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# Influence of boar breeds or hybrid genetic composition on semen quality and seminal plasma biochemical variables

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#### ABSTRACT

The enzyme concentrations of seminal plasma are important for spermatozoa metabolism and function in boars. The need has arisen for introducing a biochemical evaluation of semen, along with the usual standard semen analyses. There are no data on the influence of boar breeds on the seminal plasma biochemical variables investigated in this study. Therefore, the objective was to determine the influence of breed and hybrid genetic composition of boars on semen quality and seminal plasma biochemical variables. Semen samples of 27 boars (Swedish Landrace, German Landrace, Large White, Pietrain and Pig Improvement Company hybrid-PIC-hybrid), aged between 1.5 and 3 years, were collected. After evaluation of semen quality, the seminal plasma was separated from the spermatozoa by centrifugation of semen. The seminal plasma was subjected to spectrophotometric analysis to determine alkaline phosphatase (ALP), acid phosphatase (ACP), y-glutamyltransferase (GGT), creatine kinase (CK) and lactate dehydrogenase (LDH) and to atomic absorption spectrophotometric analysis to measure the concentration of calcium and magnesium. Conventional semen quality variables differed depending on breed and PIC-hybrid genetic composition, though these differences were typically insignificant. In the seminal plasma, significant differences were determined in enzyme activity (ALP, GGT, CK and LDH) and in calcium concentration among boars of differnt breeds. There are, therefore, differences in semen quality and significant differences in the seminal plasma biochemical variables among boars of different breeds and PIC-hybrid genetic composition. The data and differences in semen variables detected in the present study provide knowledge for enhancing evaluation and monitoring of boar reproductive potential, semen quality and explain the potential causes of boar infertility.

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## 1. Introduction

Over the past 40 years, artificial insemination in commercial pig herds has had a significant effect on genetic selection based on quantitative and qualitative traits,

http://dx.doi.org/10.1016/j.anireprosci.2015.11.027 0378-4320/© 2015 Elsevier B.V. All rights reserved. which has resulted in rapid pork production progress of the swine industry (Dyck et al., 2011). The success of artificial insemination depends on identification and selection of boars based on reproductive performance assessment, such as libido, ability for mating and above average semen quality (Okere et al., 2005). Knowledge of the physiological characteristics of semen, seminal plasma, spermatozoa and the influence of breed, lines and individual boar characteristics are prerequisites for the successful improvement

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of reproductive efficiency and for inheritance of the most desirable semen genotype traits.

The semen composition and properties, particularly of the seminal plasma, differs among species and individuals within species or breeds. Such differences are important for evaluating semen quality, and are influenced by many exogenous and endogenous factors (Ciereszko et al., 2000; Smital, 2009). The male fertility potential, semen quality and possible causes of infertility are estimated by semen analyses. Such analyses are based primarily on conventional methods, including spermatozoa counts, and assessing cell motility and morphology. Studies on domestic animals have indicated that these semen traits are often not correlated with male fertility and that the most relevant boar semen quality assessment is related to pregnancy and healthy offspring (Tsakmakidis et al., 2010; López Rodríguez et al., 2013). The causes that lead to infertility of boars are largely unknown, because boars are immediately culled without any further examination and treatment (López Rodríguez et al., 2013). In human reproduction, to verify the male reproductive potential, several analyses other than the routine seminogram are performed, including the determination of hormones and various seminal plasma biochemical variables, which are recommended by the World Health Organization (WHO, 2010). In the seminal plasma, the enzymes alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) have an important role in the metabolic processes that provide energy for spermatozoa survival, motility and the fertility potential of the cells (Diaconescu et al., 2014). The activity of enzymes such as  $\gamma$ -glutamyl transferase (GGT), creatine kinase (CK), acid phosphatase (ACP) and ALP in seminal plasma are related to semen quality and spermatozoa membrane function, and are involved in various metabolic processes during maturation (Seligman et al., 2005; Wysocki and Strzezek, 2006; Teixeira and Borges, 2012: Bucci et al., 2014). Research on boars, other animals and humans has established that several biochemical variables may be of clinical significance, such as indicators of testicular function or dysfunction and/or other accessory sex glands, or may be associated with semen quality, reproductive potential or infertility (WHO, 2010; López Rodríguez et al., 2013).

There are no data in the literature on the influence of boar breed on the seminal plasma biochemical variables investigated in the present study, while some of these biochemical variables have not been previously determined in boar seminal plasma.

The objective of the present study, therefore, was to determine the influence of boar breed and hybrid genetic composition on phenotypic reproductive variables such as: quality of semen and biochemical composition of seminal plasma, and in particular, mineral concentrations and enzyme activity.

#### 2. Materials and methods

### 2.1. Animals

The study was performed on 27 boars, aged between 1.5 and 3 years  $(2.51 \pm 0.71; \text{ mean} \pm \text{SD})$ , which were selected

| Table 1   Composition of feed for the boars. |       |
|--|-------|
| Crude protein (%)                            | 16    |
| Crude fat (%)                                | 16    |
| Crude fiber (%)                              | 7     |
| Phosphorus (%)                               | 0.6   |
| Calcium (%)                                  | 1     |
| Sodium (%)                                   | 0.25  |
| Zinc (mg/kg)                                 | 50    |
| Vitamin A (IU/kg)                            | 5 000 |
| Vitamin D <sub>3</sub> (IU/kg)               | 625   |

in the Centre for Reproduction in Croatian Livestock, Production work unit, Križevci, Croatia. Animals were divided into five groups according to breed. The German Landrace and Pietrain groups consisted of six boars, while the groups Swedish Landrace, Large White and Pig Improvement Company hybrid (PIC hybrid) each included five boars. During the experimental period, animals were kept in separate fenced compartments (size  $12 \text{ m}^2$ ), equipped with a covered outlet in which zoo-hygienic measures were regularly conducted. Animals were fed once daily, before the time of semen collection, with 3 kg of commercial feed for boars produced by Poljoprivredna zadruga Virje, Croatia (Table 1). Water was provided to boars ad libitum by automatic watering.

#### 2.2. Semen sampling and processing

Semen samples were collected using routine procedures twice weekly to obtain semen for artificial insemination. Semen samples were taken once in the early autumn (late September or early October). Before each ejaculation, boars were stimulated by taking boars into a separate room containing the "phantom" sow, and semen was obtained by manual manipulation of the penis after mounting of the "phantom" sow. The two latter semen fractions of each ejaculate were collected in graduated sperm collection containers and were strained through three layers of sterile gauze to separate the bulbourethral gland secretions from the other semen constituents. Following semen sampling, evaluation of semen purity and macroscopic properties (volume, colour, consistency and odour) was performed. Following this evaluation, which lasted several seconds, strained ejaculates were placed in a water bath heated at 37 °C, where the samples remained during the period of microscopic evaluation and determination of semen density. Fresh semen samples were evaluated microscopically for progressive spermatozoa motility and morphologic spermatozoa features by visualization using a phase-contrast system microscope with use of a warmed stage (Olympus BX50F, Tokyo, Japan). The concentration of spermatozoa was objectively determined using a Photometer SDM 5 (MiniTüb, Landshut, Germany). Semen samples meeting the criteria of macroscopic and microscopic evaluations and spermatozoa concentration (150 to  $250 \times 10^6$  spermatozoa/mL) were processed further. The total number of spermatozoa per ejaculate per boar was calculated by multiplying spermatozoa concentration and ejaculate volume.

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