The effect of the stable environment and age on the semen production in the Czech Fleckvieh bulls

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Abstract: The objective of this study is to evaluate the effect of stable environment and age on semen production in the Czech Fleckvieh bulls. During the period from March 2014 to August 2014, semen samples (n = 232) were collected using an artificial vagina. Immediately after collection, laboratory examinations were made for all samples, which included finding out ejaculate volume, sperm concentration and total sperm count. Ejaculate volume was measured using the graduated tube and sperm concentration was determined using a spectrophotometer. Total sperm count was calculated by multiplying ejaculate volume by sperm concentration. For statistic evaluation of the effect of stable environment, the average values of THI_1 (temperature-humidity index from the one day before semen collection) were used and the bulls were divided into the groups according to the age. Monitored characteristics were expressed in weighted average and standard error. The effect of the stable environment and age on bovine semen production was tested by the general linear model (PROC GLM) in SAS 9.1. Based on the results we can conclude that age had the significant influence ($P < 0.05$) on all monitored parameters. In case of THI_1, no statistically significant differences among determined classes were found ($P > 0.05$).

Key-Words: age, bovine semen, Czech Fleckvieh bulls, semen production, stable environment

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.

For the last sixty years, AI has become one of the most important tools in Czech cattle breeding programs. At present in the Czech Republic, more than 98% [1] of breeding stock of Czech Fleckvieh dairy cows is artificially inseminated. It is clear that the need of knowledge of factors affecting the sperm production and quality of bovine semen is of considerable with regard to reproductive efficiency.

Photoperiod, temperature, relative humidity, nutrition, diseases and some other factors can affect welfare of farmed animals. Cattle production can be affected by heat stress, when under high ambient temperature and relative humidity of stable air, internal thermoregulatory mechanisms are unable to increase body heat loss and body temperature increases above physiological limits [2]. In case of AI sires, heat stress can cause a decrease of quantitative and qualitative parameters of their ejaculates [3]. The size of heat stress, can be defined as a sum of external factors on the animal that act to displace body temperature from set point, is caused by the combined effects of temperature, humidity, solar radiation and wind speed [4]. A variety of indices were used to estimate the degree of heat stress affecting cattle. The temperature-humidity index (THI), uses temperature and relative humidity of stable air to estimate the size of heat stress [5].

The present study was conducted to determine the effect of stable environment and age on the semen production of a selected population of the Czech Fleckvieh bulls kept in AI center.

Material and Methods

Characterization of location

The project was realized in AI center of Chovatelské družstvo Impuls, družstvo at Vysočina region (GPS:

49°28'25.137"N, 16°4'3.303"E and 558 m above sea level). This region is characterized by mild climate throughout the all year. Average daily temperature in stable was 14.9ºC with a minimum of 3.8ºC and a maximum of 23.6ºC and average relative humidity was 65.9 % (43.1 – 94.5%), during the experimental period.

Animals and treatment

The study was carried out on a biological matherial consisting of semen samples from the 18 Czech Fleckvieh AI bulls, aged between 1.5 and 8.5 years, as Table 1 shown. 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.

Tab. 1 Division of bulls according to their age

Class of age	*n*	*Age of bulls*
1	*8*	< 2 years
2	*4*	2 – 5 years
3	*6*	> 5 years
Total 18		

Semen samples and analyses

In total, the 232 semen samples were collected in period from the 1st March, 2014 till the 31st August, 2014. A standard bovine artificial vagina at a temperature of 42°C was used to collect the semen from all bulls. The bulls were paraded around a teaser bull to increase the libido prior to semen collection [6].

Immediately after collection, macroscopic and microscopic examinations were made for all samples, which included finding out ejaculate volume, concentration of sperm and total sperm count. The ejaculate volume was read directly from the tube after semen removal from the artificial vagina. Concentration was established through spectofotometric method, using a spectrofotometer calibrated for bull semen. Total sperm count was calculated by multiplying ejaculate volume by sperm concentration.

Data of stable environment

The effect of stable environment was analyzed by using THI_1 (temperature-humidity index one day before collection) which was counted based on data of temperature and relative humidity of stable air. Data of temperature and relative humidity were measured by HOBO data logger at 30 minutes intervals. THI_1 was determined using the following equation [7]:

$$THI_1 = \frac{(0.8 \times t_{db} + ((t_{db} - 14.4) \times RH))}{100} + 46.4$$

Where:

- *t_{db} is temperature of stable air (°C)*
- *and RH is relative humidity (%).*

For statistic evaluation of the effect of stable environment, the average values of THI_1 from the one day before semen collection were used and divided, as indicated in Table 2.

