterine involution, low body condition, lameness and long luteal phase.

Ovarian examinations gave us insight in pattern of dominant follicles development and distribution during first 52 open days for cows in NP and AP group.

Levels of FSH are very important for follicular development, but also presence of corpus luteum and levels of progesterone. Dominant follicles in first two weeks, often do not react to gonadotropin stimulation, ovulation is absent, those follicles goes through atresion and their number is reduced (*Montiel et al., 2005*), which can be seen in our results too, where in same time their number is significantly lower. Both groups begin to develop DF in the first 10 days postpartum (*Leslie et al., 1983; Sheldon et al., 2002*) and had more dominant follicles in first examination in left ovary. Reason of this is probably because most pregnancies are in right uterine horn. Folicular activity in the begining is decreased in ovary ipsilateral to previously pregnant uterine horn, which can be seen also in NP and AP groups, but higher activity in same horn later is related with better conception (*Sheldon et al., 2000*).

During puerperium, through relationship hypothalamus-pituitary-ovaries, cyclic secretion of gonadotrophin hormones is restored and in NP cows, this process is finished by the end of six weeks (*Peter et al., 2009*), which prety much, may be seen in our results too, where foliculogenesis begins to stabilise and number of dominant follicles to increase in observed period of 38 – 52 days postpartum. This begins in most cows in period of 14-28 days postpartum and it is mentioned by many authors (*Sheldon et al., 2002; Sheldon et al., 2004; Williams et al., 2007*).

In our research, decrease in number of dominant follicles in both groups is most expressed in period of 14-30 days. About 2/3 of cows have restored ovarian cyclicity after 45 days postpartum (*Shretsha et al., 2004*), which coincide with our increase in number of dominant follicles and their stabilisation until the end of experimental period.

Presence of microorganisms in uterus and resorption of their products, cause disturbance in relation hipotalamus-pituitary-ovaries, which have effects to secretion of GnRH and LH hormones, decrease in number of dominant follicles and lesser secretion of estradiol (*Sheldon et al., 2004*). Cows with retention of placenta, are under higher effect from endotoxins from G- microorganisms, which changes physiological function between hipotalamus, pituitary and ovaries. In period 22-29 days the highest incidence of *endometritis*, developed after placental retention have been found (*Gilbert et al., 2005*), which is probably related with lowest number of dominant follicles found in that period in AP group. This kind of changes, delay foliculogenesis and decrease follicular grow rate during puerperium (*Peter et al., 1988*), which is not so obvious in the begining as it is during later *puerperium*, which coincide with same author.

Conclusions

1. In comparation between number of dominant follicles in NP and AP group and considering difference in number of animals, total number of dominant follicles is not signifficantly different among groups.
2. Great number of dominant follicles is found in first 14 days postpartum in NP and AP groups, which then goes undo atresion.
3. Total number of dominant follicles is larger in left ovaries in the begining of puerperium in NP and AP group.
4. Total number of dominant follicles after decline, stabilises in period 30-37 days postpartum and begin to increase.
5. Right ovaries had more dominant follicles in all observed periods, except in the begining of puerperium.
6. Cows with diagnosed endometritis had lowest number of dominant follicles.
7. Increased number of vital dominant follicles from period 38-45 days postpartum and absence of abnormal uterine content in lumen in same period postpartum is sign of upcoming fertile estrus.

Distribucija dominantnih folikula kod mlečnih krava postpartum

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Rezime

Klinički i subklinički poremećaji i obolenja, dovode do reproduktivnih neuspeha i pada mlečnosti. Etiologija poremećaja je najčešće u patološkom delovanju mikroorganizama, propusta u ishrani i smeštaju, te u menadžmentu. Nakon telenja, telo je pod stresom i mlečnost je najviša, a što pogoduje pojavi metaboličkih i infektivnih obolenja. Status puerperija, broj laktacija, telesna kondicija i godišnje doba telenja, imaju najveći uticaj na ciklične procese na jajnicima.

Pravilan razvoj dominantnih folikula, ovulacija, formiranje žutog tela i luteoliza su neophodni za uspostavljanje pravilne ovarijalne cikličnosti, koja vodi boljoj plodnosti. Eksperiment je uključio 50 crnih Holštajn krava tokom prvih 52 dana laktacije. Krave su podeljene na dve glavne grupe, one s normalnim

puerperijem - NP (n=32) i abnormalnim puerperijem - AP (n=18). Pregledi su se radili tokom perioda 6 do 52 dana postpartum. Dominantni folikuli na jajniku su uočavani dijagnostičkim ultrazvukom.

Najveći broj dominantnih folikula je uočen tokom prva dva pregleda, zatim njihov broj opada i tokom zadnja dva pregleda je ponovo u rastu. Pad broja dominantnih folikula u obe grupe je najizražajniji u periodu 14-30 dana. Prilikom prvog pregleda, levi jajnici imaju više dominantnih folikula, dok pri kasnijim pregledima, to se menja u korist desnih jajnika. Rast broja vitalnih dominantnih folikula u periodu 38-45 dana postpartum i odsustvo abnormalnog sadržaja u lumenu uterusa u istom periodu, najavljuje dolazak fertilnog estrusa.

Ključne reči: goveda, jajnici, dominantni folikuli, puerperium

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Received 23 February 2017; accepted for publication 30 April 2017

Biotechnology in Animal Husbandry 33 (2), p 193-200 , 2017
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 591.111.1:591.146'62
https://doi.org/10.2298/BAH1702193D

SERUM ENZYME ACTIVITIES IN BLOOD AND MILK IN THE DIFFERENT STAGE OF LACTATION IN HOLSTEIN DAIRY COWS

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Original scientific paper

Abstract: The objective of this study was to determine correlation between serum blood and milk enzyme activities of aspartate-aminotransferase (AST), alanine- aminotransferase (ALT), alkaline-phosphatase (ALP in the 36 dairy Holstein cows divided into three groups according to production period. Group 1 consisted cows in the start of lactation (n = 12); Group 2 -consisted of early lactation cows (n=12) and Group 3 included mid lactation cows (n=15). Statistically significant higher ($P<0.01$) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation group of cows. ALT activity showed a lower ($P<0.01$) serum activities in early lactation groups of cows than in the mid lactation cows. Higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid laltation cows, but without statistical significance ($P>0.05$). Research results showed possibility of mild degree of hepatic lesions, probably due to fat infiltration in early lactation cows. No significant difference ($P>0.05$) was observed in milk serum value for AST, ALT and ALP between the three groups of cows. No significant correlations among AST, ALT and ALP activities in blood and milk serum were determined ($P>0.05$) and shows that activity of these enzyme in the milk are not used as markers for early diagnosis of subclinical metabolic disease.

Keys words: dairy cows, enzymes activities, blood, milk, early lactation periods, mid lactation

Introduction

The metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle (*Oetzel, 2004*, *Stengärde et al*., *2008*; *Gross et al., 2011*). Evaluation of the blood and milk biochemical parameters to assess the animal health and milk yield has always been interested by authors and the various discrepancies have been observed in both blood and milk yield results (*Nozad et al. 2011; Jozwik et al., 2012*). Milk parameters originate from blood and food component and clarifying the appropriate relationships among these parameters individually in food blood and milk are useful in understanding the health and production status in animals (*Jozwik et al.,2012; Liu et al., 2012; 2013*). Major health disorders in high-yielding cows occur around parturition and during lactation. Metabolic conditions of negative energy balance (fasting, parturition and lactation) lead to an increased uncontrolled rate of mobilization of body fat and its increased accumulation in liver cells, resulting in disturbance of the physiological and morphology integrity of the liver (*Vazquez-anon et al.,1994; Overton and Waldron, 2004; Bobe, 2004*). Fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (AST, GGT, LDH), the activity there of in the blood being considerably elevated (*Oezel, 2004; Stojevic et al., 2005; Lubojacka et al., 2005*). Blood serum ALT, AST, ALP and GGT activities were reported to be useful indicator of liver function for postpartum dairy cows (*Bobe, 2004; Stojević et al., 2005*). While little information is available concerning about the activity changes of ALT, AST, GGT and ALP in milk. The activities of these enzymes were monitored in milk and blood serum of cows and results of correlation analysis and regressive models showed a close relation between them (*Liu et al., 2012, 2013; Ghadaa 2014*). More practical attention has been given to detection of enzyme activity in milk and many enzymes have been proposed and listed a reliable markers for early diagnosis of subclinical disease (*Babae et al., 2007; Katsoulos et al., 2010; Liu et al., 2012; 2013).*

The objective of this study was to determine correlation between serum blood and milk enzyme activities in the different stage of lactation in the dairy cows.

Material and methods

Animals, diets and protocol design: A total of 36 dairy cows were randomly selected from the same Holstein herd containing 445 cows (FARM: Šarulja, Knić, Central Serbia). The cows were high-yielding with a preceding lactation of about

8500 L. Three groups of clinically healthy cows were chosen from the herd. Group 1 consisted cows in the start of lactation (n = 12) in period of 5 ± 3 days after calving; Group 2 -consisted of early lactation cows (n=12) in the first month of lactation (22±15 days), and Group 3 included mid lactation cows (n=15) between 90 to 150 days of lactation (133±75 days). The experimental cows were free in open-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the energy requirement of late pregnancy, early and mid-lactation cows. Diet in early lactation consisted of of 4 kg grass hay, 10 kg corn silage (30% Dry Matter, DM), 20 kg sweet corn silage, 12 kg beet nodle silage, 4 kg concentrate (18% crude protein, CP) and 1 kg molasses. Diet in mid lactation consisted of 4.5 kg lucerne hay, 19 kg corn silage (30% Dry Matter, DM), 16 kg beet nodle silage, 9 kg concentrate (18% crude protein, CP) and 1.2 kg soybean expeller.

The chemical composition of total mixed rations offered to early lactation and mid lactation dairy cows are given in Table 1.

Table 1. Chemical composition of total mixed rations offered to early lactation and mid lactation dairy cows.

	Early lactation	Mid lactation
Dry Matter (DM) (kg)	15.60	19.58
Net Energy of Lactation (NEL) (MJ)	95.52	128.65
Crude Protein (CP) (% of DM)	11.31	16.88
Rumen undegradable protein (RUP) (% of CP)	33.91	26.33
Fat (% of DM)	3.47	4.68
Fibre (% of DM)	22.17	18.85

Sample collection: Blood and milk samples were taken simultaneously from each lactating cow during morning milking. Blood samples (10 ml) were taken by jugular puncture into a sterile tube from each animal, and the blood serum was separated by centrifugation at room temperature (1,800×g, 15 min). Milk samples were collected in sterile tube and centrifuged at 12,000×g for 30 min at 4°C and the supernatant was transferred into the new sterile tubes. Blood plasma and milk were stored at -20°C until being used for biochemical measurements.

Biochemical analysis: The blood and milk serum activities of aspartate-transaminase (AST) alanine-aminotransferasese (ALT) and alkaline-phosphatase (ALP) were measured in the biochemical laboratory „OXUS“ (Kragujevac, Serbia) by spectrophotometric techniques using a BT 1000, (Biotehnica Italia) and the corresponding commercial kits (DIALAB, YUNICOM).

Statistical analysis: The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA). The analysis of variance were used to evaluate the

probability of the significance of the statistical differences between mean serum enzyme activities in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when P values were below 0.05 or 0.01.

Results and Discussion

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to predict such disorders and eventual subclinical diseases it is necessary to determine physiological ranges of biochemical parameters in a clinically healthy herd (*Oezel, 2004; Overton and Waldron, 2004*). The present study compared the serum enzyme activities in blood and milk serum in dairy cows during early and mid-lactation period.

The results of the serum blood and milk activities of AST, ALT, ALP in cows in the early and mid-lactation period and correlations among blood an milk serum enzyme activities are given in Tables 2 and 3.

Table 2. Blood and milk serum enzyme activities in the start of lactation (Group 1), early (Group 2) and mid lactation (Group 3) dairy cows (n=12 in each group). Results are expressed as mean ± standard deviation (SD).

	Group 1	Group 2	Group 3
AST (blood) IU/l	90.81±21,98 ^{A}	84.18±16.19 ^{A}	59.72± 10.95 ^{B}
ALT(blood) IU/l	28.00±8.46 ^{A}	28.54 ±3.96 ^{A}	36.45±9.62 ^{B}
ALP(blood) IU/l	162.36±193.25 ^{a}	117.64±22. 28 ^{a}	96.81 ±31.94 ^{a}
AST (milk) IU/l	33.82±23.76 ^{a}	33,27±9.65 ^{a}	25.36±11.87 ^{a}
ALT(milk) IU/l	20.05±12.47 ^{a}	20.27±14.66 ^{a}	29.55±19.83 ^{a}
ALP(milk) IU/l	199.23±186.23 ^{a}	241.11±109.31 ^{a}	121.64±32.56 ^{a}

Legend: Mean values within a row with no common superscript differ significantly, values marked by small letter differ significantly ($p < 0.05$); values marked by capital letter differ high-significantly ($P < 0.01$).

Table 3. Correlation coefficients among the biochemical parameters in the blood and milk calculated for all cows in the present study.

	AST (blood)	ALT (blood)	ALP (blood)
AST (milk)	$r=0.14^{NS}$	$r=0.16^{NS}$	$r=-0.09^{NS}$
ALT(milk)	$r=-0.23^{NS}$	$r=-0.03^{NS}$	$r=0.17^{NS}$
ALP(milk)	$r=-0.06^{NS}$	$r=0.11^{NS}$	$r=0.15^{NS}$

Legend: NS - non-significant ($p>0.05$)

Lactation has a great impact on biochemical parameters in the blood of cows, reflecting on metabolic demands. The activity of AST in blood is very important.

AST act as a catalyst in connecting the metabolism of amino-acids and carbohydrates. Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage. AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows (*Pechova et al., 1997; Bobe, 2004; Lubojacka et al., 2005; Stojević et al., 2005*). In this study, statistically significant higher ($P<0.01$) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation groups of cows No significant difference ($P>0.05$) was observed in milk serum value for AST between the three groups of cows.

ALT activity in cows differs during certain production periods. The lowest ALT activity was measured during early lactation, while activity increased in the second and third periods of lactation. In the dry period enzyme activity decreased, but it was still statistically much higher than in the first period of lactation. The author considers that the role of ALT in predicting liver damage in ketosis is not significant (*Tainturier et al., 1984; Stojević et al., 2005*). Our results confirm this because in the period of mid lactation (third period) we measured the highest ($P<0.01$) concentration of ALT. No significant difference ($P>0.05$) was observed in milk serum value for ALT between the three groups of cows.

ALP are used as biochemical marker in diagnosis of osteoporosis, hepatobiliar disease and fatty liver in the dairy cows. The activity of ALP in blood serum are increased in periods from puerperium to mid lactation in the dairy cows, especially in cows with liver lipidosis. (*Bobe et al.,2004; Stojevic et al., 2005*). In this study, higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid laltation cows, but without statistical significance ($P>0.05$) as consequence high individual variabilites. On the basis changes in blood and milk AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration.

Significant correlations among AST, ALT and ALP activities in blood an milk serum are not determined ($P>0.05$) in this study (Table 3), and shows that activity of these enzyme are not used as markers for early diagnosis of subclinical disease. These results are in opposite with results (*Liu et al., 2012; 2013; Ghadaa 2014*), who found a strong correlation between them. Further investigations will confirm or not these statements.

