VETERINARSKI ARHIV 90 (4), 323-330, 2020 DOI: 10.24099/vet.arhiv.0804

The effects of sex and castration on the serum biochemical profile in commercial pigs

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ABSTRACT

Sex-related differences in physiological processes, pathophysiology, diagnosis and treatment of disease have great importance in human medicine, and these differences should also be investigated and implemented in studies on animals. To our knowledge, this study is the first to investigate the effects of sex and castration on the serum biochemical profile of commercial pigs. The study was conducted on clinically healthy, sexually mature fattening pigs of the Swedish Landrace breed, divided into four groups: intact males (n = 6), intact females (n = 5), castrated males (n = 6) and castrated females (n = 7). Blood samples were taken from the jugular vein using blood test tubes without an anticoagulant. After centrifugation, the serum was separated, and the values of the following parameters were determined by spectrophotometry: the activities of aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transferase, and the concentrations of total proteins, albumins, triacylglycerols, total cholesterol, glucose, urea, bilirubin and creatinine. The study results showed significantly lower ($P<0.05$) serum glucose concentrations in castrated males as compared to intact males. However, castrated males had significantly higher ($P<0.05$) urea concentration than intact ones. There were no significant between-group differences in the values of the other

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parameters investigated, however, certain patterns of occurrence were observed. Accordingly, further studies are required in order to define more accurately the effects of sex and castration on the values of biochemical parameters in the blood serum of pigs.

Key words: biochemical parameters; blood serum; castration; pigs; sex

Introduction

Pig farming is of great economic importance and one of the world's largest livestock production branches. Recently, much progress has been made in pig production, especially in the selection of different breed lines, with the main goal of improving desirable hereditary traits. Stricter, new legal provisions regarding pig welfare and protection are also in effect (ANONYM., 2008; MILLS et al., 2010).

Analysis of blood parameters in pigs and sheep can be used as a procedure for assessing the herd health status (EVANS, 1994; DURAK et al., 2015). Reference intervals for haematological and biochemical parameters can contribute to the early detection of disease or poor growth, and may assist clinicians and researchers in interpreting blood test results (KLEM et al., 2010; PERRI et al., 2017). However, despite the importance of analysing haematological and biochemical parameters, they are rarely used in assessing the health status in pig production. This may be due to a lack of data on reference intervals for commercial pigs, and the costs associated with labour and laboratory analyses, especially when compared to the low economic value of pigs (EVANS, 1994). In addition, there may be suspicion that haematological and biochemical blood analyses will not provide much useful information on pig health (PERRI et al., 2017).

Traditionally, indicators such as reproductive performance, daily weight gain, feed conversion and mortality have been used to evaluate pig herd productivity. However, measurement of serum metabolites can provide additional important data that can be used to assess pig herd health and metabolic status, and the effects of different management practices and housing conditions on animals (MORRIS, 1988; EKERT KABALIN et al., 2017). Although serum biochemical analysis is more commonly used in the routine diagnosis of cattle and sheep, it is also applicable in pigs. Yet, in order to be useful as a diagnostic tool, it is necessary to identify methods sensitive enough to detect changes in pig health status and physiological factors affecting the values of biochemical parameters (VERHEYEN et al., 2007).

Many factors can affect the values of haematological and biochemical parameters, including environmental and physiological factors, such as age, breed, sex, nutrition and housing, season, physical activity, disease and stress (EVANS, 1994; HUMANN-ZIEHANK and GANTER, 2012; PERRI et al., 2017).

Pigs have been widely used in recent decades as animal models in biomedical studies, even more than rodents (EKSER et al., 2012), due to their great similarity with humans in physiological processes and anatomical features (KANEKO et al., 2011; KAWAGUCHI et al., 2011; 2012). For example, due to the similarity of the digestive system and metabolism in pigs and humans (BAKER, 2008), genetically obese and lean pigs are often used in childhood obesity studies to elucidate the mechanism responsible for obesity development (SPEAKMAN et al., 2007; HE et al., 2012). Pigs are often the animal model of choice as the representative of large animal species used in xenotransplantation studies (COOPER et al., 2002), and mini-pigs are frequently used in studies of cardiovascular and skin diseases (KAMIMURA et al., 2003; KAWAGUCHI et al., 2013; YOSHIKAWA et al., 2013).

