THE EFFECT OF INBREEDING DEPRESSION ON SEMEN PRODUCTION IN THE CZECH FLECKVIEH BULLS

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Abstract: In this study, the relationship between inbreeding depression and semen production was examined. During the period from May 2008 to December 2014, semen samples (n = 2929) were collected using an artificial vagina. Immediately after collection, laboratory examinations were made for all samples, which included finding out volume of ejaculate, sperm activity, concentration of spermatozoa and total sperm count. Volume of ejaculate was measured using the graduated tube, sperm activity was assessed by subjective method according to the percentage of motile sperm in the native ejaculate and concentration of spermatozoa was determined using a spectrophotometer. Total sperm count was calculated by multiplying volume of ejaculate by concentration of spermatozoa. Monitored characteristics were expressed in weighted average and standard deviation. The effect of inbreeding depression on bovine semen production was tested by the general linear model (GLM) in SAS 9.4. The inbreeding coefficient (F_x) was calculated by the program FSpeed version 2.04. For comparison between each level of inbreeding Duncan´s Multiple Range Test was used. Based on the ascertained results we can conclude that inbreeding depression had the significant influence ($P < 0.001$) on all monitored semen parameters of the Czech Fleckvieh bulls.

Key Words: bovine semen, Czech Fleckvieh bulls, inbreeding depression, semen production

INTRODUCTION

Artificial insemination (AI) is one of the most powerful and the most valuable biotechnology methods that allows to dairy cattle breeders to use of quality proven AI sires and thus to improve genetic potential and increasing profitability of their herds. Knowledge of factors affecting sperm production and semen quality is of importance with regard to reproductive efficiency and thus genetic improvement as well as for the productivity and profitability of AI centers (Fuerst-Waltl et al. 2006). Evaluation of qualitative and quantitative parameters of bovine semen was done by many authors (Vilakazi et al. 2004, Sarder 2007, Igna et al. 2010) who consider external influences, namely: the effect of season, stable microclimate and sampling techniques, for the most important factors which affecting semen quality. Internal factors are described too, mainly the genetic (Mathevon et al. 1998, Brito et al. 2002). The increasing rate of AI application during the past few decades has resulted in the widespread use of only a few top sires (Behmorad et al. 2015). The best animals accumulate in pedigrees so that it is nowadays practically impossible to find animals without multiple genetic ties to certain individuals in a given dairy cattle breed (Croquet et al. 2006). The mating of related individuals is called inbreeding. The concept of inbreeding is based on the probability that two genes at one locus are identical, and could be shared witch ancestors (Falconer et al. 1996). Inbreeding is typically measured by the correlation between the parents of and individual (Thompson et al. 2000). Number of studies has shown a contrary effect on production traits and non-production traits (Cassell et al. 2003, Wall et al. 2003, Sewalem et al. 2006, Behmorad et al. 2015). Inbreeding depression is expressed as the average variation of the traits per increase in the breeding percentage (Gengler et al. 1998). In the case of traits with low heritability such as fertility are expected to be more severely affected by inbreeding, due to low genetic variation and inbreeding is expected to decrease heritability, although results from empirical studies are inconclusive (Kristensen et al. 2005). A few studies also dealt with inbreeding depression of bovine semen and mostly a negative effect was described (Maximini et al. 2011, Behmorad et al. 2015). The potentially negative effect of inbreeding can also be problem for livestock, primarily where large population often stem from a little number of founding members (Malhado et al. 2013). The objective of this study was to evaluate the effect of inbreeding depression on semen production in the Czech Fleckvieh bulls.

MATERIAL AND METHODS

Characterization of location and experiment design

The project was realized in AI center of Breeding Cooperative Impuls at Vysočina Region in the Czech Republic (GPS: 49°28'25.137"N, 16°4'3.303"E and 558 m above sea level). In period from May 2008 to December 2014, the study was carried out on a biological matherial consisting of 2929 semen samples from the 163 Czech Fleckvieh bulls. All bulls were kept intensively and were fed ad libitum of hay and 3 kg of a 14% protein concentrate diet per bull per day. Water was available ad libitum too. All ejaculate were made by the sampling team of AI center, in room specially adapted to this task, the sampling method to artificial vagina on a dummy (Louda 2001). A standard bovine artificial vagina with a temperature of 42°C was used. The bulls were paraded around a teaser bull to increase the libido prior to semen collection (Vilakazi et al. 2004).