Conclusion

Biochemical examination of blood serum showed higher activities of AST (P<0.05) and ALP (P>0.05), in groups of early lactation cows, and lower activity of ALT (P>0.05) compared to the group of mid lactation cows. No significant difference (P>0.05) was observed in milk serum value for AST, ALT and ALP between the three groups of cows. On the basis of changes in blood AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration. No significant correlation among AST, ALT and ALP activities in blood an milk serum are determined (P>0.05), showing that activity of these enzyme in the milk are not used as markers for early diagnosis of subclinical metabolic disease.

Serumske enzimske aktinosti u krvi i mleku u različitim stadijumima laktacije

Radojica Đoković, Marko Cincović, Vladimir Kurćubić, Zoran Ilić, Miroslav Lalović, Boban Jašović, Miloš Petrović

Rezime

Cilj ovog rada je bio da se utvrde korelacije između serumskih aktivnosti aspartat-amino transferaze (AST), alanin-aminoreasferaze (ALT) i alkalne-fosfataze (ALP)) u krvi i mleku kod 36 mlečnih krava rase Holštajn, koje su podeljene u tri grupe u zavisnosti od laktacionog perioda. Grupu 1 (n=12) činile su krava na samom početku laktacije, Grupu 2 (n=12) krave u ranoj laktaciji i Grupu 3 (n=12) krave na sredini laktacije. Statistički značajno (P<0,01) veće aktivnosti u krvnom serumu AST su utvrđene kod grupa krava u ranoj laktaciju u odnosu na grupu krava tokom sredine laktacije. ALT aktivnosti u krvnom serumu su bile značajno manje (P<0,01) kod grupa krava na početku laktacije u odnosu na grupu krava na sredini laktacije. Najviše vrednosti za aktivnosti ALP u krvi su utvrđene kod grupa krava u ranoj laktaciji u odnosu na grupu krava u sredinu laktacije, ali bez statističke značajnosti (P>0,05). Dobijeni rezultati ukazuju na mogućnost pojave masne infiltracije jetre blagog stepena kod grupa krava na početku laktacije. Nisu utvrđene stistički značajne razlike (P>0.05) za aktivnosti AST,ALT i ALP u mleku između ispitivanih grupa krava. Nisu utvrđene statistički značajne korelacije (P>0.05) između aktivnosti AST, ALT i ALP u krvi i mleku, što ukazuje da

aktivnosti ovih enzima u mleku se ne mogu koristiti kao markeri za ranu dijagnostiku subkliničnih metaboličkih oboljenja.

Ključne reči: mlečne krave, enzimske aktivnosti u krvi i mleku, rana laktacija, sredina laktacije

Acknowledgment

This work was financed by Ministry of Education and Science, Republic of Serbia, projects TR. 31001.

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Received 12 April 2017; accepted for publication 2 June 2017

Biotechnology in Animal Husbandry 33 (2), p 201-209 , 2017
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.52
https://doi.org/10.2298/BAH1702201D

EFFECT OF BODY WEIGHT OF LAYING HENS ON PRODUCTION TRAITS OF BROILER PARENTS

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Original scientific paper

Abstract: Certain investigations have been conducted in two broiler breeder flocks of Ross 308 and Cobb 500 hybrids. At the beginning of the production cycle (24 weeks of age), an average laying hens' body weight of 2680.40 g was found in the case of Ross 308 hybrid, and 2697.80 g in the case of Cobb 500 hybrid. During 42nd week of age (the middle of the production cycle), the body weight of laying hens was 3565.10 g (Ross 308) and 3599.05 g (Cobb 500), while at the end of the production cycle (61 weeks of age) the body weight of laying hens of Ross 308 hybrid was 3841.50 g, and 3850.00 g of Cobb 500. Identified differences in body weight of laying hens (17.40 g, 33.95 g, 8.50 g) in certain periods of the production cycle, as well as the difference in body weight of laying hens for the entire production cycle (23.26 g) were not statistically significant ($P>0.05$). More specific observation of the effect of body weight of laying hens on productive capacity of broiler breeders was determined by calculating the coefficients of phenotype correlation between the indicators studied. Thus, statistically significant ($P<0.001$, $P<0.01$, $P<0.05$) coefficients of phenotype correlation between the body weight of laying hens and the majority of production indicators have been determined, while statistically significant ($P<0.001$, $P<0.01$, $P<0.05$) correlation coefficients between the body weight of laying hens and the intensity of laying capacity for hatching and fertilized eggs have been determined, but for a shorter period of the production cycle.

Keywords: Laying hens, body weight, production traits, broiler breeders, correlation

Introduction

In addition to the age and optimal sex ratio, the body weight of laying hens during the production cycle also significantly influences the productive capacities of broiler breeders (*Savic et al., 2004; Đermanović et al., 2005; Đermanović et al., 2008; Djermanovic et al., 2009; Djermanovic, 2010; Đermanović et al., 2010;*

Đermanović et al., 2012; Mitrovic et al., 2005; Mitrovic et al., 2009; Mitrovic et al., 2010; Mitrovic et al., 2011; Pandurevic et al., 2013). Proper hormonal functioning of the endocrine system of the laying hens in addition to their age and photostimulation (*Lewis et al., 2005; Lewis and Gous, 2006, 2007; Usturoi et al., 2007*) depends very much on the physical development of breeding birds. When the body weight is optimal at the certain age, the function of the ovaries is stimulated, and hence the maturation of the ovum, i.e. the production of eggs is accelerated.

Only proper nutrition and technology of exploitation of the parent flock can provide the precondition for the necessary vitality and quality of eggs for incubation (*Suarez et al., 1997; Sahin et al., 2009*). In order to make the production of fertilized eggs last for a long time period, it is necessary to constantly keep hens in breeding condition, paying particular attention to their physical development. It should also be borne in mind that the uniformity of the flock in terms of weight is especially significant factor in the second half of the production cycle.

In the case of the majority of heavy line hybrids, the production of eggs for the purpose of incubation starts at the 24th week of age, when the intensity of laying capacity is about 5% and more. From this period on, the egg production gradually increases to the maximum, and then the productivity of broiler breeders decreases more or less. Therefore, we can say that the period of exploitation of broiler breeders significantly depends on that time period. As an indicator for estimation of the period up to which it is justified to use broiler breeders in the production of hatching eggs, the calculated coefficients of phenotype correlation between body weight and productive traits of laying hens in the final period of the production cycle, which represents a turning stage in the utilization of parent flocks, can make a significant contribution.

Material and Methods

The studies cover two parent flocks of broiler breeders of Ross 308 and Cobb 500 heavy hybrids. During the production cycle, the technology suggested by the breeders of the respective hybrids was used. Broiler parents of both flocks were kept on floor in deep litter, and feeding, watering, ventilation and lighting were automatically regulated. The studied flocks were grown up to 61st week of age, i.e. both flocks began to lay eggs at the beginning of the 22nd week, and the eggs laid from the 24th week of age, and later, until the end of the production cycle were used for incubation, because in that period they satisfied a minimum weight suitable for incubation (>50.00 g). The presented results indicate that the egg production period lasted for 38 weeks.

The total of 5200 birds of both sexes of Ross 308 hybrid and 5430 birds of broiler parents of Cobb 500 hybrid, reared in two separate buildings, were used as the initial experimental material. 4750 ♀ and 450 ♂ birds of Ross 308 hybrid were

placed in the first building, and another 4960 ♀ and 470 ♂ of Cobb 500 hybrid in the second one, so that the sex ratio was 1 : 10.56 (Ross 308) and 1 : 10.55 (Cobb 500). In the preparatory period between 21st and 24th week of age of flock, mortality and culling of the laying hens of the hybrid Ross 308 was 13 birds (0.279 %), and in the case of the Cobb 500 hybrid, it was 12 birds (0.24 %). This means that there were 4737 laying hens in the flock of broiler parents of Ross 308 hybrid, i.e. 4948 laying hens of Cobb 500 hybrid, at the beginning of the use of eggs for incubation.

In order to control body weight, 200 laying hens of Ross 308 and Cobb 500 hybrids, selected randomly, were weighed individually every week. By the means of these measurements, the uniformity of laying hens of the tested flocks was observed in the production cycle, after which the effect of the weight of the laying hens on the productive parameters of the broiler parents was examined: the intensity of laying capacity for hatching eggs (%), the intensity of laying capacity for fertilized eggs (%), egg weight (g), daily consumption of food per bird (g/day), food consumption per hatching egg (g/egg) and food consumption per fertilized egg (g/egg). Primary data processing was performed using variation - statistical methods, and testing of the difference between hybrids was done using the t -test. In addition, the obtained results were used to calculate the correlation of the tested characteristics per week of production in the last third of the production cycle, i.e. from 50th to 61st week of age, using the correlation analysis. Statistical data processing was performed using the software program SAS/STAT (*SAS Institute, 2000*).

Results and Discussion

The average values, variability and significance of differences in body weight of laying hens during certain periods of the production cycle, as well as for the entire egg production period, are shown in Table 1.

Table 1. The average values, variability and significance of differences in body weight of laying hens (g) at certain periods of the production cycle (*Pandurevic et al., 2013*)

Production cycle period	Weeks of age (production)	Hybrid	$\bar{x}$ ±SEM	S	$\bar{d}$
Beginning	24 (1)	Ross 308	2680.40±14.63	206.93	17.40^{ns}
		Cobb 500	2697.80±17.09	241.66	
Middle	42 (19)	Ross 308	3565.10±19.86	280.92	33.95^{ns}
		Cobb 500	3599.05±20.12	275.28	
End	61 (38)	Ross 308	3841.50±21.39	302.56	8.50^{ns}
		Cobb 500	3850.00±21.68	306.59	
Entire production cycle	61 (38)	Ross 308	3411.15±61.58	394.33	23.26^{ns}
		Cobb 500	3434.41±61.03	390.76	

^{ns} P>0.05.

The data in Table 1 show that the average body weight of laying hens of each hybrid was gradually increasing during the production cycle. Body weight of hens at 24th week was 2680.40 g (Ross 308) and 2697.80 g (Cobb 500), and at the end of exploitation, it was 3841.50 g in the case of Ross 308 and 3850.00 g in Cobb 500 hybrids. During the production cycle, laying hens of Cobb 500 hybrid compared to the hens of Ross 308 hybrid had a higher average body weight which was not statistically confirmed ($P<0.05$). The average body weight of laying hens of Ross 308 hybrid for the entire period of exploitation was 3411.15 g, and of Cobb 500 hybrid, it was 3434.41 g, where the difference in body weight between the laying hens (23.26 g) of the studied hybrids was not statistically significant ($P>0.05$), indicating that genotype had no significant effect on body weight of laying hens.

Body weight of lying hens of the studied hybrids was slightly higher than the norms predicted by the genetic potential. *Djermanovic et al. (2009), Djermanovic (2010), Mitrovic et al. (2010)* and *Pandurevic et al. (2013)* came to the similar results, in terms of average body weight of laying hens. *Usturoi et al. (2007)*, in the course of rearing of broiler parents of Ross 308 hybrid, found slightly lower average body weight of laying hens, which, depending on the groups of hens, varied between 3988.95 g and 3990.44 g in the 60th week of age. *Lewis et al. (2005)* and *Lewis and Gous (2006)*, in the 60th week of age of Cobb 500 laying hens, found a significantly higher average body weight of laying hens, between 4.21 and 4.25 kg, while *Lewis and Gous (2007)*, in the 59th week of age, found a significantly higher average body weight of laying hens of hybrids Ross 308 (4.43 kg) and Cobb 500 (4.56 kg).

In addition to the established measures of variation in body weight of laying hens belonging to the analyzed parent flocks, and in order to better analyze the impact of body weight of laying hens on productive performances, the coefficients of phenotypic correlation relationship between the examined traits in the last third of the production cycle (Table 2) were calculated.

Table 2. Phenotypic correlation relationship between body weight of lying hens and productive traits

Age (weeks)	Hybrid	BW, g	Coefficients of phenotypic correlation					
			r_1	r_2	r_3	r_4	r_5	r_6
50	Ross 308	3685.50	0.617^{***}	0.618^{***}	0.989^{***}	0.763^{***}	-0.643^{***}	-0.644^{***}
	Cobb 500	3710.00	0.663^{***}	0.662^{***}	0.989^{***}	0.797^{***}	-0.658^{***}	-0.659^{***}
51	Ross 308	3703.50	0.580^{***}	0.581^{***}	0.992^{***}	0.749^{***}	-0.625^{***}	-0.627^{***}
	Cobb 500	3722.00	0.633^{***}	0.632^{***}	0.994^{***}	0.792^{***}	-0.642^{***}	-0.643^{***}
52	Ross 308	3710.50	0.539^{**}	0.540^{**}	0.991^{***}	0.743^{***}	-0.605^{***}	-0.607^{***}
	Cobb 500	3732.50	0.603^{***}	0.602^{***}	0.994^{***}	0.788^{***}	-0.626^{***}	-0.628^{***}
53	Ross 308	3743.00	0.499^{**}	0.499^{**}	0.989^{***}	0.732^{***}	-0.585^{***}	-0.586^{***}
	Cobb 500	3755.00	0.570^{***}	0.569^{***}	0.993^{***}	0.786^{***}	-0.608^{***}	-0.610^{***}
54	Ross 308	3754.00	0.459^{**}	0.458^{**}	0.989^{***}	0.727^{***}	-0.563^{***}	-0.564^{***}
	Cobb 500	3767.50	0.535^{***}	0.534^{***}	0.992^{***}	0.785^{***}	-0.589^{***}	-0.590^{***}
55	Ross 308	3770.00	0.410^{**}	0.411^{**}	0.987^{***}	0.708^{***}	-0.538^{***}	-0.541^{***}
	Cobb 500	3777.50	0.495^{**}	0.494^{**}	0.991^{***}	0.786^{***}	-0.567^{***}	-0.568^{***}
56	Ross 308	3782.50	0.349^{*}	0.347^{*}	0.986^{***}	0.673^{***}	-0.507^{**}	-0.509^{**}
	Cobb 500	3792.50	0.451^{**}	0.450^{**}	0.991^{***}	0.790^{***}	-0.540^{***}	-0.541^{***}
57	Ross 308	3797.00	0.293^{*}	0.291^{*}	0.988^{***}	0.624^{***}	-0.480^{**}	-0.481^{**}
	Cobb 500	3805.00	0.402^{**}	0.401^{**}	0.991^{***}	0.792^{***}	-0.509^{**}	-0.509^{**}
58	Ross 308	3805.50	0.232^{ns}	0.230^{ns}	0.986^{***}	0.562^{***}	-0.447^{**}	-0.448^{**}
	Cobb 500	3812.50	0.347^{*}	0.346^{*}	0.989^{***}	0.792^{***}	-0.473^{**}	-0.473^{**}
59	Ross 308	3812.50	0.174^{ns}	0.172^{ns}	0.985^{***}	0.486^{**}	-0.415^{**}	-0.416^{**}
	Cobb 500	3825.00	0.278^{ns}	0.277^{ns}	0.987^{***}	0.790^{***}	-0.419^{**}	-0.418^{**}
60	Ross 308	3827.50	0.110^{ns}	0.109^{ns}	0.983^{***}	0.429^{**}	-0.367^{*}	-0.368^{*}
	Cobb 500	3835.00	0.206^{ns}	0.205^{ns}	0.984^{***}	0.777^{***}	-0.355^{*}	-0.354^{*}
61	Ross 308	3841.50	0.046^{ns}	0.045^{ns}	0.986^{***}	0.376^{**}	-0.305^{*}	-0.307^{*}
	Cobb 500	3850.00	0.122^{ns}	0.122^{ns}	0.981^{***}	0.669^{***}	-0.272^{*}	-0.270^{*}

BW – Body weight (g). * P<0.05; ** P<0.01; *** P<0.001; ^{ns} P>0.05.

r_1 – Body weight of lying hens (g) x Intensity of the laying capacity for hatching eggs (%); r_2 – Body weight of the laying hens (g) x Intensity of the laying capacity for fertilized eggs (%); r_3 – Body weight of the laying hens (g) x Egg weight (g); r_4 – Body weight of laying hens (g) x Daily consumption of food per bird (g/day); r_5 – Body weight of laying hens (g) x Consumption of food per hatching egg (g/egg); r_6 – Body weight of laying hens (g) x Consumption of food per fertilized egg (g/egg).