Knowledge of the physiological values of haematological and biochemical parameters in different animal species used in biomedical studies is of the utmost importance. However, it is also important to know the effects of physiological traits, such as age, sex and hormonal status, on these parameters. Since sex-related differences are increasingly important in physiological processes, pathophysiology, diagnosis and treatment of

diseases in human medicine (LEGATO, 1997), these differences should also be considered in animal studies (CHRISTOFFERSEN et al., 2007).

To date, the few studies conducted only on mini-pigs have shown that age and sex have certain effects on the serum biochemical profile (KAWAGUCHI et al., 2012; 2013). The aim of this study was to investigate the effects of sex and castration on the values of biochemical parameters in the blood serum of commercial pigs.

Materials and methods

Animals and farm management. The study was conducted at a state-owned pig farm in Požega, Croatia. It included 24 clinically healthy, sexually mature fattening pigs of the Swedish Landrace breed, aged 9 months, and kept for economic purposes: 6 intact males, 5 intact females, 6 castrated males and 7 castrated females. After weaning up to four months of age, each group of pigs was housed in a 3 × 2 m pen, with *ad libitum* water and feed, and fed with a complete feed mixture for weaners (20% pig starter pellets, TSH Ltd., Čakovec, Croatia). Then, the pigs were transferred to 2.5 × 4.3 m pens, with *ad libitum* water and restricted feed 3 times *per* day with a complete feed mixture for growing and fattening pigs up to 60 kg (BEK-1, TSH Ltd., Čakovec, Croatia). From the eighth month of age until slaughter, the pigs were housed in 3 × 6 m pens. Water was *ad libitum* and feed restricted to 3 times *per* day with a complete feed mixture for growing and fattening pigs over 60 kg (BEK-2, TSH Ltd., Čakovec, Croatia). In the final, ninth month, the pigs were fed additionally with a feed mixture for fattening and breeding pigs (40% pig super concentrate, TSH Ltd., Čakovec, Croatia).

Experimental procedure. At four months of age, six male and seven female pigs were castrated. Castration was performed by bilateral orchiectomy or ovariectomy, using acepromazine (0.2 mg/kg i.m.). Upon completion of the procedure, the animals were given a tetanus antitoxin (5 mL, s.c.) and a combination of procaine benzylpenicillin and benzathine benzylpenicillin with prolonged effect (0.07 mL/kg i.m.) to prevent secondary bacterial infections. All preparations used were authorized for use in the Republic of Croatia. At the end of the fattening period, blood samples were collected from the jugular vein into tubes without anticoagulant (BD Vacutainer™ SST™ II Advance Tubes, Plymouth, UK). After blood coagulation, the samples were centrifuged at 3500 rpm for 10 minutes. Serum samples were transported to the laboratory at +4 °C and stored at -20 °C for 24 hours, and then at -80 °C until analysis. The following parameters were determined in serum samples: the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT), and concentrations of total proteins, albumins, triacylglycerols, total cholesterol, glucose, urea, total and conjugated bilirubin, and creatinine. The values of all parameters were determined by spectrophotometry on a SABA 18 automatic analyser (AMS, Italy) using complete kits (Dijagnostika, Sisak, Croatia; Randox, Ireland, UK) according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed using the Statistica v. 13.4 software package (TIBCO Software Inc., 2017). The normality of data distribution was tested by the Kolmogorov-Smirnov test, and the significance of differences between the groups by analysis of variance (ANOVA) and the Tukey HSD test. Statistical difference was set at the level of $P<0.05$. The values are expressed as Mean ± SEM.

Ethical approval. The study trials were approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb (Class: 640-01/18-17/83; Record number: 251-61-41-18-01).

Results

Serum enzyme activity. AST activity was lowest in the serum of intact males (27.69 ± 1.46 U/L), followed by castrated females (28.80 ± 2.99 U/L) and intact females (30.07 ± 4.02 U/L), with the highest value determined in the serum of castrated males (37.75 U/L ± 4.35 U/L). The highest ALT activity was determined in the serum of castrated males (68.00 ± 5.22 U/L), and lowest in intact males (51.91 ± 3.59 U/L). Similar values of the ALT activity were found in the serums of intact females (59.16 ± 4.65 U/L) and castrated females (60.98 ± 4.72 U/L). The lowest GGT activity was determined in the serum of castrated females

(30.68 ± 3.30 U/L), followed by intact females (31.09 ± 2.99 U/L), intact males (32.43 ± 3.95 U/L) and finally castrated males (33.22 ± 3.06 U/L). However, none of the between-group differences in enzyme activities were significant (Fig. 1).