Laboratory evaluation of bovine semen

Immediately after collecting, macroscopic and microscopic examination of all samples was performed in laboratory of AI center. Which included the measure of the volume of ejaculate, concentration of spermatozoa and sperm activity. The volume of ejaculate was detected directly, reading from the scale calibrated collection containers. Sperm activity was assessed by subjective method according to the percentage of motile sperm in the native ejaculate. We evaluated the percentage of sperm with progressive direct movement after the head (Louda 2001) and concentration

of spermatozoa was evaluated by spectrophotometer calibrated for bovine semen. Total sperm count was determined by calculation of the concentration of spermatozoa per mm^3 and a total volume of ejaculate (Fuerst-Waltl et al. 2006).

Input data and statistical analysis

For statistic evaluation of the effect of inbreeding depression on semen production, based on the calculated inbreeding coefficients (F_x), bulls were divided into 8 groups, as indicated in Table 1. Statistical analyses of the input data were done using the general linear model (GLM) procedure of SAS software 9.4 (SAS Institute Inc. 2005). For comparison between each level of inbreeding Duncan´s Multiple Range Test was used. Pedigree data with an average depth of seven complete generations back per bull was provided to calculate of inbreeding coefficients using by program FSpeed 2.04 (Tenset Technologies Ltd. 2009) on the basis of the following formula (Mrode 1996).

$$\boldsymbol{F_x = \sum \left(\frac{1}{2}\right)^n (1 + F_A)} \qquad (1)$$

Legend: n = number of generations to a common ancestor, F_A = inbreeding coefficient of common ancestor.

To estimate the effect of inbreeding depression on semen production was used following model:

$$\boldsymbol{y_{ijklmn} = \mu + age_i + fx_j + interval_k + season_l + year_m + int_{ijklm} + a_n + e_{ijklmn}} \qquad (2)$$

Legend: y = observed parameter of semen; age = fixed class of age; fx = coefficient of inbreeding; interval = interval between successive collections; season = season of collection; year = year of collection; int = interactions between each fixed affects age, fx, interval, season and year; a = effect of each animal and e = residual error.

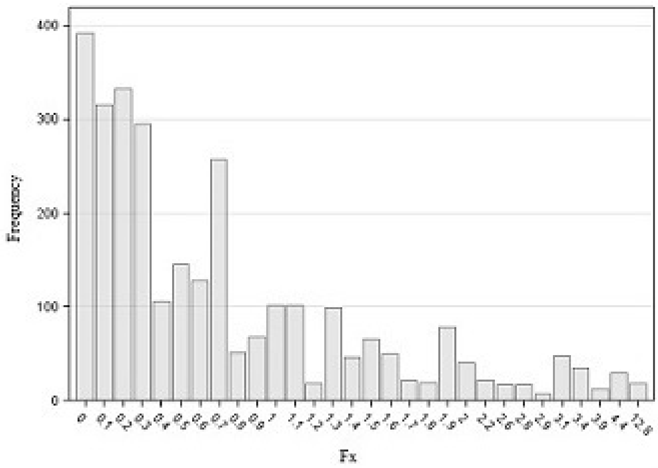
Table 1 Division of the Czech Fleckvieh bulls into the individual classes of inbreeding

F CLASS	Unit	1	2	3	4	5	6	7	8
F_x	%	0.0	0.1–0.2	0.3–0.4	0.5–0.9	1.0–1.4	1.5–1.9	2.0–2.9	>3.0
Frequency	Pcs	391	647	401	648	365	232	103	142
Percent	%	13.4	22.1	13.7	22.1	12.5	7.9	3.5	4.9

RESULTS AND DISCUSSION

The effect of inbreeding depression on bovine semen production was expressed by inbreeding coefficients (F_x). In order to investigate, bulls were divided into 8 classes allow meaningful statistical analysis were defined. Distribution of inbreeding coefficients in observed group of Czech Fleckvieh bulls are presented in Figure 1.