Statistically significant (P<0.001; P<0.01; P<0.05) correlation relationship between the body weight of laying hens and the intensity of the laying capacity for hatching eggs, i.e. fertilized eggs in the case of Ross 308 hybrid has been determined by the 34th week of the production cycle (57 weeks of age), and in the case of Cobb 500 hybrid, it was determined by the 35th week of the production cycle (58 weeks of age). From that period until the end of the production cycle no statistically significant (P>0.05) correlation relationship has been determined between the examined parameters. However, the absolute correlation relationship (P<0.001) has been determined between the body weight of laying hens and the weight of eggs in both cases of the studied hybrids. During the production cycle, Cobb 500 hybrid

hens were consuming more food compared to the hens of Ross 308 hybrid, which indicates a statistically significant correlation relationship (P<0.001 - Cobb 500 and P<0.001, P<0.01 - Ross 308) between the studied parameters. Similar to the food consumption per bird, in both analyzed hybrids, food consumption per hatching and the fertilized egg was statistically significant (P<0.001, P<0.01, P<0.05) during the entire production cycle (Table 2).

In their researches, most of the authors dealt more with the effect of the age of laying hens on the productive indicators of broiler parents, and somewhat less with the effect of the body weight of laying hens. However, *Djermanovic et al. (2009), Djermanovic (2010), Djermanovic et al. (2012), Đermanović et al. (2005), Djermanovic et al. (2008), Djermanovic et al. (2010), Mitrovic et al. (2005), Mitrovic et al. (2009), Mitrovic et al. (2011), Sahin et al. (2009), Savic et al. (2004)* and *Suarez et al. (1997)* came to the similar, but also to the conflicting results, regarding the correlation relationship between body weight of laying hens and production indicators.

Conclusion

In comparison with the technological standards of the studied hybrids, the average body weight of the laying hens was also lower, both at the beginning and at the end of the production cycle. However, the differences between the body weights of laying hens belonging to both hybrids were not statistically significant (p>0.05), i.e. a genotype did not significantly affect the body weight of hens.

Based on the calculated coefficients of phenotypic correlations and their significance, it can be concluded that the body weight of laying hens significantly affected the production performances because in both cases of parent flocks, and for the entire production cycle, statistically significant (P<0.001, P<0.01, P<0.05) correlation coefficients have been determined between body weight of laying hens and the majority of the observed indicators, while for the shorter period, statistically significant (P<0.001, P<0.01, P<0.05) correlation coefficients have been determined between the body weight of laying hens and the intensity of the laying capacity for hatching and fertilized eggs. Based on the aforesaid, it can be seen that the productive capacity of laying hens decreases with the increase in body weight. Also, the aforementioned indicates that the increase in body weight of laying hens causes shortening of the production cycle than anticipated, i.e. the existence of the turning phase in the last third of the production cycle.

Uticaj telesne težine nosilja i proizvodnih osobina brojlerskih roditelja

Vladan Djermanovic, Sreten Mitrovic, Milena Milojević

Rezime

Ispitivanja su sprovedena na dva jata brojlerskih roditelja hibrida Ross 308 i Cobb 500. Na početku proizvodnog ciklusa (24. nedelja starosti) kod hibrida Ross 308 utvrđena je prosečna telesna težina nosilja 2680.40 g, a hibrida Cobb 500 2697.80 g. U 42. nedelji starosti (sredina proizvodnog ciklusa) telesna težina nosilja iznosila je 3565.10 g (Ross 308) i 3599.05 g (Cobb 500), dok je na kraju proizvodnog ciklusa (61. nedelja starosti) telesna težina nosilja hibrida Ross 308 iznosila 3841.50 g, a Cobb 500 3850.00 g. Utvrđene razlike telesne težine nosillja (17.40 g, 33.95 g i 8.50 g) u određenim periodima proizvodnog ciklusa, kao i razlika u telesnoj težini nosilja za ceo proizvodni ciklus (23.26 g) nisu bile statistički signifikantne (P>0.05). Konkretnije sagledavanje uticaja telesne težine nosilja na proizvodne sposobnosti brojlerskih roditelja utvrđeno je izračunavanjem koeficijenata fenotipske korelacije između ispitivanih pokazatelja. Tako su između telesne težine nosilja i većine proizvodnih pokazatelja utvrđeni statistički signifikantni (P<0,001; P<0,01; P<0,05) koeficijenti fenotipske korelacione povezanosti, dok su između telesne težine nosilja i intenziteta nosivosti priplodnih i oplođenih jaja utvrđeni statistički signifikantni (P<0,001; P<0,01; P<0,05) koeficijenti korelacije, ali za nešto kraći period proizvodnog ciklusa.

Ključne reči: kokoši nosilje, telesna masa, proizvodne osobine, odgajivači brojlera, korelacija

Acknowledgement

The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for sponsoring part of the study within project № TR–31033.

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Received 17 May 2017; accepted for publication 14 June 2017

Biotechnology in Animal Husbandry 33 (2), p 211-219 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.05'65
https://doi.org/10.2298/BAH1702211D

EFFECT OF DIETARY PROTEIN LEVEL AND LENGTH OF FATTENING PERIOD ON DRESSING PERCENTAGE AND CARCASS CONFORMATION IN BROILER CHICKENS

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Original scientific paper

Abstract: This study analyses the effect of different protein levels in broiler feeds (supplemented with protease) and different lengths of fattening period on some parameters related to dressed carcass quality. Medium-growing Master Gris broiler chickens were used in a fattening trial lasting 63 days. At slaughter, dressing percentages and abdominal fat percentages were determined based on traditionally dressed carcass weights and abdominal fat weights of broilers at 49 and 63 days, and conformation indices were calculated based on absolute conformation measurements. Results showed that dietary treatment had an effect only on one relative conformation measurement – body weight/shank length in chickens at 49 days, as control broilers had higher values of this index compared to chickens receiving feeds containing reduced levels of crude protein and protease supplementation (0.2% or 0.3%). Length of fattening period affected almost all studied parameters, except breast angle, dressing percentage of traditionally dressed carcass and abdominal fat percentage.

Key words: broiler chickens, protease, length of fattening period, traditionally dressed carcass, conformation measurement

Introduction

Knowledge of genetic and non-genetic factors affecting broiler meat quality and production is the key to successful poultry meat production. The main goal of modern broiler production is maximum utilisation of the genetic potential of fast-growing broiler strains.

Nutrition is an important factor governing meat quality, with dietary protein level having the greatest influence. The need for reducing the negative environmental impact

of poultry nutrition requires feed quality improvement to minimise the entry of waste products of the digestive process (ammonia, phosphates, etc.) into the environment.
Broiler slaughter weight has a large effect on body conformation traits (*Pavlovski and Mašić, 1983*), which are, moreover, very often directly associated with the weight and distribution of muscles, primarily those of the breast, thighs and drumsticks *(Pavlovski et al., 2006).*
Moderate- and slow-growing broilers have been selected for long fattening periods under semi-intensive and free-range production systems; therefore, they have longer shanks. Furthermore, prolonged fattening leads to a considerable improvement in body conformation traits in these broilers compared to young chickens, with the only significant difference, in most cases, found in shank length (*Pavlovski et al., 2007).*
With aging, body weight and dressed carcass weight increase, while dressing percentage decreases *(Bogosavljević-Bošković et al., 2008)*. In addition, prolonged fattening causes an increase in the proportion of major primal cuts i.e. breast, thighs and drumsticks (*Milićević, 2006*), which is, however, not always the case in slow-growing strains *(Grashorn and Clastermann, 2002)*.
As found by *Zerehdaran et al. (2005)*, the genetic correlation between body weight and abdominal fat percentage at 70 days of age was higher than at 48 days; the increase in growth at 48 days was accompanied by an increase in valuable parts, whereas this increase at 70 days was accompanied by an increase in abdominal fat percentage.
There is a relatively large body of literature on the quality of meat obtained from fast-growing broilers. However, meat quality of medium-growing broilers has not been extensively studied. There is also a scarcity of literature data on the nutritional requirements of moderate growth broilers and medium-growing broilers. Crude protein requirements of slow-growing chickens are lower than those of fast-growing broilers *(Morris and Njuru, 1990)*, whereas dietary lysine requirements are the same regardless of growth rate *(Han and Baker, 1993).*
It is for the above reason that this study focused on the effect of protein levels in diets supplemented with protease on the dressing percentage of the traditionally dressed carcass and body conformation in medium-growing Master Gris broilers at 49 and 63 days of age.

Materials and methods

During 63 days of the experimental fattening period, 300 day-old medium-growing Master Gris broilers were randomly allocated to 3 groups, each consisting of 100 chickens. Stocking density was 10 chickens/m^2. Broilers were fed ad libitum. During the experiment, optimum air temperature and humidity conditions were provided in the poultry house.

Dietary treatments

Broiler feeding involved three stages: starter (the first 3 weeks), grower (22-35 days) and finisher (from 36 days until the end of the experiment). Broilers received complete feeds based on maize and soybean products (soybean meal and full-fat soybean grits), designed for fast-growing broilers (Table 1). Across groups, at all fattening stages, control broilers were fed diets containing standard levels of crude protein, whereas experimental E-I and E-II chickens received diets with crude protein amounts reduced by 4% and 6%, respectively, compared to the normal protein level (through reduction in the amount of soybean meal used), and supplemented with protease (Ronozyme ProAct, DSM, The Netherlands) at 0.2 % and 0.3%, respectively.

Table 1. Nutrient composition of diets for treatments[1]

	Starter phase			Grower phase			Finisher phase		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
ME, kcal/kg	3.081	3.100	3.112	3.157	3.174	3.183	3.181	3.198	3.207
Crude proteins, %	22.59	21.72	21.24	18.99	18.22	17.84	17.16	16.45	16.09
Crude fats, %	5.59	5.55	5.70	5.67	5.73	5.76	5.55	5.61	5.64
Ca, %	0.96	0.95	0.95	0.91	0.91	0.90	0.90	0.89	0.89
Available P, %	0.44	0.44	0.43	0.40	0.40	0.40	0.39	0.39	0.39
Total lysine, %	1.33	1.27	1.24	1.15	1.10	1.08	1.05	1.00	0.98
Total methionine+cystine, %	0.92	0.90	0.89	0.91	0.89	0.88	0.86	0.84	0.83
Total threonine, %	0.90	0.87	0.85	0.75	0.72	0.70	0.67	0.64	0.63
Total tryptophane, %	0.30	0.29	0.28	0.23	0.22	0.21	0.20	0.19	0.18

[1]Treatments: C-control group, standard broiler diet, without protease; E-I- broilers fed a diet with 0.2% protease (Ronozyme ProAct) supplementation; E-II broilers fed a diet with with 0.3% protease (Ronozyme ProAct) supplementation

Data collection

Ten male and 10 female broilers at 49 and 63 days of age were randomly chosen from each group, individually tagged, weighed after 10 hours of fasting and slaughtered.

At slaughter, weights of traditionally dressed carcass and abdominal fat were measured. Thereafter, the carcasses were dissected into breast, drumsticks, thighs, wings, back and pelvis as primal cuts (*Commission Regulation (EC) No. 543/2008*), and evaluated for conformation traits: breast angle BA (degrees), shank length SL (mm), keel length KL (mm), breast depth BD (mm) and thigh girth TG (mm) as indicators of major carcass parts and their development (*Pavlovski and Mašić, 1983*). To eliminate the influence of body weight on these traits, conformation indices (body weight/shank length (g/mm) BW/SL, body weight/keel length (g/mm) BW/KL, body weight/breast depth (g/mm) BW/BD and body weight/thigh girth (g/mm) BW/TG) were determined.

Statistical analysis

Data were statistically analysed by conventional methods, using the statistical software *Statistica for Windows Release 6.0 (1995)*.
The mathematical model of a two-way analysis of variance (3x2 design – 3 feeding treatments-FT and 2 fattening periods -FP) was used to test the significance of differences for meat quality parameters.
The significant differences detected by the analysis of variance (ANOVA) and the results of the expected value of the F-ratio were assessed by the LSD test ($P<0.05$).

Results and Discussion

Table 2 presents slaughter body weights of Master Gris broilers, traditionally dressed carcass weights, dressing percentages, abdominal fat weights and abdominal fat percentages of traditionally dressed carcasses.

Table 2. Weights and dressing percentages of traditionally dressed carcasses of broilers across experimental groups

Treatment			Slaughter weight, gr	Traditionally dressed carcass weight, gr	Dressing percent of TD carcass, %	Adbominal fat, gr	Abdominal fat, %
Protease	Fattening period, days						
No	49	$\bar{x}$	2570.25^{b}	2200.53^{b}	85.61	43.34^{b}	1.71
		Sd	192.76	165.87	1.97	13.57	0.58
	63	$\bar{x}$	3387.00^{a}	2903.50^{a}	85.72	63.82^{a}	1.91
		Sd	406.19	382.80	1.99	12.60	0.43
0.2%	49	$\bar{x}$	2452.00^{b}	2125.70^{b}	86.69	40.82^{b}	1.68
		Sd	210.21	184.15	1.20	11.46	0.50
	63	$\bar{x}$	3334.00^{a}	2851.66^{a}	85.53	64.32^{a}	1.96
		Sd	361.21	338.71	1.80	15.03	0.53
0.3%	49	$\bar{x}$	2513.50^{b}	2156.87^{b}	85.81	46.08^{b}	1.86
		Sd	227.32	192.04	1.25	11.73	0.54
	63	$\bar{x}$	3302.50^{a}	2830.97^{a}	85.72	61.85^{a}	1.91
		Sd	388.12	354.65	1.40	15.58	0.58
p-value							
Source of variation							
Protease			0.423	0.544	0.446	0.891	0.777
Fattening period			0.001	0.001	0.114	0.001	0.067
Protease x fattening period			0.790	0.920	0.196	0.433	0.602

TD- "traditionally dressed"
[a-b] Means followed by different superscript letters within columns differ significantly ($P<0.05$)

As indicated by Table 2, dietary treatments showed no differences in carcass quality parameters ($p>0.05$). Prolonged fattening resulted in an increase in body weights of Master Gris broilers by about 830 gr on average ($p<0.05$). Also, traditionally dressed

carcass weights increased by about 700gr on average ($p<0.05$), while similar values were recorded for the dressing percentage of traditionally dressed carcass regardless of broiler age ($p>0.05$). Prolonged fattening caused an increase in abdominal fat weight ($p<0.05$), while no significant changes were found in abdominal fat percentage, similarly to the dressing percentage ($p>0.05$).

Body weight of broilers is largely affected by rearing system and diet. In the present study, Master Gris broilers achieved somewhat higher body weights compared to the performance data provided by the producer (*Master Gris, 2004*), which was most likely due to much more intensive nutrition (broilers received feeds designed for fast-growing strains). However, *Blagojević (2011)* reported considerably lower average body weights of Master Gris broilers at 49 days (1434.25 g) and at 63 days (1626.40 g) under extensive free range production conditions.