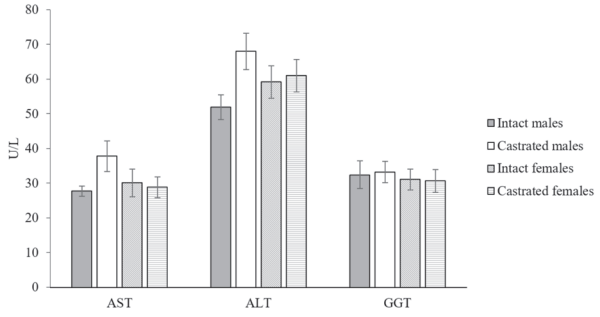

Fig. 1. Activities of enzymes AST, ALT and GGT in the serum of intact and castrated male and female pigs (Mean ± SEM). AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - γ-glutamyl transferase

Serum protein concentration. The lowest total protein concentration was found in the serum of intact males (81.88 ± 4.95 g/L), followed by castrated females (84.49 ± 3.22 g/L), while the concentrations of the total proteins in the serum of castrated males (88.09 ± 3.96 g/L) and intact females (88.24 ± 3.01 g/L) were highest and approximately equal. As with total proteins, the mean albumin concentration was highest in the serum of intact females (49.20 ± 0.73 g/L), then castrated males (47.00 ± 2.19 g/L), with the lowest and similar values found in intact males (46.40 ± 2.44 g/L) and castrated females (46.71 ± 1.44 g/L). None of these differences were significant (Table 1).

Serum lipid concentration. The concentrations of the triacylglycerols and total cholesterol in the serum of all the investigated groups of pigs were near equal and did not differ significantly. Similar triacylglycerol concentrations were determined in the serum of castrated males (0.44 ± 0.06 mmol/L) and intact females (0.43 ± 0.08 mmol/L). Castrated females had the highest concentration of triacylglycerols (0.54 ± 0.05 mmol/L), and intact males the lowest (0.35 ± 0.05 mmol/L). The lowest total cholesterol concentration was also found in the serum of intact males (1.47 ± 0.19 mmol/L), and the highest in castrated females (1.61 ± 0.03 mmol/L). In-between concentrations were determined in castrated males (1.51 ± 0.08 mmol/L) and intact females (1.58 ± 0.14 mmol/L), Fig. 2.

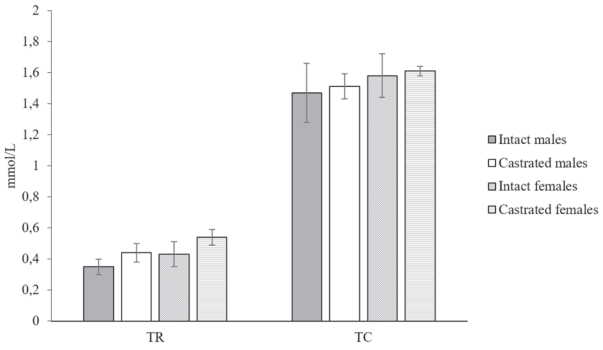

Fig. 2. Concentrations of triacylglycerols and total cholesterol in the serum of intact and castrated male and female pigs (Mean ± SEM). TR - triacylglycerols; TC - total cholesterol

Serum glucose and urea concentrations. Glucose concentrations were significantly lower ($P<0.05$) in the serum of castrated males (3.95 ± 0.16 mmol/L) as compared to intact males (4.78 ± 0.16 mmol/L), and intact females (4.98 ± 0.31 mmol/L). In castrated females, the serum glucose concentration was 4.30 ± 0.07 mmol/L, which did not differ significantly from the other pig groups. The mean urea concentration was significantly higher ($P<0.05$) in the serum of castrated males (6.34 ± 0.41 mmol/L) compared to intact males (4.33 ± 0.52 mmol/L). The urea concentration in the serum of intact females was 5.10 ± 0.24 mmol/L, similar to the value determined in the serum of castrated females (5.61 ± 0.31 mmol/L), Fig. 3.

