ASSESSMENT OF EJACULATE QUALITY IN ROOSTERS OF THREE LAYING LINES

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Abstract: The aim of this study was to examine the semen quality in roosters of three laying lines used in breeding in Czech Republic. The maternal lines Bar Plymouth Rock (BPR – 08) and Rhode Island Red (RIR – 05), and the maternal line Light Sussex (SU – 07) were used. Ejaculates were collected four times during laying period of hens by dorso-abdominal massage. The following parameters were determined: volume, concentration of spermatozoa, motility and total sperm abnormality. Statistically significant differences were found in volume of ejaculate between all three lines (from 0.55 to 0.80 cm^3). The highest volume as well as the highest concentration of spermatozoa ($2.39 \times 10^6/mm^3$) were found in BPR. Statistically significant differences were found also in motility of spermatozoa between BPR and RIR (68.59 vs. 77.19%). However, very high percentage of sperm abnormality were found in all three lines (from 56.29 to 72.63%). This phenomenon may be caused by transportation of ejaculate into laboratory. It was concluded that ejaculate quality varied widely among examined lines, although the maternal line SU seemed to be the weakest of all three lines.

Key Words: Roosters, semen evaluation, Bar Plymouth Rock, Rhode Island Red, Light Sussex

INTRODUCTION

Many methods of semen evaluation and estimation of fertilizing potential were invented during years in farm animals. Some of these methods are highly subjective, others require special laboratory devices (Lukaszewicz et al. 2008). The fertilizing potential of the rooster semen is dependent upon the quality and quantity of spermatozoa produced by the testes. Because each rooster is responsible for mating with several hens, sperm characteristics can have a great impact on the fertility of a flock (McDaniel et al. 1998).

The traditional evaluation of the poultry semen quality is mainly based on monitoring of motility, viability, concentration of spermatozoa, semen morphology and acrosomal integrity (Bansal, Cheema 2014). The utilization of artificial insemination, in combination with ejaculate dilution, reduces the number of males needed at each breeding level and enables a high degree of genetic selection (Long 2014). However, ejaculates of poultry are ordinarily pooled to ensure sufficient volume, and males are not evaluated individually. At time of collection, only color and volume are visually assessment (Holsberger et al. 1998).

In this study, rooster ejaculate of three Czech laying lines used in breeding was assessment. Ejaculate was assessment individually and these data were used for evaluation of whole line.

MATERIAL AND METHODS

Animal and semen collection

A total of 48 adult healthy roosters of two initial paternal lines Bar Plymouth Rock (BPR – 08; n = 16) and Rhode Island Red (RIR – 05; n = 16), and one initial maternal line Light Sussex (SU – 07, n = 16) from Integra, a.s. Bantice were used in this study. Each rooster was placed into one cage and fed with complete feed mixture. There was 15 h light and 9 h dark in the hall. The samples of semen were collected four times in age of 175, 230, 295 and 336 days (RIR) or in age of 182, 237, 302 and 343 days (BPR, SU). Semen was collected by the dorso-abdominal massage (reported by Burrows and Quinn 1935, 1937).

Semen evaluation

Immediately after semen collection, spermatozoa motility was evaluated under light microscopy at 400× magnification on a warm plate. After this evaluation, the semen was transported to the laboratory within 2 h at 15°C (Kozumplik 1992). Analyses of samples were carried out in the laboratory of Department of Animal Breeding. A drop of each ejaculate was placed on a slide and eosin nigrosin stain was performed (Blom 1981). At least 200 spermatozoa were examined under oil emulsion (1000× magnification) and abnormal morphology was investigated. Volume of ejaculate was measured using calibrating pipette. Concentration of spermatozoa was estimated by the hemocytometer method with 3% NaCl.

Statistical analysis

The results were statistically analysed using one-way ANOVA in STATISTICA Cz software, version 10 (StatSoft, Inc., Prague, Czech Republic). Differences at $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The average values of qualitative and quantitative parameters of ejaculate collected from Bar Plymouth Rock (BPR), Rhode Island Red (RIR) and Light Sussex (SU) roosters are showed in Table 1.

The highest mean volume of ejaculate was found in BPR (0.80 cm^3) and the lowest in SU (0.55 cm^3). The mean volume of ejaculate in RIR was 0.67 cm^3. There were found statistically significant differences between these values of all three lines. Jarinkovičová et al. (2012) reported the values of volume 0.66 cm^3, 0.46 cm^3 and 0.55 cm^3 in BPR, SU and RIR, respectively. Our results suggest a moderate improvement in this ejaculate parameter. Compared to study Malik et al. (2013) who investigated ejaculate of Malaysian Red jungle fowl, domestic chicken and Bantam chicken, our values are comparatively high. The mean volumes of ejaculates of mentioned breeds were 0.33 cm^3, 0.29 cm^3 and 0.10 cm^3 (respectively). On the other hand, a relatively large volume was found by Hrnčár et al. (2013) in Brown Leghorn (0.72 cm^3).

