THE EFFECT OF BORAX ON INHIBITION OF MICROORGANISMS AND SPERM MOTILITY DURING LIQUID PRESERVATION IN SHORT-TERM BOAR SEMEN EXTENDER

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Abstract

The objective of this study was to investigate the effect of borax on inhibition of microorganisms and their influence on sperm motility during liquid preservation in short-term boar semen extender. The amounts tested borax added to boar semen extender was not affected on initial sperm motility ($p<0.05$) unlike higher amounts of borax. Six ejaculates from 3 healthy and fertile AI boars were used for this study. Tested amount of borax BA-0.5 g/l and BB-0.73g/l were added to BTS extender without antibiotics in dilution rate of 1+2, 1+4 and 1+8 and stored at 17°C up to 48h, for everyday evaluation. Sperm motility was evaluated according CASA program at 0h and with thermo-resistance survival test (TRT test). Sperm motility were affected by storage time, the dilution ratio, hours of evaluation and different amount of borax ($p<0.05$). Significant differences ($p<0.05$) of total mean values of sperm motility in TRT test were found between samples BTS 59.11% and BTS+BB 57.94% vs. BTS+BA 47.86%. In sperm motility 0h was not found any differences ($p>0.05$) between tested samples. Sperm motility 0h was affected dilution ratio and storage time in total sperm motility and in the proportion of immotile sperm ($p<0.05$). Microorganisms were not significantly inhibited by any amounts of borax ($p>0.05$). In conclusion, the tested amounts of borax decreased sperm motility during TRT test. Use of the tested amounts of borax as a potential substitute for antibiotics in boar semen extender is not appropriate because their low activity in the reduction of microorganisms was found.

Key Words: Boar semen, short extender, borax, microorganisms, sperm motility

In the modern swine production industry, successful artificial insemination with boar semen subjected to liquid storage at 17°C is commonly used to facilitate pig breeding (Shaoyong et al., 2019). Bacteriospermia is a frequent finding in freshly extended porcine semen and can result in detrimental effects on semen quality and longevity if left uncontrolled (Althouse et al., 2005). Bacterial contamination of the boar semen is associated with a decrease in sperm motility, viability (Bussalleu et al., 2011) and membrane integrity (Sepúlveda et al., 2014). Martín et al. (2010) found in their study that the presence of E. coli either alone or together with other gram-negative bacteria may have an important influence on the agglutination of boar spermatozoa and hence negatively affects the litter size obtained from sows inseminated with such semen samples. Recently many microorganisms have become resistant to the most antibiotic used in semen extenders (Schulze et al., 2015; Morrell and Wallgren, 2014) and now we are looking possible alternatives which could be used to reduce this resistance.

Borax, also known as sodium borate, sodium tetraborate, or disodium tetraborate (Gainsford et al., 2008) contain 21% boron by weight (Plumlee, 2004). Borax is a naturally occurring alkaline compound that is a precursor in the manufacture of boric acid. Used as a preservative, buffer, antiseptic and fungicide (Gattey, 2008). Boron is a mineral that has been

supported with data from research spanning many decades to impact animal health, ranging across various species (Green, 2020).

The objective of this study was to investigate the effect of borax on inhibition of microorganisms and their influence on sperm motility during liquid preservation in short-term boar semen extender.

Material and Methods

Six sperm rich ejaculate fraction with motility ≥80% and number of morphologically abnormal spermatozoa ≤25% from three fertile AI boars of Přeštice black-pied pig aged 3.5 to 5 years were collected using the gloved-hand technique. The boars were kept in the same housing, feeding and breeding conditions.

The following parameters were evaluated in the fresh native boar semen: semen volume, sperm motility, sperm concentration, morphologically abnormal spermatozoa (MAS), pH and osmolality. The sperm motility was assessed subjectively using phase contrast microscopy with a heating stage (38°C) at 200× magnification. Sperm concentration was measured with Chroma Colorimeter 254 (Sherwood Scientific Ltd., Cambridge, England). Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1500× magnification. The pH was assessment using the Hanna precision pH meter at 20°C (Sigma-Aldrich, Czech Republic) and osmolality (mOsmol/kg) with the Marcel Osmometr OS 300 (2THETA ASE, Czech Republic).