In this study, values for the dressing percentage of the traditionally dressed carcass were somewhat higher than those determined by *Blagojević et al. (2009)* - 83.68% in Master Gris broilers at 91 days of age, along with a somewhat higher abdominal fat content (3.16%).

Absolute body conformation measurements (shank length, keel length, breast depth, breast angle and thigh girth) for broilers belonging to different age groups are provided in Table 3.

Table 3. Body conformation (absolute values) of broilers across experimental groups

Treatment			SL mm	KL mm	BD mm	BA degrees	TG mm
Protease	Fattening period, days						
No	49	$\bar{x}$	78.85[b]	117.10[b]	101.55[b]	127.10	145.65[b]
		Sd	4.28	4.65	5.50	3.04	6.88
	63	$\bar{x}$	87.65[a]	126.30[a]	109.55[a]	127.75	160.40[a]
		Sd	7.58	6.21	7.35	2.63	10.50
0.2%	49	$\bar{x}$	79.85[b]	117.95[b]	100.20	128.20	149.75[b]
		Sd	5.21	5.55	7.71	1.51	7.25
	63	$\bar{x}$	88.75[a]	126.10[a]	108.80[a]	127.45	157.65[a]
		Sd	7.64	4.85	6.77	2.39	9.66
0.3%	49	$\bar{x}$	82.95[b]	117.05[b]	102.70[b]	127.00	145.20[b]
		Sd	5.24	4.58	5.57	2.73	7.80
	63	$\bar{x}$	88.75[a]	126.55[a]	108.50[a]	128.90	156.00[a]
		Sd	7.50	5.25	6.52	7.41	7.50
p-value							
Source of variation							
Protease			0.192	0.960	0.704	0.812	0.224
Fattening period			0.001	0.001	0.001	0.389	0.001
Protease x fattening period			0.470	0.831	0.610	0.300	0.190

SL-shank length, KL-keel length, BD-breast depth, BA-breast angle, TG-thigh girth

[a-b] Means followed by different superscript letters within columns differ significantly ($P<0.05$)

Reduced dietary protein levels and protease supplementation had no effect on absolute conformation traits in the tested broilers ($p>0.05$). The 14-day prolongation of the fattening period led to increased values for all absolute conformation measurements taken ($p<0.05$), except for breast angle, which had similar values at both 49 and 63 days ($p>0.05$), consistently with the results of *Pavlovski et al. (2007)*. As the result of higher values for body weight and dressed carcass weight, body conformation scores for broilers in this study were better than in *Blagojević (2011)*, who found similar shank lengths, but much lower values for the other absolute conformation traits in Master Gris broilers at 91 days.

Relative conformation measurements – body conformation indices in Master Gris broilers across dietary treatments, are given in Table 4.

Table 4. Body conformation indices in broilers across experimental groups

Treatment			BW/SL g/mm	BW/KL g/mm	BW/BD g/mm	BW/TG g/mm
Protease	Fattening period, days					
No	49	$\bar{x}$	32.61[b]	21.93[b]	25.34[b]	17.66[b]
		Sd	1.90	1.11	1.87	1.18
	63	$\bar{x}$	38.58[a]	26.80[a]	30.87[a]	21.06[a]
		Sd	2.48	2.77	2.59	1.41
0.2%	49	$\bar{x}$	30.71[c]	20.79[b]	24.52[b]	16.36[b]
		Sd	1.65	1.45	1.85	0.89
	63	$\bar{x}$	37.55[a]	26.40[a]	30.61[a]	21.12[a]
		Sd	2.16	2.22	2.28	1.50
0.3%	49	$\bar{x}$	30.34[c]	21.46[b]	24.47[b]	17.32[b]
		Sd	2.56	1.58	1.77	1.38
	63	$\bar{x}$	37.18[a]	26.04[a]	30.38[a]	21.12[a]
		Sd	2.62	2.28	2.44	1.75
p-value						
Source of variation						
Protease			0.001	0.188	0.333	0.114
Fattening period			0.001	0.001	0.001	0.001
Protease x fattening period			0.612	0.487	0.836	0.071

BW – body weight at slaughter, SL – shank length, KL – keel length, BD – breast depth, TG – thigh girth
[a-c] Means followed by different superscript letters within columns differ significantly ($P<0.05$)

As shown in Table 4, relative conformation traits were significantly affected by length of fattening period ($p<0.05$), similarly to absolute conformation traits, carcass weight and abdominal fat weight. The prolongation of the fattening period for 2 weeks led to an increase in all absolute conformation measurements, which was in agreement with *Pavlovski and Mašić (1983)* and *Pavlovski et al. (2006).* The conformation indices were much higher, as the result of intensive production

of this strain of broilers. There are very few literature data regarding body conformation of Master Gris broilers. *Blagojević et al. (2009)* reported the following values for the same strain of broilers at 91 days of age: body weight/shank length 22.57, body weight/keel length 19.41, body weight/breast depth 19.33, body weight/thigh girth 15.27. Dietary protein level had no significant effect on body conformation indices, except on body weight/shank length in broilers at 49 days. Specifically, C broilers had significantly higher values for this index compared to E-I and E-II chickens ($p<0.05$), due to somewhat greater body weights and somewhat shorter shanks compared to E-I and E-II broilers.

Conclusion

Results showed similar responses of Master Gris broilers to dietary treatments in terms of the carcass traits analysed, given that significant difference occurred only at 49 days of the fattening trial in one relative conformation measurement – body weight/shank length (between C and E-I broilers and between C and E-II broilers). The parameters tested were considerably more affected by another factor studied – length of fattening period, which produced significant effects on all traits, except the dressing percentage of traditionally dressed carcass, abdominal fat percentage and breast angle. The prolongation of the fattening period for two weeks led to an increase in all absolute and relative conformation measurements taken, except breast angle, which had similar values for both lengths of fattening period.

Uticaj nivoa proteina u hrani i dužine trajanja tova na randman i mere konformacije trupova pilića

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Rezime

U radu je analiziran uticaj različitog nivoa proteina u hrani za tov pilića (uz dodatak enzima proteaze) i dužine trajanja tova na neke parametre kvaliteta obrađenih trupova. U ogledu je korišćen medium-growing linijski hibrid Master Gris, a ogled je trajao 63 dana. Na liniji klanja, na osnovu mase klasično obrađenog trupa i mase abdominalne masti pilića uzrasta 49. i 63.dana tova utvrđen je randman klasimično obrađenog trupa i udeo abdominalne masti, a na osnovu apsolutnih mera konformacije izračunati su indeksi mera konformacije. Rezultati ogleda su pokazali da je uticaj ispitivanih obroka ispoljio efekat samo na jednu

relativnu meru konformacije trupova – telesna masa/dužina piska kod pilića starosti 49.dana, jer su pilići iz kontrolne grupe imali veću vrednost ovog indeksa u odnosu na piliće koji su hranjeni smešama sa nižim nivoima sirovih proteina, uz dodatak enzima proteaze (0,2% ili 0,3%). Različita dužina trajanja tova uticala je na skoro sve ispitivane parametre, izuzev na veličinu grudnog ugla, randman klasično obrađenog trupa i udeo abdominalne masti.

Ključne reči: pilići, proteaza, dužina trajanja tova, klasično obrađen trup, mere konformacije

Acknowledgment

This study is part of Project No. 31033 titled “Sustainable Conventional and Revitalised Traditional Production of Value-Added Poultry Meat and Eggs” funded by the Ministry of Science and Technological Development of the Republic of Serbia.

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Received 17 May 2017; accepted for publication 10 June 2017

Biotechnology in Animal Husbandry 33 (2), p 221-231 , 2017
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.086:637.05'65
https://doi.org/10.2298/BAH1702221Z

EFFECT OF EXTRUDED FLAXSEED ENRICHED DIET ON PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF BROILER MEAT

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Original scientific paper

Abstract: The aim of this experiment was to examine the effect of the addition of extruded flaxseed to chicken feed on physico-chemical and sensory characteristics of breast and leg-thigh meat. The basic chemical composition, pH value, instrumental colour and sensory characteristics of white (breast) and dark meat (leg-thigh) were examined by feeding two groups (both comprising males and females) of 500 Ross 308 hybrid line chickens by standard feed (control group) and with the addition of 6% of extruded flaxseed mixture (experimental group). Instrumental characteristics of colour were changed, especially in white meat. Both breast muscles of male broilers were significantly lighter, but a* values were significantly lower in *m. pectoralis profundus* and b* values higher in *m. pectoralis superficialis* of both genders. In dark meat, a* values were significantly lower in the meat of males, while b* values were higher in the meat of females. The addition of extruded flaxseed to chicken feed did not led to significant changes in the sensory characteristics of meat.

Key words: chicken meat, extruded flaxseed, instrumental colour, sensory evaluation

Introduction

Meat is an important source of high biological value proteins, minerals, vitamins and other nutrients. On the other hand, meat lipids do not have such beneficial characteristics, but rather properties such as high fat and cholesterol content, high energy and atherogenic value, bad ratio of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), low content of ω-3 PUFA, as well as a poor ratio of ω-6/ω-3 FA (*Delgado-Pando et al., 2010*).

Fatty acid profile of meat lipids can be improved by a diet rich in ω-3 PUFA especially in monogastric animals (pigs, poultry and fish) since their organism absorbs fatty acids in their intact form (*Bou et al., 2009*).

Poultry meat has become very important in the last several decades and nowadays accounts for about 33% of global meat production (*FAO, 2010*). Until recently, flaxseed oil was predominantly used in chicken feed, as the use of flaxseed is limited by antinutritional factors such as: cyanogenic glycosides, phytic acid, linatin dipeptide (vitamin B6 antagonist) (*Anjum et al., 2013*). The extrusion process reduces the content of antinutritional factors by more than 93%, at the same time retaining useful components and rendering extruded flaxseed adequate for use in animal diet (*Anjum et al., 2013*).

In the process of increasing the level of ω-3 PUFA in meat, it is important to achieve positive nutritional and functional effects on meat and at the same time not diminish the sensory quality. Meat with a higher content of unsaturated fatty acids has a higher nutritional value; however, the higher content of unsaturated fatty acids, especially in dark meats, results in their oxidative instability and poorer sensory characteristics of taste and odour. Poorer taste and odour can be ascribed to the presence of compounds from, for instance, marine products used in animal feed and the oxidation of unsaturated fatty acids (*Bou et al., 2009*). More pronounced oxidative instability of meat means that sensory changes will also be more noticeable (*Palmquist, 2009*).

The goal of this experiment was to examine the effect of the addition of extruded flaxseed to chicken feed on physic-chemical and sensory characteristics of breast and leg-thigh meat as the most valuable parts in nutritional and economic terms.

Materials and methods

Experimental design and animal management

One thousand unsexed one-day chickens of the Ross–308 hybrid line had *ad libitum* access to water and to the diets (starter to 28 days, followed by 28–45 day finisher). The experiment was carried out at the chicken farm of meat company "Union MZ", Svilajnac, Serbia. The chicks were reared under standard conditions of housing and management in floor pens with wooden shavings as litter material. Ventilation, lighting and relative humidity were automatically regulated. During the starter period chickens were placed in a single floor pen. On day 28 two equal groups were randomly formed in three replicates each, having an approximately equal sex ratio: control group (CON), fed by standard feed, and experimental group (EXP), fed with the addition of 6% of extruded flaxseed mixture (Croquelin, Walorex SAS, La Messaayais-35210 Combourtille, France). Some research studies indicate that extruded flaxseed level in poultry diet of 5% and more could adversely influence some sensory characteristics of broiler meat such as flavour and aroma (*Anjum et al., 2013*). In view of these data, the addition of 6% of extruded flaxseed mixture (3% of extruded flaxseed) to the diet was chosen to

avoid such changes. The composition of their diet is presented in Tables 1 and 2. At the age of 45 days, 15 male (CONM & EXPM) and 15 female (CONF & EXPF) chickens from each group (5 from each replicate) were selected by the average weight of the group (± 5%) and marked before being slaughtered and then eviscerated. The carcasses were air-chilled for about 2 hours until the temperature of below 4 °C was reached within the breast muscle. After weighing, 12 male and 12 female processed carcasses from each group were randomly selected (4 from each replicate); the meat was apportioned into cuts: back, two leg-thighs, two wings and breasts. Breasts (white meat) and leg-thighs (dark meat) were then deboned, the skin was removed and the meat was used for physicochemical analysis. From each group 3 male and 3 female processed carcasses were used for the sensory evaluation of the meat.

Physicochemical analysis of meat

It was explained in *Experimental design and animal management* section. The chemical composition of meat was determined in the following manner: water content by drying samples at 105 °C (*ISO 1442, 1997*); protein content by the Kjeldahl method and multiplying by factor 6.25 (*ISO 937, 1978*); fat content by the Soxhlet method (*ISO 1443, 1973*); ash content by sample mineralization at 550–600 °C (*ISO 936, 1998*). pH value was measured by pH-meter Hanna, HI 83141 (Hanna Instruments Srl, Sarmeola di Rubano, Italy).

Table 1. Ingredients and composition (%) of diets (finisher)

Ingredients	CON	EXP
Corn	61.00	57.00
Soybean meal	12.00	12.00
Soybean grit	13.00	13.00
Sunflower meal (33% protein)	4.00	4.00
Croquelin [1]	-	6.00
Livestock yeast	3.00	1.00
Monocalcium phosphate	0.80	0.80
Limestone	1.30	1.30
Soybean oil	3.00	3.00
Premix[2]	1.00	1.00
Iodized Salt	0.30	0.30
Lysine	0.10	0.10
Methionine	0.19	0.19
Threonine	0.16	0.16
Tryptophan	0.02	0.02
Phytase	0.01	0.01
Mineral clay (Minazel)	0.20	0.20
Crude protein	17.53	17.43
Moisture	10.75	10.58
Crude fat	7.83	7.82
Ash	4.68	4.47
Calcium	0.66	0.60
Total phosphate	0.44	0.52

[1] extruded flaxseed (TRADI-LIN®) 50%, wheat flour 30%, sunflower meal 20%. [2] Composition of premix provided (per kg of premix): vitamin A1, 200,000 IU; vitamin D3, 300,000 IU; vitamin E, 3,000 mg; vitamin K3, 250 mg; vitamin B1, 200 mg; vitamin B2, 600 mg; pantothenic acid, 1,500 mg; nicotinic acid, 2,500 mg; vitamin B6, 600 mg; folic acid, 100 mg; choline chloride, 31,000 mg; vitamin B12, 2,000 µg; biotin, 3,000 µg; Fe, 6,000 mg; Cu, 800 mg; Zn, 6,000 mg; Mn, 8,000 mg; J, 60 mg; Se, 15 mg; Ca, 100 mg; BHT (E321), 10,000 mg.

