THE EFFECT OF DIFFERENT CONCENTRATION OF GALLIC ACID ON SPERM MOTILITY AND INHIBITION OF MICROORGANISMS IN LIQUID PRESERVED BOAR SEMEN

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Abstract

The objective of this study was to investigate the effect of different concentration of gallic acid added to boar semen extender on inhibition of microorganisms and their influence on sperm motility. The selected concentrations added to boar semen extender had no effect on initial sperm motility ($p<0.05$) unlike higher concentrations of gallic acid. Nine ejaculates from 4 healthy and fertile AI boars were used for this study. Different concentration of gallic acid (GA1, 2, 3) was added to boar extender BTS without antibiotic (BTS0) 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 affected by storage time, the dilution ratio and concentration of GA ($p<0.05$). Significant differences ($p<0.05$) from total mean values of sperm motility were found between samples BTS0 70.21% vs. BTS0+GA2 57.87% and BTS0+GA3 58.89%. Microorganisms were not significantly inhibited by any of tested concentration of GA1, 2 and 3 ($p>0.05$). In conclusion, utilization of tested concentration of gallic acid as a potential substitute for antibiotics in boar semen extender is not possible because their low activity in the reduction of microorganisms was found in the tested samples and also GA2 and GA3 concentrations decreased sperm motility during preservation. Therefore, it is necessary to research other potential substances for a possible replacement for antibiotics in boar semen extender.

Key Words: Boar semen, gallic acid, microorganisms, sperm motility

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is a bioactive phytochemical that commonly occurs in a wide range of land plants (Aruoma et al., 1993). Gallic acid is a type of phenolic acid found in hornbeam and oak bark, hazelnuts, tea leaves, hops and other plants. Gallic acid has antioxidant, antibacterial and antifungal activity (Daneshfar et al., 2008). The mechanism of the antimicrobial action of phenolic compounds including gallic acid is based mainly on their ability to disrupt the integrity of the bacterial cytoplasmic membrane and interfere with the metabolism of bacteria (Mazurova et al., 2015).

Due to the increasing resistance of microorganisms in the boar semen, possible alternatives are looking which could be used to reduce this resistance. Therefore, the objective of this study was to investigate the effect of different concentration of gallic acid added to boar semen extender on inhibition of microorganisms and their influence on sperm motility.

Material and Methods

Nine sperm-rich ejaculate fractions with motility ≥80% and number of morphologically abnormal spermatozoa ≤25% from four 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, 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 micros copically 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 and BTS without antibiotics (Minitüb, Germany) – control samples. Gallic acid (Sigma-Aldrich, USA) was added to extender BTS without antibiotics (BTS0) in concentration 0.008 mol/l (BTS0+GA1), 0.009 mol/l (BTS0+GA2) and 0.010 mol/l (BTS0+GA3). These selected concentrations added to boar semen extender had no effect on initial sperm motility ($p<0.05$) unlike higher concentrations of gallic acid. Samples were diluted in the same dilution rate as a control samples and were stored at a temperature of 17°C up to 48h. Sperm motility was evaluated at 0h, 24h and 48h storage time. Sperm motility (%) was estimated with the use Computer Assisted Semen Analysis (CASA). For this study, value of sperm motility was expressed as a progressive sperm motility according CASA program. The pH was evaluated in all tested samples at 0h.The assessment antibacterial activity of gallic acid was in a microbiological laboratory. Each sample was diluted 100× in physiological saline solution (Penta s. r. o., Czech Republic) and then 100 µl 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 and significance (p) were calculated by the QC Expert program (TriloBite Statistical Software s. r. o., Pardubice, Czech Republic). Statistical significance ($p<0.05$) was determined using ANOVA-Fisher test.