Tab. 2 Division of observation according to THI_1

Class of THI_1	*n*	*Range of THI_1*
1	*17*	< 50
2	*59*	50.1 – 55
3	*57*	55.1 – 60
4	*40*	60.1 – 65
5	*59*	> 65.1
Total 232		

Statistical analyses

Statistical analyses of the data were done using the general linear model (GLM) procedure of the Statistical Analysis System [8]. The least square means (LSM) option of general linear model (GLM) was used for analysing of effect of age and THI_1 for each trait separately, as follows:

$$y_{ij} = age_i + thi_j + e_{ij}$$

Where:

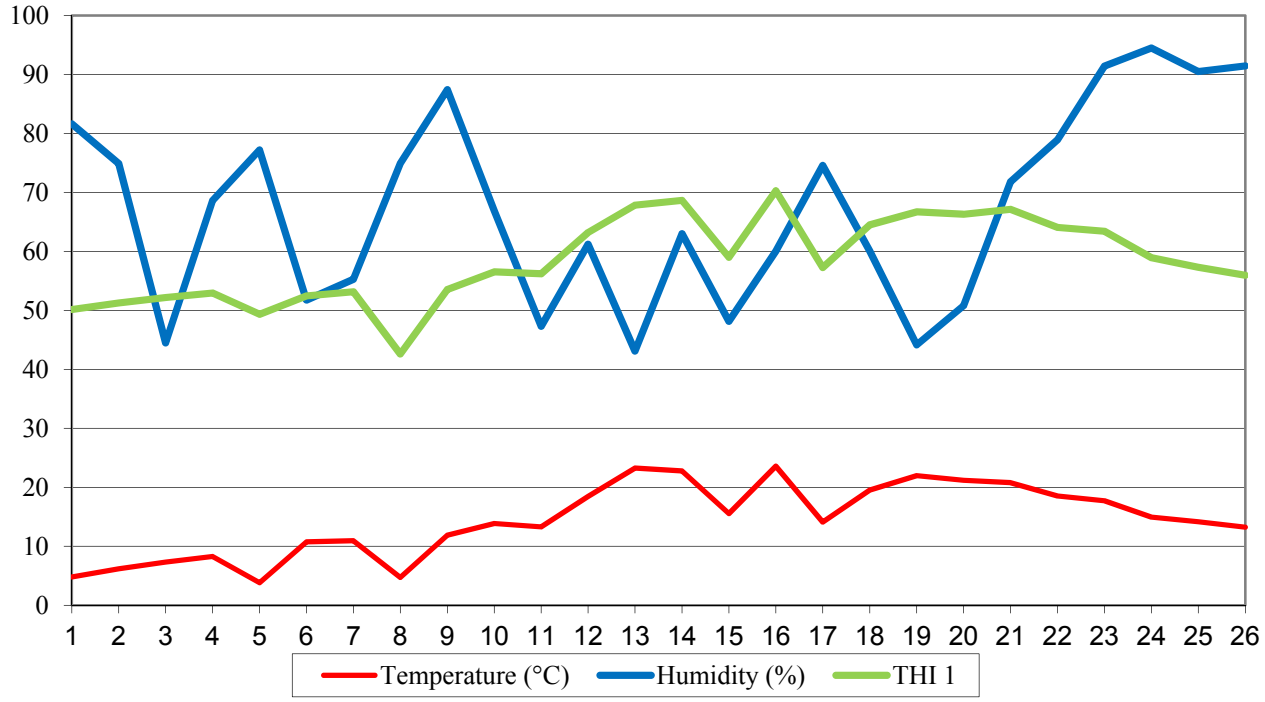
- *y is observations of ejaculate volume, sperm concentration and total sperm count;*
- *age is fixed effect of i^{th} group of age of bulls ($i = 1 – 3$);*
- *thi is fixed effect of j^{th} group of THI_1 index ($j = 1 – 5$);*
- *and e was residual error.*

Results and Discussion

Analysis of temperature, humidity and THI_1

In the course of observation period, the trend of temperature and relative humidity responded the typical climate of Vysočina region, when extreme values were not found, as indicated in Figure 1. The same trend was observed in the case of THI_1.

Fig. 1 Trend of temperature, relative humidity and THI_1 during the observation period



Effect of age on the bovine semen production

The results recorded indicated that age had a statistically significant influence ($P < 0.05$) on all monitored characteristics.