The boar semen was diluted in dilution rate 1+2, 1+4 and 1+8 in extender BTS without antibiotics - control samples (Minitüb, Germany). Borax BA-0.5g/l, BB-0.73g/l, (Lachema Brno, Czech Republic) was added to in extender BTS without antibiotics in the dilution rate 1+2, 1+4, 1+8. These selected amounts of substance added to boar semen extender was not affected on initial sperm motility ($p<0.05$) unlike higher amounts of substances. Samples were stored at a temperature of 17°C up to 48h. Sperm motility was evaluated at 24h and 48h storage time.

Sperm motility (%) was estimated with the use Computer Assisted Semen Analysis (CASA) 0h. Values of sperm motility was expressed as a motility (M), progressive sperm motility VCL>25µm/s and STR≥45% (PM), non-progressive motility VCL>25µm/s and STR<45% (NP) and immotile (IM) according CASA program. Sperm motility was evaluated with thermo-resistance survival test (TRT test). The TRT test was performed on 3 ml samples kept at 38°C in water bath after storage time 24h and 48h and motility of spermatozoa was evaluated at the 1h, 3h and 5 h during the incubation.

The assessment antibacterial activity of borax was in a microbiological laboratory. Each sample was diluted 100× in physiological saline solution (Penta s.r.o., Czech Republic) and then 100µl of the sample was inoculated on blood agar with 5% defibrinated ram blood (HiMedia Laboratories, USA). Samples were incubated for 48h at 37°C in a biological thermostat BT 120MR (EKOM s.r.o., Czech Republic). The number of colonies was determined by colony counter STC 1000 (VWR, Switzerland) and the total number of microorganisms was determined according to the formula and expressed in colony-forming unit (CFU/ml).

Basic statistical characteristics of the results of arithmetic means, standard deviations (SD) and significance (p) were calculated by the QC Expert program (TriloBite Statistical Software s.r.o., Pardubice, Czech Republic). The data were analysed by statistical analysis of variance (ANOVA) followed by the Fisher test ($p<0.05$). Statistical significance was determined using a two-way ANOVA to assess the effect of diluent, dilution ratio, evaluation day, evaluation hour and interactions of these factors, always in combination with extender on sperm motility.

Results and Discussion

The initial quality of native semen was as follows: semen volume 226.00±57.27ml, sperm motility 81.00±4.18%, sperm concentration 366.000±81.74×10^3/mm^3, MAS 24.36±1.45%, pH 7.93±0.34 and osmolality 317.40±10.41 mOsmol/ kg. Values of pH and osmolality extenders are presented in the Table 1.

Comparison of total values of sperm motility 0h (%) according CASA program in tested samples are presented in the Figure 1. There were not noted differences between sperm motility in tested samples of borax to BTS ($p>0.05$).

However, sperm motility 0h was affected dilution ratio and storage time in total sperm motility and in the proportion of immotile sperm ($p<0.05$). Dilution rate 1+2 and 1+4 had higher total sperm motility than 1+8 by 13,5% and 12,5% (87.53% and 86.44% vs. 73.95%) and in the proportion of immotile sperm it was conversely ($p<0.05$). In storage time was values total sperm motility at 24h 78.53% and at 48h it was 87.92% ($p<0.05$). Significant differences ($p<0.05$) of total mean values of sperm motility in TRT test were found between samples BTS 59.11% and BTS+BB 57.94% vs. BTS+BA 47.86%. (Figure 2).

The effect dilution rate on sperm motility in TRT test are noted in the Figure 3. There were found differences in dilution rate 1+4 and 1+8 in BTS vs. BTS+BA and in the different dilution rates in the same extender $p<0.05$.

The effect hours of evaluation on sperm motility in TRT test are presented in the Figure 4. There were found differences at 1h of evaluation in BTS vs. BTS+BA and in hours of evaluation in the same extender $p<0.05$.