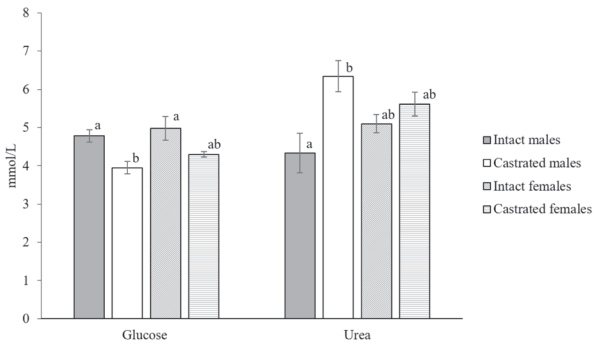

Fig. 3. Concentrations of glucose and urea in the serum of intact and castrated male and female pigs (Mean ± SEM). [a,b] Values referring to the same biochemical parameter marked by a different letter differ significantly ($P<0.05$)

Serum bilirubin concentration. The lowest conjugated bilirubin concentration was found in the serum of intact males (4.66 ± 0.49 μmol/L), followed by intact females (5.36 ± 0.34 μmol/L) and castrated males (5.75 ± 0.56 μmol/L), and the highest in the serum of castrated females (5.87 ± 0.43 μmol/L). As with conjugated bilirubin, the concentration of total bilirubin was lowest in the serum of intact males (5.10 ± 0.47 μmol/L), followed by intact females (5.51 ± 0.31 μmol/L) and castrated males (6.36 ± 0.52 μmol/L), with the highest value found in the serum of castrated females (6.43 ± 0.38 μmol/L). However, there were no significant between-group differences (Table 1).

Serum creatinine concentration. The mean serum creatinine concentration was similar in all groups of pigs, without significant differences (Table 1). The lowest concentration was found in the serum of intact females (162.36 ± 4.15 μmol/L), followed by castrated males (167.47 ± 7.14 μmol/L) and castrated females (172.11 ± 12.18 μmol/L), and the highest in intact males (184.84 ± 19.40 μmol/L).

Table 1. Concentrations of total proteins, albumins, total and conjugated bilirubin, and creatinine in the serum of intact and castrated male and female pigs (Mean ± SEM)

Parameter	Intact males	Castrated males	Intact females	Castrated females
TP (g/L)	81.88 ± 4.95	88.09 ± 3.96	88.24 ± 3.01	84.49 ± 3.22
ALB (g/L)	46.40 ± 2.44	47.00 ± 2.19	49.20 ± 0.73	46.71 ± 1.44
TBL (μmol/L)	5.10 ± 0.47	6.36 ± 0.52	5.51 ± 0.31	6.43 ± 0.38
CBL (μmol/L)	4.66 ± 0.49	5.75 ± 0.56	5.36 ± 0.34	5.87 ± 0.43
CR (μmol/L)	184.84 ± 19.40	167.47 ± 7.14	162.36 ± 4.15	172.11 ± 12.18

TP - total proteins; ALB - albumins; TBL - total bilirubin; CBL - conjugated bilirubin; CR - creatinine

Discussion

Serum biochemical parameters are used in assessment of animals' condition and disease detection, monitoring and evaluation of disease treatment outcomes, in the interpretation of nutritional disorders, in animal productivity studies and the collection of pathophysiological data (DURAK et al., 2015). The values of the biochemical parameters obtained in this study are generally in line with the reference values for pigs reported in previous studies (VERHEYEN et al., 2007; KANEKO et al., 2008; WANG et al., 2009; CHMIELOWIEC-KORZENIOWSKA et al., 2012; KAWAGUCHI et al., 2012; 2013; KOZERA et al., 2016; PERRI et al., 2017). However, to our knowledge, the effect of castration on the values of the serum biochemical parameters in pigs has not been investigated to date. Moreover, there are no data on the effect of sex on the serum biochemical profile in commercial pig breeds.

The study results showed no significant difference in serum urea concentration between intact males and females. This finding is in agreement with the study of KAWAGUCHI et al. (2013), who also found no difference in the urea concentration between sexes in mini-pigs. The study of KAWAGUCHI et al. (2012) revealed contradictory results. These authors determined significantly higher urea concentrations in the serum of male mini-pigs as compared to females. This was possibly due to the differences in the pigs' ages and the analytical methods used in the different studies. In the present study, significantly higher serum urea concentrations were found in castrated males as compared to intact ones. This can be explained by the fact that testosterone reduces hepatic urea production, thus reducing its concentration in the serum (LAM et al., 2017).