The values of ejaculate volume ranged from 7.26 ± 0.25 to 11.91 ± 0.24 ml with an overall average of around 9.67 ± 0.21 ml. Figure 2 illustrates that the highest ejaculate volume was obtained from the class of age 3 (> 5 years) and the smallest values was found in class of age 2 (2 – 5 years). Class of age 3 had a significantly higher ejaculate volume than class of age 1 (< 2 years) and class of age 2 ($P < 0.01$). Mathevon *et al.* [9] reported that the the volume of the ejaculate improves with an increase in the age of bulls. The same trend was recorded in our study (Figure 2).

In case of sperm concentration, values were moving in variation ranging from 1055.70 ± 31.22 × 10^6 to 1244.42 ± 56.64 × 10^6 with an overall average of around 1101.04 ± 22.21 × 10^6 spermatozoa/ml of ejaculate. Statistically significant difference ($P < 0.05$) was found between class of age 2 and the class of age 1, and highly statistically significant difference ($P < 0.01$) was proved between class of age 2 and the class of age 3, as indicated in Table 3.

In terms of total sperm count, values of monitored groups were very balanced (8649.53 ± 404.54 × 10^6 and 8699.79 ± 502.39 × 10^6) except the class of oldest bulls (12427.05 ± 427.65 × 10^6) with an overall average value of around 10464.02 ± 292.10 × 10^6 spermatozoa per ml of ejaculate. In case of this class, a highly statistically significant difference ($P < 0.01$), between it and the remaining classes, was found.

Tab. 3 Effect of age on semen production

Efekt		*n*	*Ejaculate volume (ml)*		*Sperm concentration (×10⁶ spermatozoa/ml)*		*Total sperm count (×10⁶ spermatozoa/ml)*	
			Mean	*Std.*	*Mean*	*Std.*	*Mean*	*Std.*
Age	**1**	*88*	7.76^{A}	2.31	$1104.45^{a,A}$	343.28	8649.53^{A}	3794.88
	2	*33*	7.26^{A}	2.24	$1244.42^{b,A}$	325.39	8699.79^{A}	2886.04
	3	*111*	$11.91^{A,B}$	2.57	1055.70^{B}	328.89	$12427.05^{A,B}$	4505.60

A, B – between values with different letters in a column were proved statistical highly significant differences ($P < 0.01$)
a, b – between values with different letters in a column were proved statistical evidential differences ($P < 0.05$)

Our results are confirmed by Fuerst - Waltl *et al.* [10] who analyzed effect of age and environmental factors on semen production too and state, that total number of sperm per ejaculate followed those for ejaculate volume. This trend was also observed in our study (Table 3) and was probably due to the size of the bull´s testicles, which continue to grow up to five years of age [11].

Fig. 2 Effect of age on ejaculate volume (ml)

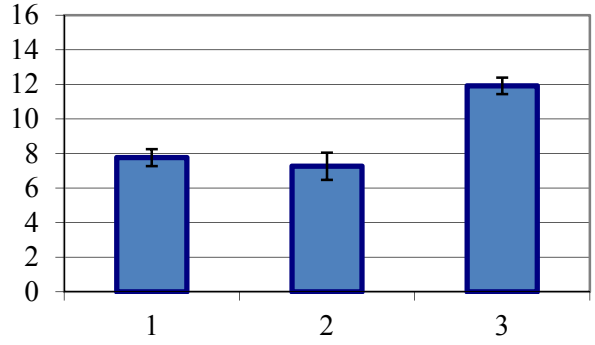


Fig. 3 Effect of age on concentration ($\times 10^6$)

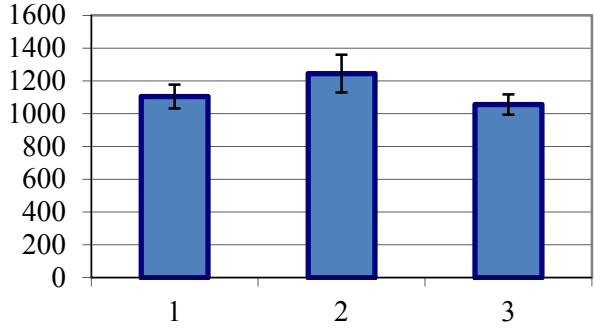
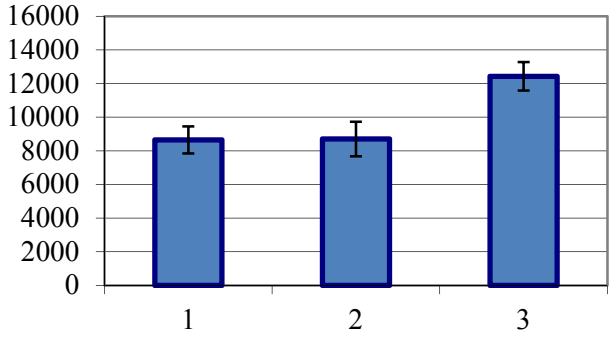