We found that amounts of tested borax had negative influence on sperm motility by 1 - 11% in TRT test to BTS as control sample. Krishnan et al. (2019) noted in their study that dietary boron supplementation increased the sperm output, sperm motility and enhanced the immune and antioxidant defence capacity in male goats. Similar results also found Elkomy et al. (2015) in adult male rabbit where boron doses result in a significant improvement in semen quality characteristics and had a positive effect on their physiological status.

The most common microorganisms found in boar semen samples: *E. coli (*especially in native semen)*, Proteus* sp.*, Staphylococcus aureus, Staphylococcus cohnii* subsp. *Cohnii, Staphylococcus simulans, Staphylococcus cohnii* subsp. *urealyticum, Staphylococcus capitis* subsp. *capitis, Staphylococcus haemolyticus, Corynebacterium* sp.*, Bacillus* sp.*, Moraxella canis, Chryseobacterium gleum.* A similar representation of microorganisms was reported by Bresciani et al. (2014) and Gaczarzewicz et al. (2016). The mean value of microorganisms in native boar semen was 3.0×10^3 CFU/ml. Typical bacterial concentrations in the boar semen are presented range from 10^3 to 10^5 CFU/ml (Morrell and Wallgren, 2011; Stojanov et al., 2020). BTS+BA and BTS+BB did not inhibit microorganisms in samples of diluted semen $p<0.05$ (Figure 5, 6 and 7). Yilmaz (2012) found that the MICs and the MBCs of borax were obtained as 23.80 mg/mL, 23.80 mg/mL, 47.60 mg/mL, and 47.60 mg/mL the bacterial activities of *Staphylococcus aureus, Acinetobacter septicus, Escherichia coli,* and *Pseudomonas aeruginosa*, respectively.

Table 1. *Mean values and standard deviation (SD) of pH and osmolality of extender BTS, BTS+BA and BTS+BB.*

	pH	**Osmolality (mOsmol/kg)**
BTS	8.14±0.10	331.50±8.34
BTS+BA	7.75±0.14	331.00±5.29
BTS+BB	7.80±0.18	339.67±4.04

Figure 1. *Comparison of total sperm motility (%) 0h according CASA program in BTS, BTS+BA and BTS+BB. (M-total motility, PR-progressive motility, NP-non-progressive motility and IM-immotile).*

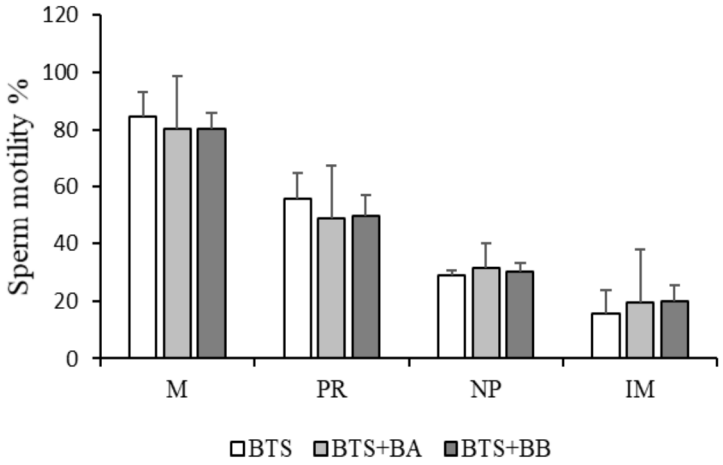
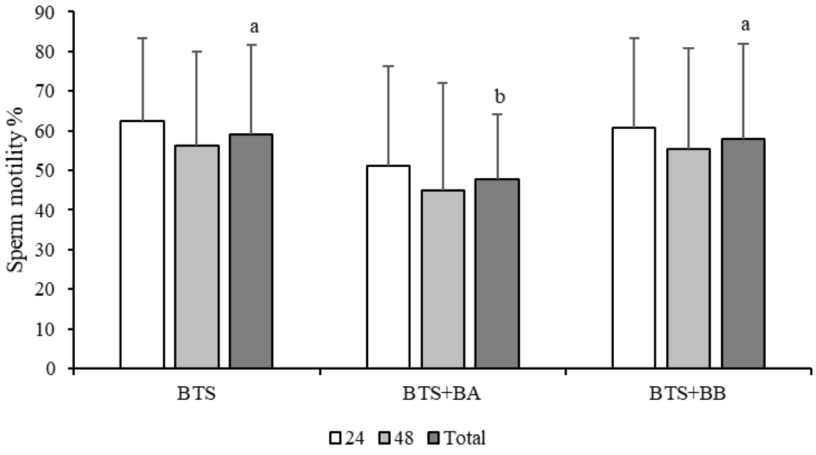
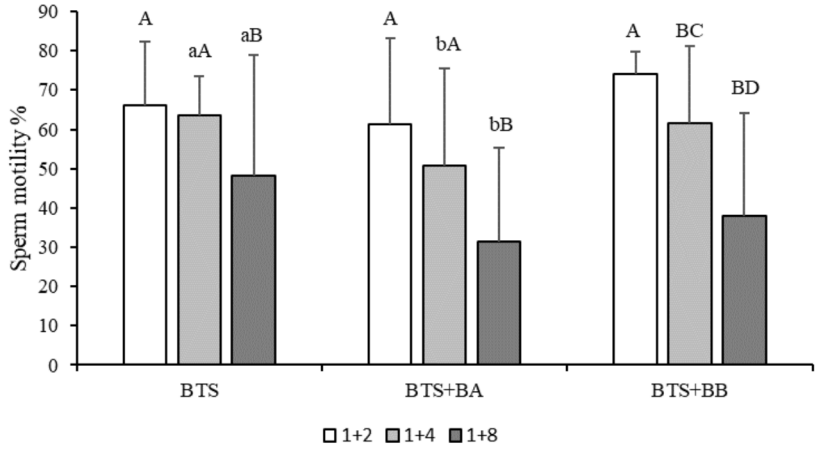