Figure 1 Distribution of inbreeding coefficients in the observed group of the Czech Fleckvieh bulls



In the 8 analyzed classes, the inbreeding coefficient ranged from 0.0 to 12.8%. The largest number of bulls (648 pcs.) represented the 4th class (F_x = 0.5–0.9%). The smallest 7th class (F_x = 2.0–2.9%) was represented by 103 bulls. Although almost every bull was inbred to some extent, the 50.0% of all tested bulls did not exceed the 0.5% value of inbreeding level and only 10.0% of them had inbreeding coefficients higher than 2.0%. Still a highly significant effect ($P < 0.001$) of inbreeding coefficient on semen production was found (Table 2). In the similar publications, the effect of inbreeding depression on bovine semen production only a small number of authors solved (Maximini et al. 2011, Behmorad et al. 2015).

Table 2 Effect of inbreeding coefficient on monitored parameters of bovine semen

TRAIT	Unit	N	Mean	SD	SE	P value
Volume of ejaculate	ml	2897	71.96	6.37	0.12	< 0.0001
Sperm activity	%	2914	7.95	3.15	0.06	< 0.0001
Concentration of spermatozoa	$\cdot 10^6$/ ml	2838	1311.82	484.56	9.09	< 0.0001
Total sperm count	$\cdot 10^9$/ ml	2837	10.38	5.62	0.11	0.0002

Legend: N = number of observation; SD = standard deviation; SE = standard error; P value = statistical significance.

In the case of all monitored parameters, statistically significant negative effect ($P < 0.05$) of inbreeding depression on semen production of the Czech Fleckvieh bulls was found (Table 3), when downward trend of values was observed with increasing inbreeding coefficient. Despite the quite low inbreeding level, the effect of inbreeding depression on semen quality traits was observed in earlier studies in cattle (Smith et al. 1989, Flade et al. 1992). Results of studies in other mammal species (Van Eldik et al. 2006, Asa et al. 2007) suggested that inbreeding depression would be more severe in higher inbreeding levels, a non-linear relation is assumed (Bezdíček et al. 2010).

Table 3 Significant differences between average means of monitored parameters of bovine semen

CLASS OF INBREEDING COEFFICIENT	V (ml) Mean	A (%) Mean	C ($\cdot 10^6$/ml) Mean	TSC ($\cdot 10^9$/ml) Mean
F_X 1	8.98 [a]	72.16 [bc]	1352.85 [ab]	10.81 [b]
F_X 2	8.34 [a]	72.48 [ab]	1364.01 [ab]	9.82 [c]
F_X 3	7.93 [b]	73.11 [a]	1317.21 [bc]	11.19 [ab]
F_X 4	7.33 [cd]	71.48 [cd]	1314.12 [bc]	11.78 [a]
F_X 5	7.41 [c]	72.52 [ab]	1261.91 [cd]	10.87 [b]
F_X 6	7.83 [b]	71.83 [bc]	1208.47 [de]	8.51 [d]
F_X 7	6.98 [d]	71.52 [cd]	1195.63 [de]	8.90 [d]
F_X 8	7.18 [cd]	70.71 [d]	1154.26 [e]	9.04 [d]

Legend: V =volume of ejaculate; A = sperm activity; C = concentration of spermatozoa; TSC = total sperm count; a, b, c, d, e = means with the same letter are not significantly different ($P < 0.001$).

CONCLUSION

Based on the ascertained results we can conclude that the inbreeding level and the inbreeding depression do not seem to be alarming currently in the case of the Czech Fleckvieh cattle. Still, the negative influence of inbreeding coefficient on chosen parameters of bovine semen was found, when downward trend of values was observed with increasing coefficient of inbreeding. However, all parameters of spermatozoa, in all levels of evaluated effects, reached values necessary for producing of insemination doses. Completing the pedigree of AI bulls and monitoring the effect of inbreeding depression on semen production is recommended to avoid unrecognized deterioration of such traits. It would also be useful to breeders assembles parental couples with regard to the inbreeding level of the offspring due to prevent increased incidence of genetically abnormalities and illnesses, increased proportion of abortions and other reproduction and production problems.

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