Table 2. Fatty acid composition* of diets (finisher)

Fatty acid	CON	EXP
C14:0	0.06	0.06
C16:0	10.90	11.10
C16:1	0.07	0.07
C17:0	0.07	0.07
C18:0	4.22	4.49
C18:1cisω-9	26.25	24.71
C18:2ω-6	49.96	51.49
C20:0	0.32	0.31
C18:3ω-3	6.72	8.22
C20:2ω-6	0.04	0.04
C22:0	0.33	0.34
C20:3ω-6	0.05	0.07
C20:5ω-3	0.03	0.00
SFA	15.90	16.37
MUFA	26.32	24.78
PUFA	56.80	59.82
ω-6	50.05	51.60
ω-3	6.75	8.22
ω-6/ ω-3	7.41	6.28

*g/100g total fatty acids

Determination of meat colour

The CIE L*a*b* colour coordinates were determined by MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) using an 8 mm aperture size, illuminant D65 and a 2º standard observer angle. The Chroma Meter was calibrated using a Minolta calibration plate (Y=87.2, x=0.3173; y=0.3348;). C* (chroma) and h (hue angle) were calculated according to *Tapp et al. (2011)*. The colour was measured on two breast muscles (*m. pectoralis superficialis* – MPS and *m. pectoralis profundus* – MPP) and one leg muscle (*m. biceps femoris* – MBF), with two measurements on each sample.

Sensory evaluation

Meat is sensory evaluated by a quantitative descriptive analysis (*ISO 6658, 2005*) using the scales with 5 or with 8 points, depending on the sensory property. Sensory evaluation was performed by eight assessors, previously trained for detection and recognition of various tastes (*ISO 3972, 2011*) and odours (*ISO 5496, 2006a*). Samples of leg-thigh and breast meat with bones and skin were thermally treated in the oven at 150 °C until the temperature of 80 °C was achieved in the

inner part of the meat pieces. Then, samples were served to the assessors in an identical way on white plastic plates.

Meat colour

Meat colour was evaluated by a 5-point scale: 5 – very good, 4 – good, 3 – acceptable, 2 – slightly acceptable, 1 – unacceptable; **Meat structure** was evaluated by a 5-point scale: 5 – very fine, 4 – fine, 3 – neither fine nor rough, 2 – rough, 1 – very rough; **Meat juiciness** was evaluated by an 8-point scale: 8 – very juicy, 7 – juicy, 6 –moderate juicy, 5 – slightly juicy, 4 – slightly dry, 3 – moderately dry, 2 – dry, 1 – very dry; **Meat softness** was evaluated by an 8-point scale: 8 – very soft, 7 – soft, 6 – moderately soft, 5 – slightly soft, 4 – slightly tough, 3 – moderately tough, 2 – tough, 1 – very tough; **Acceptability of taste and odour** of meat was evaluated by an 8-point scale: 8 – very good, 7 – good, 6 – moderate, 5 – still good, 4 – slightly bad, 3 – moderately bad, 2 – bad, 1 – very bad.

Statistical analysis

The results of the chemical composition, pH value, instrumental colour measurement and sensory analysis were processed by a two-factorial analysis of variance (ANOVA). Tukey's HSD test was used to identify significant ($P < 0.05$ and $P < 0.01$) differences between groups. Calculations were done with software Statistica 6.0 (2001).

Results and discussion

Chemical composition

Chemical composition is an important meat characteristic, not only because of the nutritional value, but also because of its effect on the sensory quality. The results of the analysis of the impact of nutrition and gender on the chemical composition of white and dark meat of broilers are presented in Table 3.

The diet to which extruded flaxseed was added did not have a large influence on the chemical composition. Statistically significant influence was established only in terms of ash content in the white meat of male broilers and protein in the dark meat of females. *Crespo and Esteve-Garcia (2002)* reached rather similar results and concluded that the protein content in meat was somewhat higher in animals fed by the control diet.

Table 3. Basic chemical coposition (%)of broiler meat

	Control group (CON)		Experimental group (EXP)		P-values of model effects		
	Male (CONM)	Female (CONF)	Male (EXPM)	Female (EXPF)	tmt	gen	tmt*gen
White meat							
Protein %	21.19±0.53^{ab}	22.02±0.57^{b}	21.06±0.63^{a}	21.65±0.51^{ab}	NS	0.006	NS
Moisture %	76.16±0.54^{A}	74.77±0.52^{B}	76.09±0.62^{A}	75.65±0.62^{AB}	NS	<0.001	NS
Fat %	1.02±0.34^{a}	0.94±0.17^{a}	0.68±0.41^{a}	0.81±0.59^{a}	NS	NS	NS
Ash %	1.30±0.08^{a}	1.44±0.09^{ab}	1.50±0.16^{b}	1.48±0.12^{ab}	0.021	NS	NS
Dark meat							
Protein %	17.89±0.77^{a}	19.27±0.7^{B}	17.59±0.84^{A}	17.76±0.44^{A}	0.005	0.013	0.047
Moisture %	74.27±0.81^{a}	72.72±1.16^{a}	74.43±1.41^{a}	73.39±1.31^{a}	NS	0.015	NS
Fat %	4.75±1.05^{a}	6.30±1.05^{a}	5.23±1.24^{a}	6.36±0.83^{a}	NS	0.050	NS
Ash %	1.19±0.23^{a}	1.22±0.18^{a}	1.26±0.21^{a}	1.23±0.11^{a}	NS	NS	NS

^{A,B} Values (mean±SD) within the same row with different uppercase letter in superscript differ significantly at $P < 0.01$; tmt – treatment, gen – gender.
$^{a, B}$ Values (mean±SD) within the same row with different small or small and upper letter in superscript differ significantly at $P < 0.05$.

Instrumental colour and pH value

Colour is one of the most important sensory characteristics of meat; it is the first thing we perceive about food, it attracts buyers or puts them off, and is often related to flavour, tenderness and safety (*Girolami et al., 2013*). A whole series of factors affect the colour of meat, some independently, and some through mutual interactions. The physical and chemical characteristics of meat have an additional impact on colour (*King and Whyte, 2006*), while the pH value of meat also plays an important role. It is known that animal feed can affect meat colour, but there are few data about the effect of the addition ω-3 FA to the feed. The results of instrumental colour measurements and pH values are presented in Table 4.

Lightness (L* values) increased in light muscles due to the addition of extruded flaxseed, though significantly only in males (P < 0.05). No differences were established between genders in either group or in either light or dark muscles. Measured pH values were between 5.67 and 5.77 (MPS) and somewhat higher in MPP, 6.00–6.15, while the differences in either case were not statistically significant which indicates that the pH value was not significantly changed by the diet and that different values of lightness can be most likely attributed to nutrition. Our results correspond to the data of *Betti et al. (2009)* who established that the addition of flaxseed, as well as the length of the feeding period, affects the increase of lightness of white meat. The redness, a* values, in the MPS of CON groups was somewhat lower (2.48 and 2.08) relative to MPP (3.77 and 3.54). The addition of extruded flaxseed to the diet resulted in a decrease of a* values in both of the tested muscles of both genders, but significantly (P < 0.01) only in MPP – "the redder" muscle. The yellowness, b* values, was higher in test groups, though considerably

only in MPS (both genders). In general, the white meat of test groups of both genders had slightly poorer characteristics in terms of colour – it was lighter, less red and more yellow.

The influence of diet on changes in parameters of instrumental colour of dark meat (MBF) differs relative to white meat. Significant changes were not determined in terms of lightness and hue angle. The a* values in EXP were considerably lower in the meat of males, while b* values were significantly higher in the meat of females. pH values of dark meat were higher (6.11–6.26) than those of white meat, which contributed (in addition to higher myoglobin content) to lower lightness and a more intensive red colour (*Dadgar et al., 2012*). As was the case with both white meat muscles, the diet did not significantly affect the change in the pH value ofMBF.

Table 4. Colour characteristics (CIE L*a*b*) and pH values of broiler white and dark meat

	Control group (CON)		Experimental group (EXP)		P-values of model effects		
	Male (CONM)	Female (CONF)	Male (EXPM)	Female (EXPF)	tmt	gen	tmt*gen
m. pectoralis superficijalis (MPS)							
L*	53.53±1.97^{a}	52.95±2.02^{a}	56.83±3.86^{b}	54.70±2.33^{ab}	0.020	NS	NS
a*	2.48±0.35^{c}	2.08±0.12^{ab}	2.26±0.31^{bc}	1.94±0.23^{a}	0.024	<0.001	NS
b*	5.15±0.64^{AB}	5.10±0.58^{a}	6.07±0.63^{C}	5.85±0.71^{bc}	<0.001	NS	NS
C*	5.72±0.66^{a}	5.51±0.54^{a}	6.49±0.56^{b}	6.17±0.69^{ab}	<0.001	NS	NS
h	64.17±3.24^{A}	67.68±2.53^{B}	69.42±3.70^{BC}	71.49±2.64^{C}	<0.001	0.003	NS
pH	5.67±0.08^{a}	5.77±0.07^{a}	5.67±0.18^{a}	5.72±0.15^{a}	0.033	NS	NS
m. pectoralis profundus (MPP)							
L*	51.89±1.58^{a}	51.27±3.18^{a}	55.45±3.56^{b}	53.09±2.65^{ab}	0.002	NS	NS
a*	3.77±0.33^{A}	3.54±0.27^{A}	2.88±0.32^{B}	2.73±0.39^{B}	<0.001	0.047	NS
b*	5.41±0.96^{a}	5.74±1.23^{a}	5.96±0.65^{a}	6.05±0.56^{a}	NS	NS	NS
C*	6.62±0.81^{a}	6.78±1.04^{a}	6.62±0.69^{a}	6.64±0.63^{a}	NS	NS	NS
h	54.65±5.21^{A}	57.56±6.22^{A}	64.11±2.13^{B}	65.75±2.42^{B}	<0.001	NS	NS
pH	6.04±0.12^{a}	6.12±0.11^{a}	6.00±0.16^{a}	6.15±0.22^{a}	NS	0.010	NS
m. biceps femoris (MBF)							
L*	50.80±2.68^{a}	49.89±2.02^{a}	49.14±0.59^{a}	49.32±1.09^{a}	NS	NS	NS
a*	14.63±1.33^{b}	13.05±1.25^{ab}	12.35±0.93^{a}	13.22±1.43^{ab}	0.051	NS	0.026
b*	10.58±0.87^{ab}	9.05±1.38^{a}	10.39±0.32^{ab}	10.74±1.07^{b}	NS	NS	0.030
C*	18.08±1.10^{B}	15.95±0.93^{A}	16.14±0.75^{a}	17.07±1.28^{ab}	NS	NS	0.002
h	35.95±3.63^{a}	34.80±5.74^{a}	40.14±2.27^{a}	39.15±4.18^{a}	0.050	NS	NS
pH	6.26±0.21^{a}	6.20±0.14^{a}	6.11±0.30^{a}	6.21±0.18^{a}	NS	NS	NS

^{A,B} Values (mean±SD) within the same row with different uppercase letter in superscript differ significantly at $P < 0.01$; tmt – treatment, gen – gender.

$^{a, B}$ Values (mean±SD) within the same row with different small or small and upper letter in superscript differ significantly at $P < 0.05$.

Sensory Evaluation

When assessing white meat, no statistically significant differences were detected in any of the observed parameters (data not shown). The panellists

assessed the colour of white meat with relatively high grades, from 4.19 (CONF) to 4.31 (EXPM), while the structure of meat of all groups was assessed as fine [3.69 (CONF)–4.06 (EXPF)]. Juiciness and softness were assessed as being between juicy and moderately juicy [6.06 (CONF)–6.50 (EXPF)] and soft and moderately soft [6.13 (CONF)–6.56 (EXPF)], respectively. Taste and odour were given high grades, between good and very good (6.81–7.31).

Dark meat grades (data not shown) were very similar to the grades for white meat, and none of the observed parameters of the sensory evaluation showed statistically significant differences.

Our results confirm to the findings of *Betti et al. (2009)* who compared the sensory characteristics of pates made from the white meat of broilers fed by flaxseed, and concluded that texture, flavour, aftertaste, liking and the overall opinion of breast samples were not different across the range of treatments compared. *Stanaćev et al. (2014)* also concluded that the addition of 4% of flaxseed oil in finisher diet did not lead to major changes of sensory characteristics of breast meat. *Schreiner et al. (2005)* observed that panellists noticed the differences between the sampled variants of chicken meat quite rarely and with difficulty, while *Lopez-Ferrer et al. (1999)* believe that the use of fish oil can have bad sensory effects, and that the use of plant oils considerably improves the sensory parameters of meat compared to fish oil.

Conclusion

The addition of extruded flaxseed to broiler diet affected the instrumental characteristics of colour, most notably in white meat. Both breast muscles of male broilers were significantly lighter. Breast muscles (*m. pectoralis superficialis* and *m. pectoralis profundus*) of both EXP groups were more yellow – higher b* values and significantly higher h values, whereas a* values were significantly reduced in *m. pectoralis profundus*. However, these changes did not cause significant differences during the sensory evaluation of the colour of white meat. As for the dark meat of males, a* values were significantly higher in CON, while b* values in the meat of females were significantly higher in EXP. Based on the results of this experiment, we may conclude that the addition of extruded flaxseed to chicken feed did not led to major changes in the sensory characteristics of meat.

Uticaj ishrane obogaćene ekstrudiranim lanenim semenom na fizičko-hemijska i senzorna svojstva mesa brojlera

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Rezime

Cilj ovog ogleda je bio da ispita efekat dodavanja ekstrudiranog lanenog semena u ishranu pilića, na fizičko-hemijska i senzorna svojstva mesa. Na početku tova 1000 jednodnevnih pilića linijskog hibrida Ros-308 imali su *ad libitum* pristup vodi i hrani. Metodom slučajnog uzorka 28. dana su formirane dve jednake grupe (sa po tri ponavljanja svaka) i približno jednakim udelom polova: kontrolna grupa (CONM – mužjaci, CONF – ženke) hranjena standardnom hranom i ogledna (EXPM – mužjaci, EXPF – ženke) hranjena sa dodatkom 6% komercijalne mešavine ekstrudiranog semena lana. Ispitivan je osnovni hemijski sastav, pH vrednost, instrumentalna boja i senzorna ocena belog mesa (grudi) i tamnog mesa (batak sa karabatkom). U pogledu osnovnog hemijskog sastava utvrđen je jedino značajno manji sadržaj proteina tamnog mesa EXPF u odnosu na CONF. Način ishrane nije uticao na pH vrednost. Oba mišića (*m. pectoralis superficialis* i *m. pectoralis profundus*) muških pilića brojlera statistički su značajno svetlija ($P < 0.05$). Udeo crvene boje značajno se smanjuje kod *m. pectoralis profundus*, a žute povećava kod *m. pectoralis superficialis*, oba pola ($P < 0.001$). U tamnom mesu (*m. biceps femoris*) udeo crvene boje značajno se smanjuje ($P < 0.05$) kod mesa mužjaka, a udeo žute značajno raste ($P < 0.05$) kod mesa ženki. Nije utvrđen značajan uticaj načina ishrane na senzorsku ocenu belog i tamnog mesa.

Ključne reči: pileće meso, ekstrudirano seme lana, instrumentalno određena boja, senzorna ocena

Acknowledgment

The research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project III–46009. We would also like to thank Mrs. Marija Stajić MA for assistance with translation and copy editing.

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Received 27 January 2017; accepted for publication 15 March 2017

Biotechnology in Animal Husbandry 33 (2), p 233- 241 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 632.4'633.15
https://doi.org/10.2298/BAH1702233K

FUNGAL CONTAMINATION OF MAIZE GRAIN SAMPLES WITH A SPECIAL FOCUS ON TOXIGENIC GENERA

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Original scientific paper

Abstract: In this study, the total fungal count and contamination with toxigenic fungi from *Aspergillus, Fusarium* and *Penicillium* genera of 127 maize grain samples collected from animal farms in subrbs of Belgrade area during 2012-2015, were determined. The total fungal count was determined using a dilution method, and standard mycological procedures were used to identify potential toxigenic fungi genera.