Figure 2. *Total sperm motility (%) and during 24h a 48h storage time in TRT test in tested samples.*



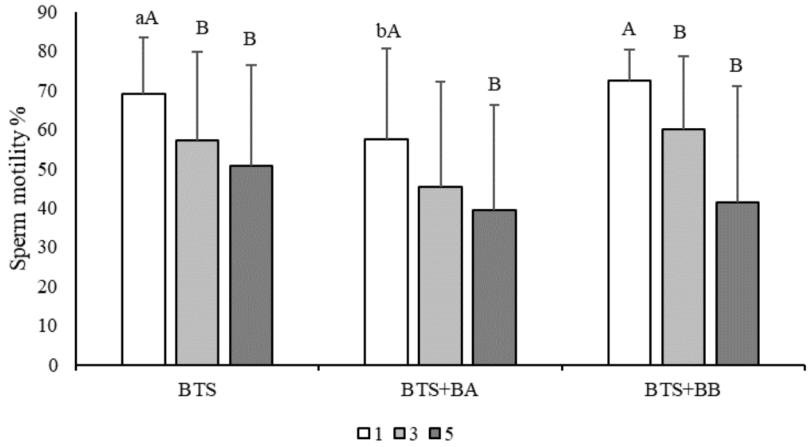
[a,b]p<0.05

Figure 3. *The effect dilution rate 1+2, 1+4 and 1+8 in BTS, BTS+BA and BTS+BB on boar sperm motility in TRT test.*



Different letters indicate significant differences between BTS, BTS+BA and BTS+BB in the same dilution rate [a,b] p<0.05 and in the different dilution rates in the same extender [A, B;C, D] p<0.05.

Figure 4. *The effect hours of evaluation in BTS, BTS+BA and BTS+BB on boar sperm motility in TRT*



Different letters indicate significant differences between BTS, BTS+BA and BTS+BB in the same hours of evaluation [a,b] $p<0.05$ and in hours of evaluation in the same extender [A, B] $p<0.05$.