Table 1. pH and osmolality of extenders

Extender	pH	osmolality (mOsmol/kg)
BTS0	8.16	333
BTS0+GA1	7.34	312
BTS0+GA2	7.27	314
BTS0+GA3	7.00	310

Table 2. pH of tested samples in different extenders and dilution rates

Extender	1+2	1+4	1+8
BTS0	7.31	7.55	7.66
BTS0+GA1	7.32	7.14	7.37
BTS0+GA2	7.40	7.48	7.31
BTS0+GA3	7.29	7.25	7.12

Table 3. Comparison of mean values of sperm motility (%) in different gallic acid concentration to control sample (BTS0) up to 48h

Extender	0h			24h			48h		
	1+2	1+4	1+8	1+2	1+4	1+8	1+2	1+4	1+8
BTS0	79.38^{a}	79.38^{a}	76.88^{a}	70.63^{b}	70.01^{b}	68.13^{b}	$63.75^{A,b}$	$62.52^{A,b}$	$61.25^{A,b}$
BTS0+GA1	77.51^{a}	75.02^{a}	77.50^{a}	70.12^{a}	70.05^{a}	70.01^{a}	$60.22^{A,b}$	$50.18^{A,b}$	$30.25^{B,b}$
BTS0+GA2	77.50^{a}	75.31^{a}	77.51^{a}	70.05^{a}	65.12^{a}	65.22^{a}	$40.09^{B,b}$	$30.43^{B,b}$	$20.32^{B,b}$
BTS0+GA3	75.21^{a}	75.06^{a}	72.50^{a}	67.50^{a}	65.02^{a}	62.50^{a}	$47.52^{B,b}$	$40.36^{B,b}$	$25.02^{B,b}$

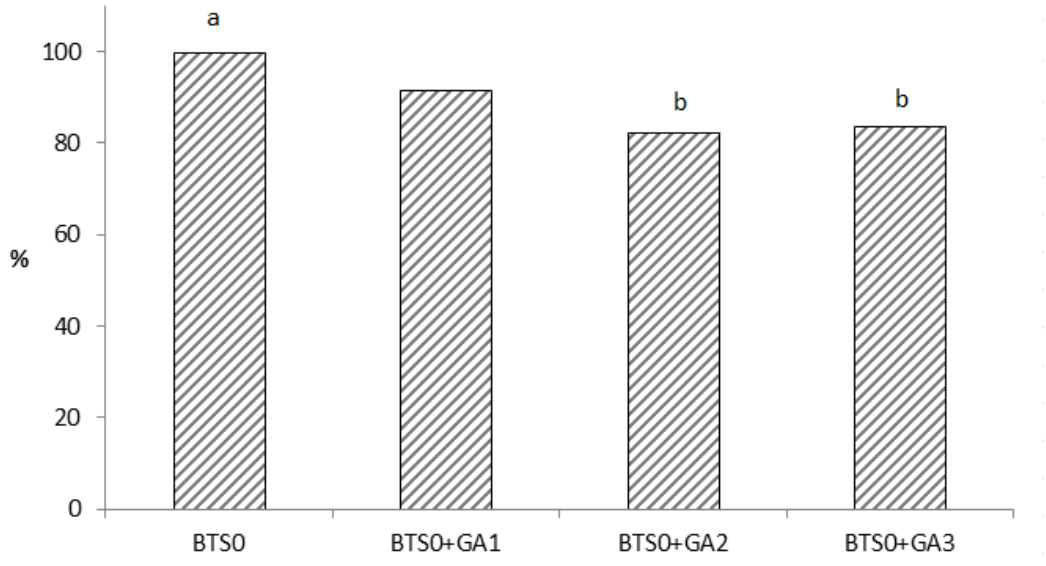
^{a,b} means within row ^{a,b} $p<0.05$ ^{A,B} means within colum ^{A,B} $p<0.05$

Results and Discussion

The initial quality of native semen was as follows: semen volume 259.56±82.59 ml, sperm motility 83.33±2.5%, sperm concentration 379.33±80.69×10^3/mm^3, MAS 22.36±1.42%, pH 7.87±0.26 and osmolality 315.29±12.26 mOsmol/kg. Values of pH and osmolality extenders are presented in the Table 1.