In the tested samples of maize grains, the total fungal count was from 1 x 10^1 to 3 x 10^6 cfu g^{-1}. No statistically significant differences between investigated years in regard to the mean total fungal count were determined. According to the Regulation on the quality of animal feed of the Republic of Serbia, the total fungal count above permitted limit (2 x 10^5 cfu g^{-1}) was established in 9.52, 7.89, 20.69 and 55.56% tested samples in 2012, 2013, 2014 and 2015, respectively.

Potentially toxigenic fungi from *Aspergillus, Fusarium* and *Penicillim* genera have been identified as the most common in all the samples. In most of the samples, based on the average value for the four-year period (2012-2015), *Fusarium* species (92.22%) have been identified, followed by the species of the genera *Aspergillus* (80.83%) and *Penicillium* (48.68%). A weak positive correlation was established between the moisture content of the samples and the total fungal count in 2012 (r=0.41), in 2013 (r=0.27) and in 2014 (r=0.36) and the medium positive correlation (r=0.61) in 2015.

Based on the results of mycological analysis of grain maize it can be concluded that the test samples in a relatively large number did not meet the criteria of hygienic quality. Therefore, regular and continuous control of the mycological quality of maize grain as the most important nutrient in animal nutrition is necessary as a preventive measure to reduce and control contamination of grain with mycotoxigenic fungi.

Key words: maize, total fungal count, toxigenic fungi

Introduction

Maize is a major food crop for people and also uses as feed for livestock. It makes about 50-70% of poultry feeds *(Jokić et al., 2004)* and it is the most important source of energy for pig feeding *(Edwards, 2002)*. However, maize grains are suitable substrate for fungal infection. There are a many potential toxigenic fungi species that contaminate grain, from which species from *Aspergillus, Fusarium* and *Penicillium* genera are dominant species, as contaminants of maize and producents mycotoxins. The contamination of maize by toxigenic fungi and their mycotoxins is the process that it can occur yet in the field during harvest and later during the storage until the consumption *(Zorzete et al., 2008)*.

Environmental conditions and climatic factors are the most important to the contamination of maize grains before and after harvest. The moisture content of grain and temperature are the most important factors for the growth of potentially mycotoxigenic fungi and spread of infection to the maize grain before and after harvest (*Kana et al., 2013*). Species of the genera *Fusarium, Aspergillus* and *Penicillium* are the most important pathogens isolated from the animal feed in Serbia, from the mycotoxicological aspect. In the environmental conditions of Serbia, *Fusarium* species are usually isolated from the maize grain which, in any given year, may cause a significant reduction in yield, increased mycotoxins contamination and the mass occurrence of mycotoxicosis in animals, particularly pigs *(Lević, 2008)*.

Given the prevalence of the most common types of toxin-producing fungi of the genera *Fusarium, Aspergillus* and *Penicillium* in Serbia, of particular importance to the health of animals is to consider the presence of mycotoxins as secondary metabolites of fungi. In the production of poultry, in particular, the presence of aflatoxin and ochratoxin, produced by *Aspergillus* and *Penicillium* species, is examined, as the most important carcinogenic contaminants of poultry feed (*Leggieri et al., 2015*). *Fusarium* mycotoxins (T-2 toxin and zearalenone) cause disorders in the reproduction of pigs and estrogenism (*Zain, 2011*). Fumonisins are *Fusarium* mycotoxins which are known as causes of equine and porcine leukoencephalomalacia pulmonary edema (*Placinta et al., 1999*). The mycotoxins commonly co-occur in the maize grains, so the presence of a range of mycotoxins is not an unusual occurrence in prepared animal mixtures (*Streit et al., 2012)*.

The aim of this paper was to determine the total fungal count and to identify potentially mycotoxigenic fungi genera in maize grain samples during the fourth-year period (2012-2015) and also to assess the potential danger of the presence of these contaminants in the food chain.

Materials and Methods

During the four-year period (2012-2015), the total fungal count was determined and toxin-producing fungal species identified in a total of 127 samples of maize grains which were collected successively (multiple times) every year (during harvest and storage) from different farms in the vicinity of Belgrade. In 2012, 2013, 2014 and 2015 analyzed a total 42, 38, 29 and 18 samples respectively. The size of laboratory sample was 1 kg. After laboratory admission, the samples were analysed for fungal contamination immediately, or were stored 2-3 days at controlled temperature prior the analysis. The moisture content of the tested maize grain samples was determined using a laboratory moisture meter (OHAUS MB35, USA), and mycological analysis was performed according to the method ISO 21527-2 (2008).

Identification of toxigenic genera of fungi was performed according to Watanabe (2002). The frequency of positive, i.e. samples contaminated by toxigenic fungi, was calculated according to the formula: Fr (%) = the number of samples were a fungal genus occurred/the total number of samples x 100.

Statistical analysis was performed with nonparametric test, using the SPSS software (IBM, Statistic 20). To determine the normality, the Shapiro-Wilk (SW) test was used, and to determine homogeneity of variance, the Levene's test. Because the Shapiro-Wilk test showed significant difference compared to the normal distribution, the significance of differences was tested using the Mann-Whitney U - test.

The correlation among individual values for moisture content and total fungal count was determined using the Pearson correlation coefficient.

Results and Discussions

The total fungal count and identification of toxigenic fungi are important indicators of hygienic quality of maize grain as feed of plant origin that is used as an important component in animal feed.

The average moisture content in tested samples of maize grain was 12.19% (2012), 12.37% (2013), 13.81% (2014) and 12.42% (2015). Mycological analysis of tested maize samples established the total fungal count in the range from 1 x 10^1 to 3 x 10^6 cfu g^{-1}. The tested samples of maize showed 9.52% (2012), 7.89% (2013), 20.69% (2014) and 55.56% (2015) of the samples with total fungal count above the limit (2 x 10^5 cfu g^{-1}) according to the Regulation on the quality of animal feed for the feed of vegetable origin of Republic of Serbia (*Official Gazette, 4/2010, and 27/2014 113/2012*) (Table 1). A large number of samples with total fungal count above allowed limit in 2015 (55.56%) can be explained by the

extremely favorable climatic conditions during the maize growing season in 2014 (April- October), when, according to the data of Republic Hydro-meteorological Service of Serbia for the Belgrade area, the total precipitation of 675.3 mm was recorded and the mean daily temperatures were >20°C. Those conditions had a favorable impact on the increase of the infective potential of toxigenic fungi in maize in the field, and later during the storage in 2015. In tested maize samples, an average total fungal count was high and was not statistically significant ($P \leq 0.05$) between the studied years (2012 - 2015) (Table 2). This is probably due to the high moisture content (> 15%) in samples of maize grains which were analyzed during the harvest and due to the poor storage conditions (uncontrolled conditions of temperature and humidity).

Similar to our results, in Turkey, analyzing 30 samples of maize grains originating from different locations, *Alptekin et al. (2009)* have determined the total fungal count of 5 x 10^5 cfu g^{-1} to 5.2 x 10^7 cfu g^{-1}. In Argentina, *González Pereyra et al. (2012)*, during a two-year study (2006-2007), in the analysis of samples of feed for cattle which contained 60 to 70% of maize grain, have found that the total fungal count ranged from 0 to 2.10 x 10^8 cfu g^{-1}, and statistically significant differences were determined in regard to the average total fungal count in samples of tested mixtures for cattle between the investigation years. These statistical differences are explained by suitable climatic factors and environmental variations during the sampling period (May to November).

Table 1. Level of fungal contamination of investigated maize grain samples during 2012-2015

Fungal counts		Frequency (%)			
cfu g^{-1} *	log_{10}cfu	Year 2012	Year 2013	Year 2014	Year 2015
2.1 x 10^5 - 3 x 10^6	5.32-6.48	9.52	7.89	20.69	55.56
1.1 x 10^4 – 2 x 10^5	4.04-5.30	66.67	71.05	68.97	16.67
1 x 10^1 - 1 x 10^4	2 - 4	23.81	21.06	10.34	27.77

*Colony forming units per g of sample

Table 2. Mean of total fungal counts (log_{10}cfu g^{-1}) in investigated maize grain samples during 2012-2015

Maize grain samples	cfu g^{-1} (log10)	SD
Year 2012	4.41	0.74
Year 2013	4.57	0.58
Year 2014	4.82	0.72
Year 2015	4.52	0.94
Level of significance	0.087 (ns)	

cfu g^{-1} - colony forming units per g of sample; ns - not significant - P>0.05

The mycological analysis of maize grain showed toxigenic species of the genera *Aspergillus, Fusarium* and *Penicillium*. On average for all investigation

years (2012-2015), the most of samples (92.22%) was contaminated with toxigenic species of the genus *Fusarium*, followed by 80.83% of samples contaminated with *Aspergillus* and 48.68% of the samples contaminated with *Penicillium* species. In 2012 and 2013, the most of samples were contaminated with *Aspergillus* species, 98.48 and 94.74%, respectively; while in 2014 and 2015 the majority of samples were contaminated with *Fusarium* species, 96.55 and 100%, respectively. A larger number of samples was contaminated with *Penicillium* species in 2012 (76.19%) and 2013 (63.16%) compared to 2014 (27.59%) and 2015 (27.78%) (Table 3). Similar to our results, in Italy, *Covarelli et al. (2011)*, in the analysis of the maize grains originating from different locations of Umbria region, within a two year period (2006-2007), have isolated *Fusarium* species in the highest percentage (up to 76.8%), *Aspergillus* (up to 14.5%) and *Penicillim* species (up to 9.2%). Likewise, in Saudi Arabia, from 20 samples of grain of yellow maize originating from different markets, *Mahmoud et al. (2013)* have isolated most commonly the species from the *Fusarium* (31.74%) and *Aspergillus* (30.83%) genera, and the *Penicillium* (13.75%) and *Alternaria* species (1.66%) have also been isolated but to a lesser extent. Also, *Mudili et al. (2014),* in the analysis of 150 freshly harvested maize samples during 2010-2012, have determined that the *Fusarium* species have been the most present in the investigated sites in India, followed by some species of the genera *Aspergillus* and *Penicillium*. Contrary to the above results, *Alptekin et al. (2009)* has found that the occurrence of species of the genus *Penicillium* was significantly higher than the species of the genera *Aspergillus* and *Fusarium,* in 30 tested samples of maize originating from different localities in Turkey. Furthermore, in Republic Srpska, *Trkulja et al. (2014),* from 83 samples of maize grain intended for human and animal consumption sampled before harvest in 2013, have isolated in almost all samples the *Aspergillus* species, and species of the genera *Fusarium, Penicillium* and *Alternaria* isolated in up to 33, 23 and 18% of the samples, respectively.

Table 3. Frequency of contaminated maize grain samples with potentially toxigenic fungi from *Aspergillus*, *Fusarium* and *Penicillium* genera

Fungal genus	Frequency of fungal contaminated samples (%)				
	Year 2012	Year 2013	Year 2014	Year 2015	Average (2012-2015)
Aspergillus	98.48	94.74	68.97	61.11	80.83
Fusarium	88.10	84.21	96.55	100	92.22
Penicillium	76.19	63.16	27.59	27.78	48.68

Using Pearson's correlation coefficient, in the tested samples of maize, a weak positive correlation was determined between the moisture content and the total fungal count, r=0.27 (2013), r=0.36 (2014) i r=0.41 (2012) and medium

positive correlation of r=0.61 (2015). Similarly, in the studies of *Alptekin et al. (2009),* a positive correlation (r = 0.378) was determined between the relative humidity (RH) and fungal count which was not statistically significant (P> 0.05).

Conclusion

Based on the results obtained in this four-year study, it can be concluded that most of the samples of maize grain were contaminated with mycotoxigenic fungi of the genera *Aspergillus, Fusarium* and *Penicillium,* and total fungal count above permitted limit was established in 9.52% (2012), 7.89% (2013) 20.69% (2014) and 55.56% (2015) of the samples. On average for all years of investigation, *Fusarium* species were identified in most maize samples (92.22%), followed by 80.83% of the samples contaminated with *Aspergillus* and 48.68% of the samples contaminated with *Penicillium* species. These results are due to agro-ecological and climatic conditions, especially the total precipitation and temperature, suitable for fungal infection of maize grains still in the field in the investigated area. Similarly, poor environmental conditions of storage after maize harvest contribute to the development of unwanted biological contaminants into the grain. Consequently, the permanent control of the mycological condition of maize during harvest and during storage is the main preventive measure against undesirable consequences to the health of humans and animals arising from the consumption of food with a high content of harmful contaminants such as mycotoxigenic fungi.

Kontaminacija uzoraka zrna kukuruza gljivama s posebnim osvrtom na toksigene rodove

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Rezime

Ukupan broj gljiva i kontaminacija s potencijalno toksigenim vrstama iz rodova *Aspergillus, Fusarium* i *Penicillium* određivani su u 127 uzoraka zrna kukuruza koji su sakupljeni na farmama u okolini Beograda tokom četvorogodišnjeg perioda (2012-2015). Primenom metode razređenja određen je ukupan broj gljiva, dok su standardne mikološke metode korišćene za identifikaciju potencijalno toksigenih rodova gljiva.

U ispitivanim uzorcima zrna kukuruza ukupan broj gljiva je bio od 1 x 10^1 do 3 x 10^6 cfu g^{-1}. Između ispitivanih godina nisu ustanovljene statističke značajne

razlike u prosečnim vrednostima ukupnog broja gljiva. Prema Pravilniku Republike Srbije o kvalitetu hrane za životinje, u hranivima biljnog porekla, ukupan broj gljiva iznad dozvoljenog limita (2 x 10^5 cfu g^{-1}) ustanovljen je u 9,52, 7,89, 20,69 i 55,56% ispitivanih uzoraka u 2012., 2013., 2014. i 2015. godini, respektivno.

Od potencijalno toksigenih gljiva identifikovane su *Aspergillus, Fusarium* i *Penicillim* vrste kao najučestalije u svim ispitivanim uzorcima. U najvećem broju uzoraka, na osnovu prosečnih vrednosti u četvorogodišnjem periodu (2012-2015), identifikovane su *Fusarium* vrste (92.22%), zatim vrste iz rodova *Aspergillus* (80.83%) i *Penicillium* (48.68%). Između sadržaja vlage ispitivanih uzoraka i ukupnog broja gljiva ustanovljena je slaba pozitivna korelacija u 2012. (r=0.41), 2013. (r=0.27) i 2014. godini (r=0.36) i srednje pozitivna korelacija (r=0.61) u 2015. godini.

Na osnovu dobijenih rezultata mikološke analize zrna kukuruza može se zaključiti da ispitivani uzorci u relativno velikom broju ne zadovoljavaju kriterijume higijenskog kvaliteta. Zbog toga, redovna i stalna kontrola mikološkog kvaliteta zrna kukuruza kao najvažnijeg hraniva u ishrani životinja je neophodna preventivna mera za smanjenje i kontrolu kontaminacije zrna s mikotoksigenim gljivama.

Ključne reči: kukuruz, ukupan broj gljiva, mikotoksigene gljive

Acknowledgment

Research was supported by Ministry of Education, Science and Technological Development, Republic of Serbia, projects TR 31023, TR 31033 and TR 31053.