Fig. 4 Effect of age on total sperm count ($\times 10^6$)



Effect of THI_1 on the bovine semen production

Salah *et al.* [12] reported in their study, that association among the temperature-humidity index (THI) and sperm concentration and total sperm count, was negative and significant ($P < 0.01$) where it was non-significant with ejaculates volume. As our results indicate, in case of effect of THI_1 on ejaculate volume, sperm concentration and total sperm count, the measured values were very balanced (Figure 5, 6, 7) and the differences recorded among them statistically insignificant (Table 4). This fact could be cause by the small variation in range of monitored climate parameters.

Fig. 5 Effect of THI_1 on ejaculate volume (ml)

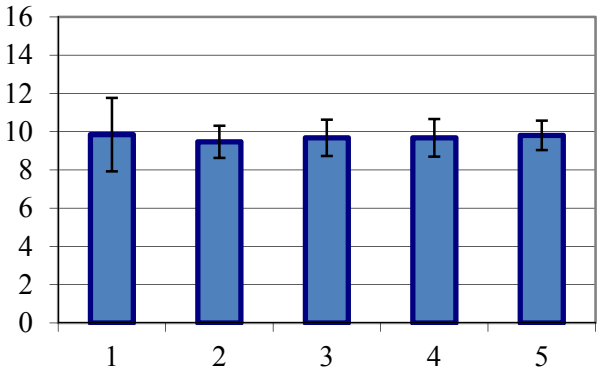


Fig. 6 Effect of THI_1 on concentration ($\times 10^6$)

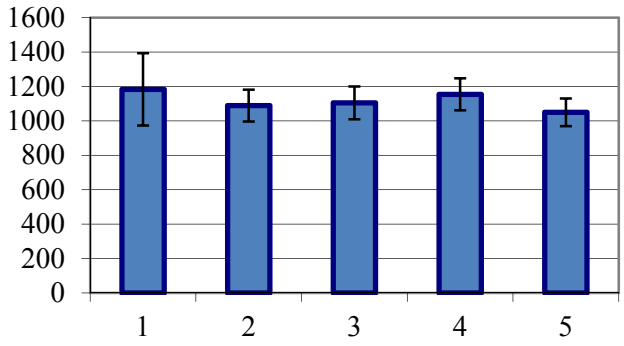
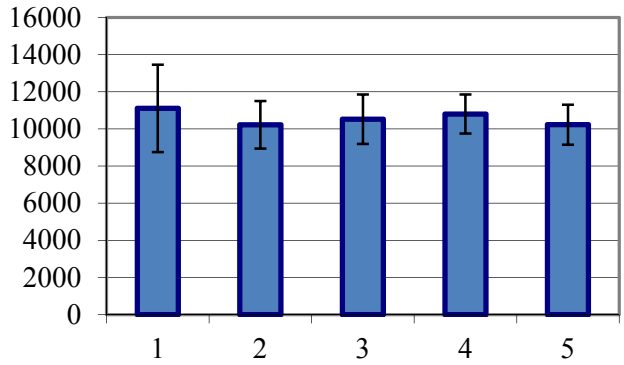


Fig. 7 Effect of THI_1 on total sperm count ($\times 10^6$)



Conclusion

According our results, the class of age has a significant influence on the observed parameters of the bovine semen. Oldest bulls have highest volume of ejaculate and total sperm count. In case of sperm concentration, the class of age 2 shows highest values. All these differences were statistically high significant ($P < 0.01$). In case of THI_1, no significant effect on the monitoring parameters was observed. This facts could be caused by mild climate without extreme values of daily temperature and humidity.

Tab. 4 Effect of THI_1 on the bovine semen production

Efekt		*n*	*Ejaculate volume (ml)*		*Sperm concentration (×10⁶spermatozoa/ml)*		*Total sperm count (×10⁶spermatozoa/ml)*	
			Mean	*Std.*	*Mean*	*Std.*	*Mean*	*Std.*
THI_1	**1**	*17*	9.85	3.74	1183.12	408.98	11106.50	4569.91
	2	*59*	9.47	3.24	1088.58	354.61	10224.38	4916.00
	3	*57*	9.68	3.54	1104.81	360.46	10526.61	5015.41
	4	*40*	9.68	3.06	1154.45	290.04	10802.93	3285.99
	5	*59*	9.81	2.98	1050.00	308.70	10228.31	4128.48

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