Osmolality and pH extenders were decreased according to the amount of added gallic acid. Johnson et al. (2000) mentioned that the pH of fresh boar ejaculate is between values 7.2 and 7.5. Table 2 shows that samples of boar semen dilution with different concentrations of gallic acid had the optimal pH values. Comparison of mean values of sperm motility (%) in samples with different concentrations of gallic acid to control sample BTS0 are presented in the Table 3. Sperm motility was affected by storage time, the dilution ratio and concentration of GA ($p<0.05$). Sperm motility was decreased in all samples of BTS0+GA2 and BTS0+GA3 compared to BTS0 in dilution rate 1+2 and 1+4 and in BTS0 GA1 compared to BTS0 in dilution rate 1+8 after 48h storage ($p<0.05$). Significant differences ($p<0.05$) of total mean values of sperm motility were found between samples BTS0 70.21% vs. BTS0+GA2 57.87% and BTS0+GA3 58.89%. Used concentration of GA2 and GA3 had a negative effect on sperm motility. Comparison of total sperm motility in different concentration of gallic acid to BTS0 as 100% is recorded in Figure 1. Differences in sperm motility were between BTS0 and BTS0+GA2 about 18% and BTS0+GA3 about 16% ($p<0.05$). In previously study, concentrations of gallic acid were tested from 300 to 2400 µg/ml and sperm motility was decreased after 24h storage time on 25-40% (Mazurova et al., 2015). The mean value of microorganisms in native boar semen was 2.2×10^5 CFU/ml. In the boar ejaculates, typical bacterial concentrations are represented range from 10^3 to 10^5 CFU/ml (Morrell and Wallgren, 2011). Microorganisms were not significantly inhibited by any of tested concentration of GA1, 2 and 3 ($p>0.05$). The results of assessment a number of microorganisms up to 48h are included in the Figure 2, 3 and 4. In the concentration of the BTS0+GA3 in dilution rate 1+8 was observed to reduce the number of microorganisms during 48h. In particular, the 1st day was lower the number of microorganisms in BTS0+GA3 in compared to BTS0. Massive growth of *E. coli* was observed in uncountable samples. The most common microorganisms found in boar semen samples were: *E.coli, Proteus* sp., *Staphylococcus aureus, Staphylococcus cohnii* subsp. *Cohnii, Staphylococcus simulans, Staphylococcus cohnii* subsp. *Urealyticum, 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). It was found that number of microorganisms greater than 10^3 CFU/mL damaged the sperm quality and reduced litter size (Chung et al., 2013; Maroto Martín et al., 2010).

Figure 1. Comparison of total sperm motility (%) in different gallic acid concentrations to BTS0 (100%)



[a,b] $p<0.05$

Figure 2. Determination of the colony-forming unit (CFU/ml) of microorganisms in different gallic acid concentrations at a dilution ratio 1+2

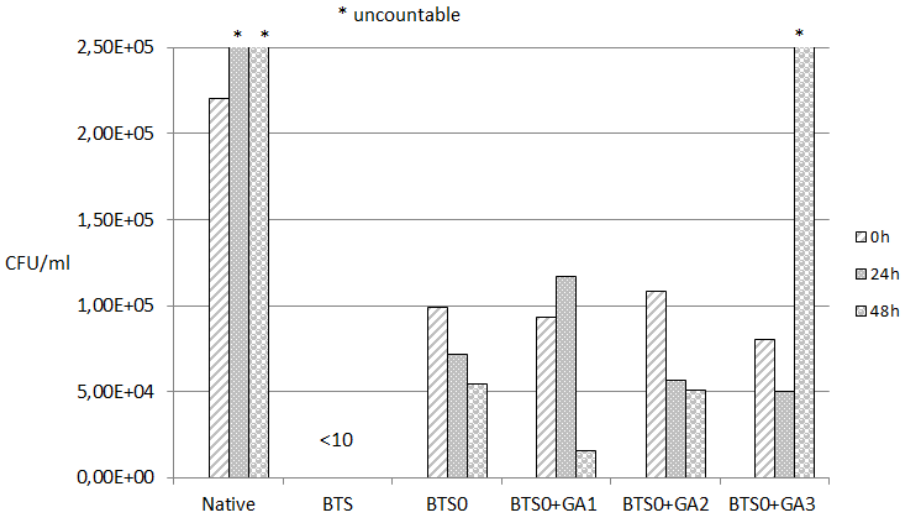


Figure 3. Determination of the colony-forming unit (CFU/ml) of microorganisms in different gallic acid concentrations at a dilution ratio 1+4