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Received 22 March 2017; accepted for publication 22 May 2017

Biotechnology in Animal Husbandry 33 (2), p 243-249 , 2017
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.085.3
https://doi.org/10.2298/BAH1702243M

PROTEIN FRACTIONS OF INTERCROPPED PEA AND OAT FOR RUMINANT NUTRITION

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Original scientific paper

Abstract: The quantification of the main crude protein (CP) fractions during the growing period of pea and oat mixtures may be used to optimize the forage management. The determination of protein fraction could improve balancing rations for ruminants. The first factor (A) is ratio of germinated seed in mixtures. The pea and oat were tested at two different mixture rates: A_1 – 50% pea + 50% oat and A_2 – 75% pea + 25% oat. The second factor (B) is a cutting time in three stages of growth: B_1 – a cutting of biomass at the start of flowering pea (10% of flowering), B_2 – a cutting of biomass at forming the first pods on 2/3 plants of pea, and B_3 – cutting of biomass at forming green seeds in 2/3 pods. Stage of growth and pea-oat ratio in mixtures are significantly related to the change in the quality and chemical composition of biomass. The highest level of crude protein was obtained in pea at flowering stage (184.85 g kg^{-1} dry matter (DM)). The high level of easily soluble protein and non-protein nitrogen compounds (over 50%) represent specific characteristics of the mixture. Unavailable fraction PC increased with plant maturation from 75.65 to 95.05 g kg^{-1} of CP.

Key words: protein fractions, pea-oat mixtures

Introduction

The goal of most dairy farmers is to use the available land to produce cheaper but high quality feed with adequate protein content for lactating cows in order to maximize milk yield. The nutritional quality of CP in forages is determined by its rate and extent of degradation in the rumen, and this can be enhanced by increasing true protein that is resistant to microbial degradation in the rumen. Feeding excess CP can result in unnecessary feed expenses with no return in milk or milk protein yield. Furthermore, the majority of excess dietary N is excreted in the urine, which is the most environmentally labile form (*Higgs et al., 2012*).

Annual legumes is mostly grown as a sole crop, but in some countries intercropping with cereals is a common practice (*Klimek-Kopyra et al*. 2014). Legume-cereal mixtures are important protein and carbohydrate sources for livestock. The most common application in practice is a mixture of field pea and oat, because of the high yield and quality of biomass (*Uzun and Asik, 2012*). This may lead to the better utilization of these mixtures as livestock feed.
The objective of the present study was to quantify the main CP fractions during the growing period of field pea and oat mixtures. Determination of protein fraction would improve balancing rations for animals, especially for dairy cows.

Materials and methods

Field pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac – Serbia (21º19′35″ E, 43º34′58″ N). Experiment was carried out in autumn in 2012, on October the 20th in a randomized block design with three replications. The first factor (A) is ratio of germinated seed in mixtures. The pea and oat were tested at two different mixture rates: A_1 – 50% pea + 50% oat and A_2 – 75% pea + 25% oat. The second factor (B) is a time of cutting of biomass with three stages of growth: B_1 – a cutting of biomass at the start of flowering pea (10% of flowering), B_2 – a cutting of biomass at forming the first pods on 2/3 plants of pea, and B_3 - cutting of biomass at forming green seeds in 2/3 pods. Both mixtures were sown on plots of 20 m^2 with three replications for each time of cutting. Plant samples were taken from the surface of 1 m^2 for chemical analysis.
The CP of the samples was determined using Kjeldahl method. The NPN, NDICP, ADICP, SolP, TP (True protein) and IP (Insoluble protein) were determined by *Licitra et al.* (*1996*). The CP, NPN, SolCP, NDICP, ADICP, TP and IP were calculated as follows:
CP = Total N x 6.25
NPN = (Total CP – Residusl CP_{NPN})/CP x 1000
SolCP = (Total CP – Residual CP $_{SolCP}$)/CP x 1000
ADICP = Residual CP_{ADICP}/CP x 1000
NDICP = Residual CP_{NDICP}/CP x 1000
TP = Residual CP_{NPN}/CP x 1000
IP = Residual CP_{SolCP}/CP x 1000
NPN_{SolCP} = NPN/SolCP x 1000
Where, CP is the crude protein, NPN - non-protein nitrogen (g kg^{-1} CP); SolCP, the soluble crude protein (g kg^{-1} CP); NDICP, the neutral detergent insoluble crude protein (g kg^{-1} CP); ADICP, the acid detergent insoluble crude protein (g kg^{-1} CP); TP – true protein (g kg^{-1} CP); IP – insoluble crude protein (g kg^{-1} CP) and NPN_{SolCP}, (g NPN kg^{-1} SolCP^{-1}).

The CNCPS (Cornell Net Carbohydrates and Protein System) crude protein fractions of the samples, PA, PB_1, PB_2, PB_3 and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to *Fox et al.* (*2004*).
PA = NPN
PB_1 = SolCP – NPN
PB_2 = CP – SolCP – NDICP
PB_3 = NDICP – ADICP
PB = 1000 – PA – PC
PC = ADICP
Where, PA refers to the non-protein nitrogen (g kg^{-1} CP); PB_1, the rapidly degraded crude protein (g kg^{-1} CP); PB_2, the intermediately degraded crude protein (g kg^{-1} CP); PB_3, the slowly degraded crude protein (g kg^{-1} CP) and PC, the bound crude protein (g kg^{-1} CP).
The data were processed by the two way ANOVA in a randomized block design. Effects were considered different based on significant ($p< 0.05$) F ratio. The significance of differences between arithmetic means was tested by Tukey test.

Results and discussion

The protein fractions of of field pea and oat mixture are shown in Table 1. The crude protein (CP) concentration decreased ($p< 0.05$) during the investigated period as the maturity advanced. The average level of CP in the mixture R_2 was for 20.59 g kg^{-1} DM higher than in mixture R_1 ($p< 0.05$). This was expected due to the higher quantity of pea in the mixture R_2. The CP content in both mixtures were significantly higher than *Kocer and Albayrak* (*2012*) reported for mixtures with similar pea : oat ratios as well as the CP values that reported *Omokanye* (*2014*) for field pea intercropping with oat and barley. The decreasing trend in CP content can be explained by increasing oat weight and reducing pea weight in mixtures with plant development from flowering to grain filling. Obtained results are consistent with results by *Uzun and Asik* (*2012*), who stated that, in the pea monoculture and in pea - barley mixtures, maturing plants decrease CP level.
In both mixtures and during maturation, except stage P_3, the soluble fraction PA was above 500 g kg^{-1} (Table 1). *Vahdani et al*. (*2014*) reported that the highest fraction of CP in DM of grass pea hay was PB_2 that is potentially degradable in rumen. These authors showed that content of PA, PB_1, PB_2, PB_3 and PC fractions were 107.8, 266.5, 511.6, 54.5 and 59.5 g kg^{-1} CP, respectively. Maturity of forage mixtures had contrasting effects on fraction PA and fraction PB_1. Fraction PA increased ($p< 0.05$) and fraction PB_1 decreased from the stage P_1 to the stage P_2, and then fraction PA decreased from stage P_2 to stage P_3, and fraction PB_1 increased. This contrasting pattern between the two soluble CP fractions can be explained by the process of accumulation and distribution of CP during the period

of rapid growth. However, fraction PA is NPN, whereas fraction PB_1 is soluble true protein. As seed makes a large fraction of total forage, these results suggest that a higher proportion of soluble true protein is accumulated in the seed as compared with NPN or that some NPN of vegetative plant parts is redistributed as true protein of the seed.

Across oat : pea ratios in mixtures and harvesting stages, result showed that intermediately degraded fractions PB_2 accounted 310.23 gkg^{-1} CP in mixture R_1, and 330.76 gkg^{-1} CP ($p< 0.05$) in mixture R_2 (Table 1).

During growing season, fraction PB_2 decreased from the stage P_1 to the stage P_2, and after that from stage P_2 to P_3 this fraction increased ($p< 0.05$). A higher proportion of seeds can be the cause of this difference between stages of growth. These results illustrate the fact that maturity had a greater effect on CP concentration than on the proportion of the main CP fractions.

Table 1. Fraction of protein by Cornell Net Carbohydrate and Protein (CNCPS)

Factors		CP g kg^{-1} DM	PA g kg^{-1} CP	PB_1 g kg^{-1} CP	PB_2 g kg^{-1} CP	PB_3 g kg^{-1} CP	PC g kg^{-1} CP
R_1	P_1	176.82b	473.70e	79.72a	279.06c	82.87a	84.65d
	P_2	152.58d	549.85b	17.98d	276.02d	52.50c	103.65a
	P_3	136.00e	500.60d	11.97e	375.60a	15.23f	96.60b
R_2	P_1	192.58a	535.55c	9.78e	350.37b	37.65d	66.65f
	P_2	167.06c	559.70a	33.10c	267.05e	63.75b	76.40e
	P_3	166.80c	459.80f	51.20b	374.85a	20.65e	93.50c
$\overline{X}_{R_1}$		155.13b	508.05b	36.55a	310.23b	50.20a	94.97a
$\overline{X}_{R_2}$		175.72a	518.35a	31.37b	330.76a	40.67b	78.85b
$\overline{X}_{P_1}$		184.85a	504.63b	44.75a	314.74b	60.23a	75.65c
$\overline{X}_{P_2}$		160.03b	554.78a	25.54c	271.53c	58.12b	90.03b
$\overline{X}_{P_3}$		151.45c	480.20c	31.58b	375.22a	17.95c	95.05a

R_1- first mixture, 50% pea + 50% oat; R_2- second mixture, 75% pea + 25% oat; P_1- a cutting of the biomassat the start of flowering pea (10% of flowering); P_2- a cutting of biomass at forming the first pods on 2/3 plants of pea; P_3- cutting of biomass at forming gren seeds in 2/3 pods; CP – Crude Protein, PA - non-protein nitrogen; PB_1 - the rapidly degraded crude protein; PB_2 - the intermediately degraded crude protein; PB_3 - the slowly degraded crude protein; PC - the bound crude protein; Different letters denote significantly different means ($P< 0.05$)

Fraction PB_3 includes CP that is insoluble in NDF but soluble in ADF. Changes in NDF concentration of plant parts with maturity may largely explain the differences in proportions of fraction PB_3 (as percentages of total CP). Results of this investigation showed that this protein fraction was higher in mixture R_1 than in mixture R_2 ($p< 0.05$). It is assumed that higher levels of oat in the mixture R_1 contributed to these results. This is consistent with the results of the quality oat and pea that were presented (*Kocer and Albayrak, 2012*).
Unavailable fraction PC (ADICP) represent bound protein that is not degraded in the rumen and is not digested in the small intestine. This fraction increased with plant maturation from 75.65 to 95.05 gkg^{-1} of CP. Statistically significant difference was observed between stages of growth. Mixture R_1 had higher content of PC fraction than mixture R_2 ($p< 0.05$). In contrast to these results, in the biomass of grass pea (*Vahdani et al*. 2014) the largest fraction was PB_2.
One, anticipated advantage of feeding bi-crop forages of cereal and legume is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen. Choosing the most efficient combination of forage species, timing the harvest could increase CP quality for ruminant production. If protein degradation is rapid or non-protein nitrogen value is higher than the capacity of ruminal microbes to utilize released amino acids or ammonia, this could lead to inefficiencies in ruminal nutrition.

Conclusions

In conclusion, stage of growth and pea-oat ratio in mixtures are significantly related to the change in the quality and chemical composition of biomass. The highest levels of crude protein in dry matter were obtained in pea in flowering stage (184.85 g kg^{-1} DM). Knowledge of the structure of the protein fractions gives us the possibility of combining the proper balance of nutrients in the livestock diet. The high level of easily soluble protein and non-protein nitrogen compounds (over 50%) represent specific characteristics of the mixture. This allows easier mixing of pea-oat mixtures with other livestock feed which have different structure of protein fractions. Thus, mixing pea-oat biomass with other perennial legume (alfalfa, red clover) can give bulky part of a ration with high protein value and a balanced ratio and representation of all protein fractions.

Proteinske frakcije u združenom usevu ovsa i graška za ishranu preživara

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Rezime

Određivanje količine proteinskih frakcija u združenom usevu graška i ovsa može poslužiti za optimalno korišćenje ovih krmnih biljaka. Poznavanje proteinskih frakcija u krmi može poboljšati balansiranje obroka za preživare. Prvi factor u ovim istraživanjima jeste odnos klijavih semena u smešama. Odnosi klijavih semena graška i ovsa su bili: 120 semena graška i 120 semena ovsa (A_1 – 2 / 4 : 2 / 4) u prvoj smeši i 180 semena graška I 60 semena ovsa (A_2 – 3 / 4 : 1 / 4) u drugoj smeši. Drugi factor je bio košenje biomase u tri različite faze razvića: B_1 – početak cvetanja graška (10% procvetalih biljaka), B_2 – faza kada su 2 /3 biljaka formirale prve mahune i B_3 – faza kada su 2 /3 biljaka formirale zeleno seme u mahunama. Faza razvića i odnos graška i ovsa u smešama su značajno uticale na sadržaj proteinskih frakcija u biomasi. Najveći sadržaj sirovih proteina je ustanovljen u fazi cvetanja graška (184,85 g kg^{-1} SM). Visok nivo lakorastvorljivih i neproteinskih azotnih jedinjenja predstavljaju specifičnu karakteristiku ovih smeša. Sadržaj nedostupne proteinske frakcije, PC, se povećavao sa rastom I razvićem biljaka od 75,65 do 95,05 g kg^{-1} SM.

Ključne reči: proteinske frakcije, smeše graška i ovsa

Acknowledgements

The authors thank the Ministry of Education, Science and Technological Development of Republic of Serbia who funded this research as part of the project TR-31057.

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Received 20 December 2016; accepted for publication 5 February 2017

Biotechnology in Animal Husbandry 33 (2), p 251-259 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 638.15
https://doi.org/10.2298/BAH1702251A

MORPHOLOGICAL CHARACTERIZATION AND WING DESCRIPTION OF *VESPA ORIENTALIS ORIENTALIS* QUEENS

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Communication

Abstract: Oriental hornets, *Vespa orientalis*, are dangerous enemy to bee colonies in some countries of the world. There are more than one subspecies of *V. orientalis*. Few studies have investigated the morphological characteristics of these subspecies. Morphological characterization can help in confirming and discriminating between the subspecies, and to follow any changes in their morphology over time. In this study, some body characteristics of *V. orientalis orientalis* queens from Egypt were measured including head width, fore wing length and width, hind wing length and width, femur length, tibia length and approximate stinger length. Also, fore wing characteristics using wing coordinates for 20 landmarks were studied. Computer based techniques were applied to take these measurements. The data of the current study can be utilized for comparisons with other subspecies.

Key Words: hornets, *Vespa*, vespidae, morphology, queens.

Introduction

The oriental hornets (*Vespa orientalis* Linnaeus, 1771; Order: Hymenoptera, Family: Vespidae) occurs in north Africa, south-eastern Europe, Arabian Peninsula, and southwestern Asia (*Archer, 1998*). It was also found in Mexico where it was probably accidentally introduced (*Dvořák, 2006*). Oriental hornets are dangerous pests to bee colonies, especially weak ones. They can be caught with bait traps inside apiaries. In north Egypt (Dakahlia), the queens have been observed to be active from January to May and a few during December and January (*Taha, 2014*). In south Egypt (Assiut), found an increase of *V. orientalis* activity during September and October with no activity during November (*Khodairy and Awad, 2013*). Similar activity trends of *V. orientalis* have been

observed in other countries, in Pakistan hornet numbers greatly increased from August to October and decreased during November (Islam et al., 2015). The activity of *V. orientalis* was significantly correlated with temperature (r=0.137) and humidity (r=0.560) (*Taha, 2014*). In general, *V. orientalis* appears from the spring until the end of autumn and disappear during winter. During autumn, large number of queens can be trapped.