Figure 5. *Determination of the colony-forming unit (CFU/ml) of microorganisms in days of evaluation at native semen in BTS extender, BTS+BA and BTS+BB at a dilution rate 1+2.*

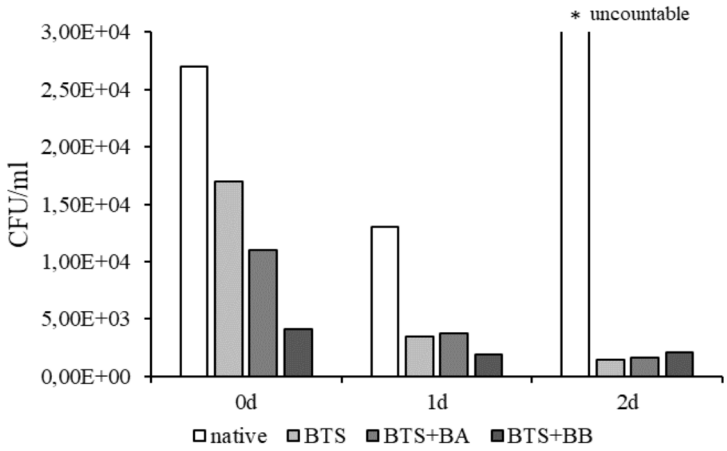


Figure 6. *Determination of the colony-forming unit (CFU/ml) of microorganisms in days of evaluation at native semen in BTS extender, BTS+BA and BTS+BB at a dilution rate 1+4.*

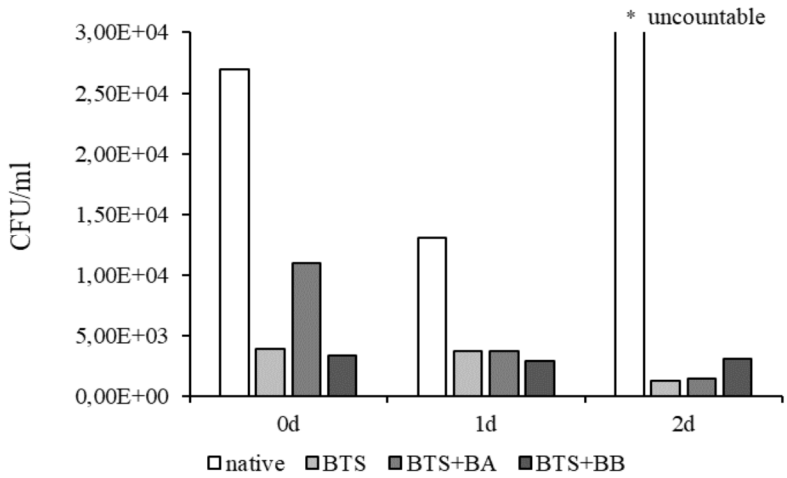
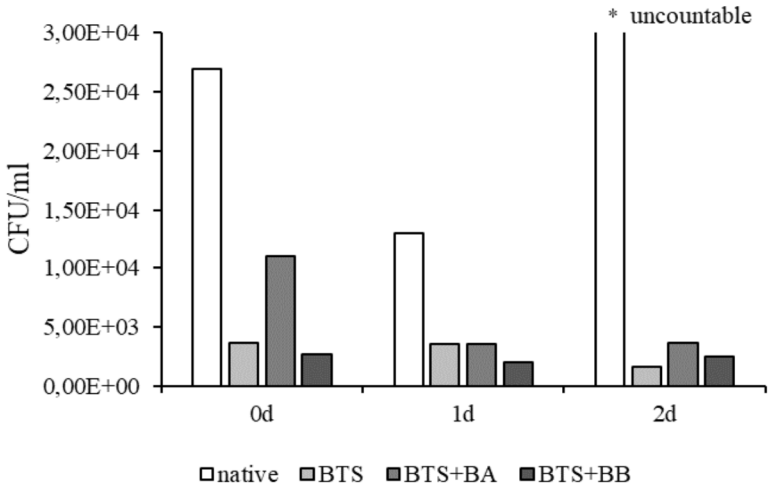


Figure 7. *Determination of the colony-forming unit (CFU/ml) of microorganisms in days of evaluation at native semen in BTS extender, BTS+BA and BTS+BB at a dilution rate 1+8.*



Conclusion

The tested amounts of borax had negative effect in sperm motility during TRT test. Use of the tested amounts of borax as a potential substitute for antibiotics in boar semen extender was not appropriate because their low activity in the reduction of microorganisms was found. Therefore, it is necessary to research other potential substances for a possible replacement for antibiotics in boar semen extender.

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