In this study, the highest mean concentration of spermatozoa was found in BPR and SU (2.39 and 2.36 × $10^6/mm^3$, respectively). In RIR, the mean concentration of spermatozoa was significantly lower (1.59 × $10^6/mm^3$). Similar values were reported by Jarinkovičová et al. (2012) as well. However, they found a higher concentration of spermatozoa in RIR (1.96 × $10^6/mm^3$). A very high concentration of spermatozoa was reported by Malik et al. (2013) in Red jungle fowl (4.44 × $10^6/mm^3$). In contradiction, a low concentration of spermatozoa was reported by Máchal and Křivánek (2002). The concentration varied from 0.84 to 1.05 × $10^6/mm^3$ in BPR, from 0.75 to 1.03 × $10^6/mm^3$ in RIR and from 0.91 to 1.06 × $10^6/mm^3$ in RIW (Rhode Island White).

Table 1 The quality of rooster ejaculate collected from three initial laying lines – from paternal BPR and RIR, and maternal SU

	BPR (n = 64)	RIR (n = 64)	SU (n = 64)
Volume (cm^3)	$0.80^a \pm 0.04$	$0.67^b \pm 0.03$	$0.55^c \pm 0.04$
Concentration ($10^6/mm^3$)	$2.39^a \pm 0.11$	$1.59^b \pm 0.09$	$2.36^a \pm 0.14$
Motility (%)	$68.59^a \pm 2.87$	$77.19^b \pm 2.56$	$73.52^{ab} \pm 2.22$
Total abnormality (%)	$56.29^a \pm 3.07$	$72.63^b \pm 2.65$	$68.09^b \pm 2.97$
Normal spermatozoa (%)	43.71 ± 3.07	27.37 ± 2.65	31.91 ± 2.97

Values are shown as mean ± SD.

Values within each column with different superscripts differ significantly at P<0.05.

Legend: BPR = Bar Plymouth Rock, RIR = Rhode Island Red, SU = Light Sussex.

Relatively low mean values of motility were found in all three lines. The lowest motility was found in BPR (68.59%) and the highest in RIR (77.19%). These values are significantly different. Compared to study Jarinkovičová et al. (2012), our results are similar but we found considerably higher

values of motility in RIR (62.7% vs. 77.19%). However, in studies Hrnčár et al. (2013) and Malik et al. (2013), motility of spermatozoa was lower. The observed motility varied from 57.51 to 64.8% and from 49.0 to 57.1% (respectively). Mixed results were reported by Máchal and Křivánek (2002). The values varied in the range from 54.1 to 86.2% in BPR, from 31.7 to 86.9% in RIR and from 49.7 to 68.2% in RIW.

Very high percentage of total sperm abnormality were found in all three lines in this study – 56.29%, 72.63% and 68.09%, respectively BPR, RIR and SU. On the other hand, a very low percentage of abnormal sperm was reported by Rakha et al. (2015) in Indian Red jungle fowl. The authors found only 8.1% of total sperm abnormalities. In study Hrnčár et al. (2013), total changes of spermatozoa varied in range from 42.75 to 46.32%. The similar results were found by Jarinkovičová et al. (2012). We assume that this high percentage of abnormal sperm found in our study may be caused by transporting of semen into laboratory. The samples of semen had to be transported due to insufficient conditions for evaluation in the place of sampling. Samples were transported at 15°C without diluent solution as reported by Kozumplik (1992). Probably only low storage temperature was not effective.

CONCLUSION

In summary, the highest volume of ejaculate as well as the highest concentration of spermatozoa was found in paternal line BPR, whereas the highest motility was found in RIR which is paternal line as well. On the other hand, the lowest volume of ejaculate was found in maternal line SU but the lowest concentration of spermatozoa was found in paternal RIR. The lowest motility was found in paternal line as well – in BPR. Regarding assessment of the sperm morphology, our finding did not respond to our previous results about motility of spermatozoa and either did not respond to results of other authors. It is likely that the values of morphology were influenced by the transportation of samples.

In conclusion, it is not possible to clearly determine which roosters of laying line had the best parameters of ejaculate quality, however the maternal line SU seemed to be the weakest of all examined lines.

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