The subspecies of *V. orientalis* are *V. orientalis* variety *aegyptica* André, *V. orientalis zavattarif* Guiglia & Capra, *V. orientalis somalica* Giordani-Soika, *V. orientalis arabica Giordani-Soika*, and *V. orientalis orientalis* (*Archer, 1998*). These subspecies can be identified using body color characteristics. Some studies have considered morphology of *V. orientalis*. The morphology of the sting organ of some hymenopterans including *V. orientalis* has been described (*Zalat et al., 1980*). Also, chemo-receptors of the antenna have been investigated (*Khodairy and Awad, 2013*). The exact measurements (i.e lengths and widths) of different body parts of *V. orientalis,* e.g. heads, wings and legs have not been widely investigated so that there are no available comprehensive databases about morphological characteristics of these subspecies. Another way to characterize or discriminate between species and subspecies is the use of wing coordinate system (geometric morphometric). By identifying the coordinates (X and Y) of specific wing landmarks (points), it is possible to analysis the shape of wings. The geometric morphometric has been used with different hymenopterans including honey bees as reviewed by *Abou-Shaara (2013)*, *Sphex* spp. (*Tüzün, 2009*), and some Vespidae hornets (*Perrarda et al., 2016*). This technique has been able to discriminate between honey bee races (*Tofilski, 2008; Francoy et al., 2008*). The objective of this study is to present a morphological database of *V. orientalis orientalis* for different body parts including heads, wings, legs and stingers, and to use wing point coordinates to present a description to fore wings. This study is likely to contribute in expanding the knowledge about the morphology of this *Vespa* subspecies, and to assist in discriminating this subspecies than others.

Materials and Methods

Fifty six queens of *Vespa orientalis* hornets were collected from traps in a private apiary at Damanhour city, Egypt during autumn 2016. The queens were then identified according to their body color to be *Vespa orientalis orientalis*. The queens were dissected to separate heads, fore wings, hind wings, legs and stingers.

Morphological characterization.

The separated body parts were scanned (Canon Scanner, k10352, CanoScan LiDE 110, Vietnam) at resolution of 1200 dpi to obtain clear images. Subsequently, the images were opened using Photoshop program according to *Abou-Shaara and Al-Ghamdi (2012)* to take the measurements as shown in Fig. 1.

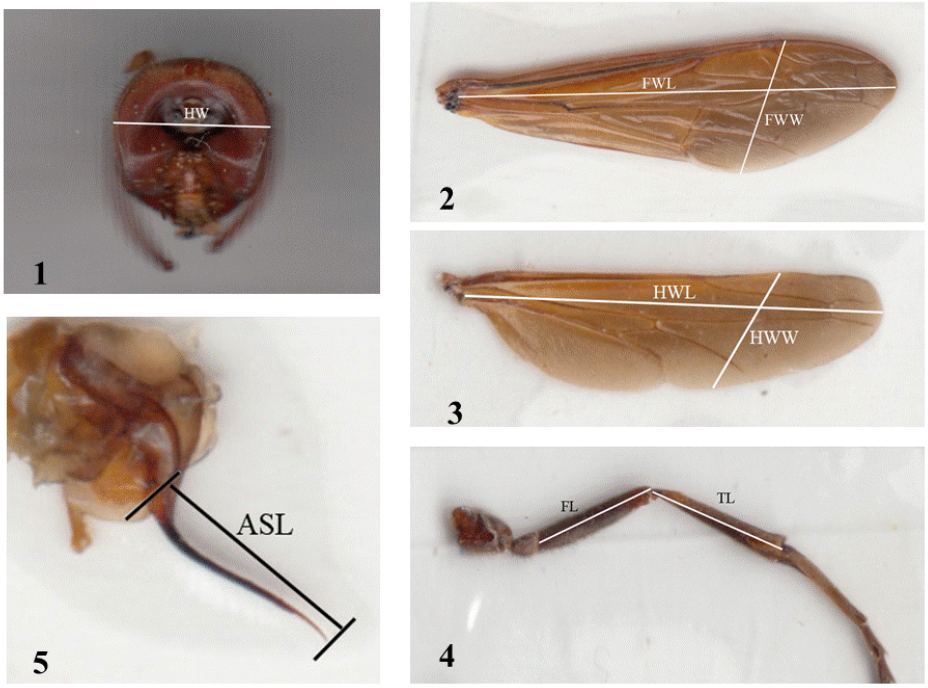

Figure 1: Characteristics measured in this study; 1) Head Width (HW), 2) Fore wing Length (FWL) and Width (FWW), 3) Hind Wing Length (HWL) and width (HWW), 4) Femur and Tibia Length (FL, TL), 5) Approximate Stinger Length (ASL).

Wing description

To present a detailed description to the fore wings, the coordinates of 20 wing landmarks (points) were obtained using ImageJ program, and these coordinates were analyzed using MorphoJ program (*Klingenberg, 2011*) according to *Abou-Shaara and Al-Ghamdi (2012).* After obtaining the X, Y coordinates for each point for 15 wings using ImageJ, the means of these coordinates were calculated. The means were analyzed using MorphoJ to obtain the transformation grid for the points after principal component analysis (PCA). Addition, procrustes sums of squares, tangent sums of squares and total variance were calculated for the points. Usually, the fore wings of dead hornets are bent (tucked). Thus, the width of bent fore wings was measured as shown in Fig. 3. The width of bent fore wings was compared with width of full fore wings to obtain measurements to fore wings

during full and bent positions. This comparison was performed on 13 fore wings, widths measured firstly for bent wings and then for full wings.

Figure 2: Twenty points of *Vespa orientalis orientalis* fore wings.

Figure 3: Bent fore wing width (BFWW).

Statistical analysis

Means and their standard errors (S.E.) were calculated for studied characteristics. The correlation coefficients (r) were calculated among measured body characteristics at 5% level of significance. The width of bent wings and full wings were compared using t-test at 5% of probability using SAS program (version 9.1.3, 2004).

Results and Discussion

Morphological characterization

The measurements in mm of different body characteristics of *V. orientalis orientalis* queens and the range of each character are listed in Table 1. The differences between minimum value and maximum one were 0.4, 0.7, 0.9, 0.8, 0.5, 0.6, 0.4, and 0.5 mm for head width, fore wing length, fore wing width, hind wing length, hind wing width, femur length, tibia length, and approximate sting length, respectively. The differences were not large and varied from 0.4 to 0.9 mm (less than 1 mm) for measured characteristics. Differences about 0.06 and 0.13 mm were

recorded in head width of emerged *Vespula vulgaris* queens during 1990-91 and 1991-92, respectively (*Harris and Beggs, 1995*). The differences between individuals of the same subspecies are normal and can be attributed to feeding quality and quantity as well as rearing conditions (e.g. cell size and nest temperature) during immature stages. A review by *Abou-Shaara (2013)* and *Abou-Shaara et al. (2013)* on wing and body characteristics of honey bees, *Apis mellifera*, highlighted factors impacting them including environmental factors, temperature and nest conditions. It's better to use body color together with body measurements to confirm the subspecies due to the variations in morphological measurements.

The correlation between all body characteristics was significant and high, with a range from 0.89 to 0.97 (Table 2). This means that body characteristics increased linearly, i.e. the increase in any character means the increase in all other body characters. Unlike honey bees, *A. mellifera*, *Kolmes and Sam (1991)* found that bees with larger overall size, corbiculate or wing measurements showed lack of possession to other morphological characters that showed high correlation in size. Therefore, size related characteristics of honey bees are not linearly correlated. Perhaps these variations are related to the ecological niche of each species; hornets are predators, therefore they need larger body characteristics while honey bees are pollinators, thus they need specific body characteristics to be larger than other characteristics to be able to perform their ecological role.

Table 1: Body characteristics of *Vespa orientalis orientalis* queens (Means ± S.E.).

Characteristics	Means ± S.E. (mm)	Range (mm)
Head width	5.52±0.01	5.4 to 5.8
Fore wing length	19.19±0.03	18.9 to 19.6
Fore wing width	6.11±0.03	5.9 to 6.8
Hind wing length	12.19±0.03	11.9 to 12.7
Hind wing width	3.26±0.02	3.1 to 3.6
Femur length	4.99±0.02	4.8 to 5.4
Tibia length	5.56±0.01	5.4 to 5.8
Sting length	3.42±0.02	3.2 to 3.7

Table 2: Correlations (r values) among body characteristics of *Vespa orientalis orientalis* queens. All correlations are significant (*P*<0.05). HW: head width, FWL: fore wing length, FWW: fore wing width, HWL: hind wing length, HWW: hind wing width, FL: femur length, TL: tibia length, and ASL: approximate stinger length.

Characteristics	HW	FWL	FWW	HWL	HWW	FL	TL	ASL
HW	--	0.94	0.95	0.95	0.96	0.92	0.92	0.92
FWL	0.94	--	0.92	0.97	0.95	0.90	0.90	0.95
FWW	0.95	0.92	--	0.95	0.96	0.95	0.93	0.89
HWL	0.95	0.97	0.95	--	0.95	0.92	0.93	0.93
HWW	0.96	0.95	0.96	0.95	--	0.91	0.91	0.93
FL	0.92	0.90	0.95	0.92	0.91	--	0.92	0.90
TL	0.92	0.90	0.93	0.93	0.91	0.92	--	0.89
ASL	0.92	0.95	0.89	0.93	0.93	0.90	0.89	--

Wing description

The transformation grid for means of the point coordinates of the fore wings obtained after principal component analysis (PCA) is shown in Fig. 4. The values of procrustes sums of squares, tangent sums of squares, and total variance were 0.104, 0.103, and 0.0069, in respect. The shape and values of the 20 fore wing landmarks could help in confirming the subspecies of *V. orientalis* because such characteristics should be unique for each subspecies. However, *Perrard et al. (2016)* did not find wing landmark configurations alone as reliable phylogenetic tools. Also, *Tüzün (2009)* found that geometric morphometric analysis alone was less successful in discriminating three species of *Sphex*. Therefore, using body color, body measurements together with wing landmark analysis is better over using one method alone to identify or discriminate *V. orientalis* subspecies.

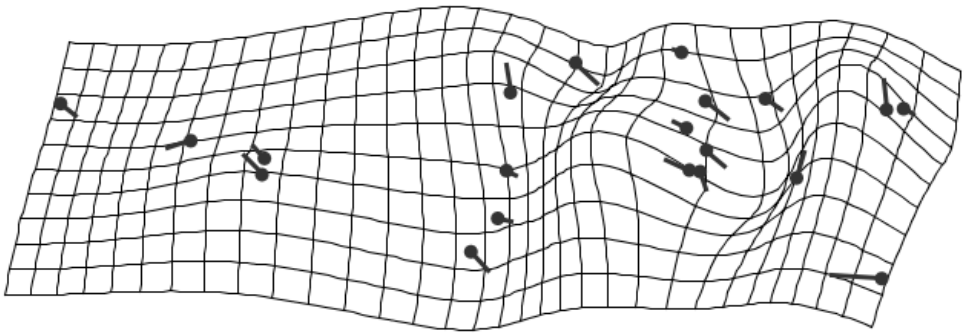

Figure 4: Transformation grid for means of 20 fore wing landmarks of *Vespa orientalis orientalis* queens.

The width average of fore wings was 6.40±0.09 mm and for bent fore wings 2.98±0.05mm of the same wings. The difference between them was about 3.42±0.06 mm, this difference was significant according to t-test (N=13, df=12, t =54.16, P<0.05). It seems that fore wings consists of two parts; upper part (Fig. 5, A) has less width with more thick cuticle, while the lower part (Fig. 5, B and C) has larger width with soft cuticle. The second part can be easily bended on the first part. The variation between the two parts is about 0.44 mm. The presence of these two fore wing parts could have a specific role in the flight of *V. orientalis*, especially since these hornets need to attack prey during flight, and to carry it to the nest. This point may worth further investigations to shade more lights on the flying mechanisms of these hornets.

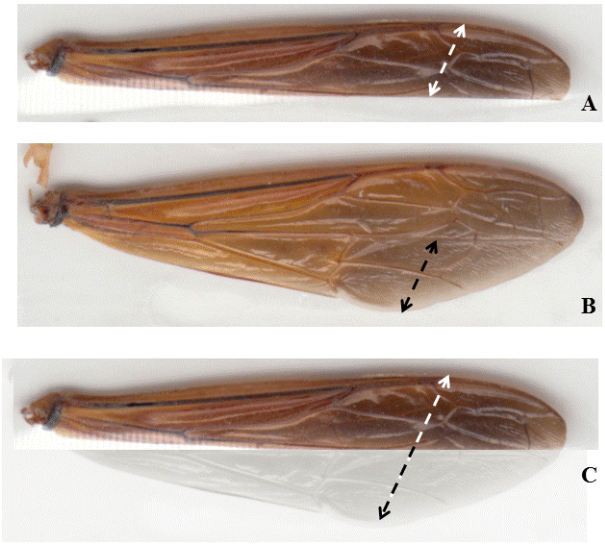

Figure 5: Fore wing at bent position (A), complete position with showing width of lower part (B), and the two positions (C). The black and white arrows show the width.

Conclusion

The study presented database for some body characteristics of *Vespa orientalis orientalis* queens of Egypt. The lengths and widths of body parts of this subspecies have not been well documented. Therefore, the results of this study have specific importance to any future comparisons either with the same subspecies or with other subspecies. Also, the coordinate analysis of 20 fore wing landmarks was done and the transformation grids of the wings were presented beside some statistical values. There was a significant difference between the width of bent wings and full wings; this suggests a special flight mechanism of these hornets. It is recommended to measure body characteristics of all *Vespa orientalis*

subspecies to facility discrimination among them and to prepare database for them. It is also better if these studies can be performed on hornets from various geographical regions to identify the potential ecotypes.

Morfološka karakterizacija i opis krila matica *Vespa orientalis orientalis*

Hossam Abou-Shaara

Rezime

Orijentalni stršljenovi, *Vespa orientalis*, opasni su neprijatelji pčelinjih kolonija u nekim zemljama sveta. Postoji više od jedne podvrste V. *orientalisa*. U nekoliko studija su se ispitivale morfološke karakteristike ovih podvrsta. Morfološka karakterizacija može pomoći u potvrđivanju i diskriminaciji podvrsta, kao i da se prate eventualne promene u njihovoj morfologiji tokom vremena. U ovoj studiji izmerene su neke karakteristike tela matica *V. orientalis orientalis* iz Egipta, uključujući širinu glave, dužinu i širinu prednjih krila, dužinu i širinu zadnjih krila, dužinu femura, dužinu tibije i približnu dužinu žaoke. Takođe, ispitivane su karakteristike prednjih krila koje koristeći krilne koordinate za 20 orijentira. Računarske tehnike su primenjene prilikom uzimanja navedenih mera.

Podaci dobijeni u ovom istraživanju mogu se koristiti za upoređivanje sa drugim podvrstama.

Ključne reči: stršljenovi, *Vespa*, *vespidae*, morfologija, matice.

Acknowledgments

I would like to thank Prof. Dr. Michael E. Archer (York St John University College, England, UK) for identifying the subspecies of Vespa hornets, and for his valuable comments on the manuscript.

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Received 24 April 2017; accepted for publication 15 June 2017

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

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[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

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

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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11th – 13th October 2017, Belgrade, Serbia

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