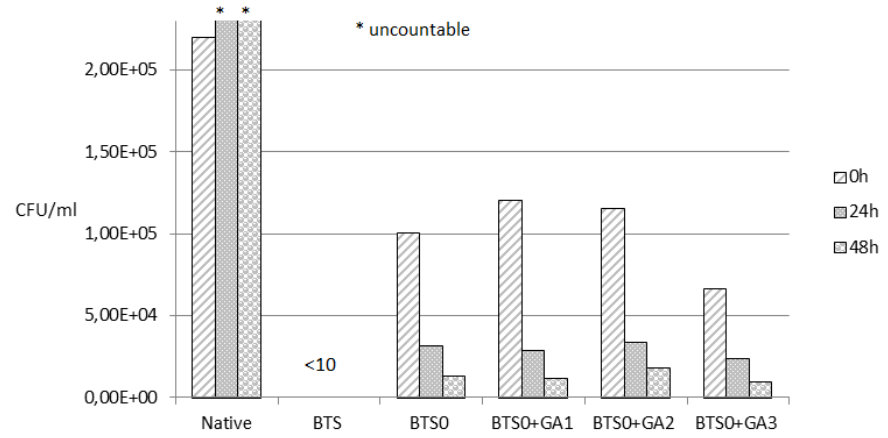
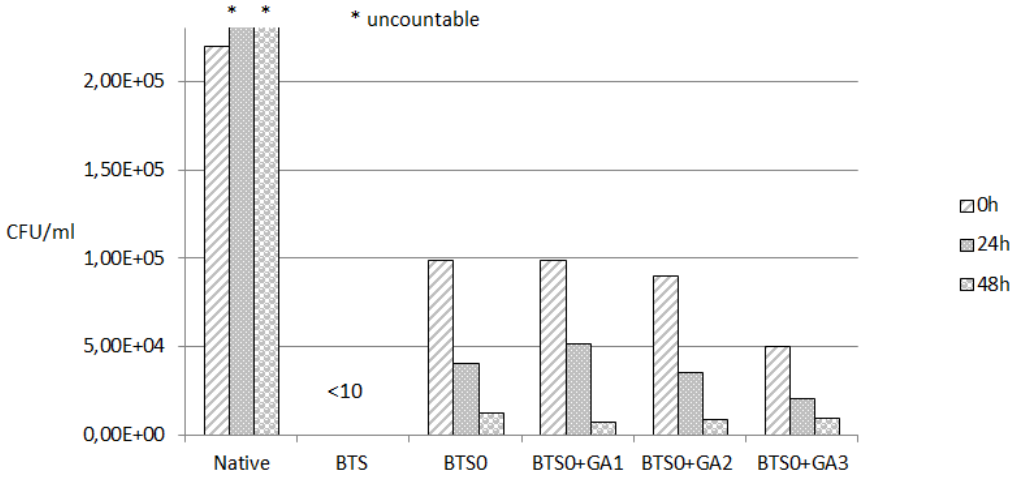


Figure 4. Determination of the colony-forming unit (CFU/ml) of microorganisms in different gallic acid concentrations at a dilution ratio 1+8



Conclusion

In conclusion, utilization of tested concentration of gallic acid as a potential substitute for antibiotics in boar semen extender is not possible because their low activity in the reduction of microorganisms was found in the tested samples and also GA2 and GA3 concentrations decreased sperm motility during preservation. Therefore, it is necessary to research other potential substances for a possible replacement for antibiotics in boar semen extender.

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