No significant difference in the serum glucose concentration was found between intact males and females, as also confirmed by the study of CHRISTOFFERSEN et al. (2007) on the effect of sex on the serum metabolites in mini-pigs. However, in this study there were significantly higher serum glucose concentrations in intact males as compared to castrated males, indicating that castration reduces glucose concentration in male pigs.

The concentrations of the triacylglycerols and total cholesterol were higher in the serum of both female groups as compared to the male groups, although not significantly. KAWAGUCHI et al. (2013) found significantly higher concentrations of total cholesterol, free cholesterol, cholesterol ester, triacylglycerols and low-density lipoprotein-cholesterol in the serum of female mini-pigs as compared to males aged 5 months. However, the differences in the serum triacylglycerol and total cholesterol concentrations between females and males disappeared at 10 to 12 months of age. This finding is supported by the results of our study. In the study of CHRISTOFFERSEN et al. (2007), female mini-pigs also had significantly higher concentrations of triacylglycerols and total cholesterol than males, with differences increasing after the onset of sexual maturity. Higher values of serum lipids in female pigs may be associated with gender-related differences in whole-body lipid metabolism (WILLIAMS, 2004).

The activities of serum AST, ALT and GGT did not differ significantly regarding sex and castration. In the study of KAWAGUCHI et al. (2013), the activities of the serum AST and ALT also did not differ between the sexes of mini-pigs aged 10 to 12 months, but ALT activity was significantly higher in females than in males aged four to six and seven to nine months. KAWAGUCHI et al. (2012) also found significant differences in serum AST activity between male and female mini-pigs, though ALT activity was significantly higher in the serum of females as compared to male pigs aged 0-34 months. This suggests that the serum ALT activity is dependent on pig age and sex.

No between-group differences in the serum concentrations of total proteins, albumins, total and conjugated bilirubin, and creatinine have been discussed, due to the lack of literature data for comparison.

In this study, while virtually none of the investigated serum biochemical parameters were significantly affected by sex or castration, certain patterns of occurrence were observed. The lowest values of AST, ALT, total proteins, albumins, triacylglycerols, total cholesterol, urea, total and conjugated bilirubin, and the highest value of creatinine were found in the blood serum of intact males. GGT activity was lowest, and the triacylglycerol, total cholesterol, total and conjugated bilirubin concentrations were highest in the serum of castrated females. The highest activities of AST, ALT and GGT, and highest urea concentration were found in the serum of castrated males, with the lowest glucose concentration. Glucose, total protein and albumin concentrations were highest, and creatinine concentration lowest in the serum of intact females. Accordingly, most of the values were lowest in the blood serum of intact males.

Conclusions

The results of this study regarding the effects of sex and castration in pigs on the values of serum biochemical parameters cannot be compared to other studies on commercial pigs due to the lack of data. Therefore, these results present new and valuable information. According to the results obtained, it can be concluded that sex and castration have certain effects on the serum biochemical profile. Further studies are required in order to define more precisely the effects of sex and castration on the values of biochemical parameters in pig blood serum.

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Received: 6 September 2019
Accepted: 31 January 2020

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SAŽETAK

Spolne razlike u fiziološkim procesima, patofiziologiji, dijagnostici i liječenju bolesti imaju sve veću važnost u humanoj medicini, a trebale bi se istražiti te uzeti u obzir i u istraživanjima na životinjama. Prema našim spoznajama u ovom je radu po prvi put istražen utjecaj spola u svinja u komercijalnom uzgoju i utjecaj kastracije općenito u svinja na biokemijski profil seruma. Istraživanje je provedeno na klinički zdravim, spolno zrelim tovljenicima pasmine švedski landras, koji su podijeljeni u četiri skupine: intaktni mužjaci (n = 6), intaktne ženke (n = 5), kastrirani mužjaci (n = 6) i kastrirane ženke (n = 7). Krv je uzorkovana punkcijom jugularne vene u epruvete bez antikoagulansa. Nakon centrifugiranja izdvojen je serum i spektrofotometrijski su određene vrijednosti sljedećih pokazatelja: aktivnosti aspartat-aminotransferaze, alanin-aminotransferaze i γ-glutamil transferaze te koncentracije ukupnih bjelančevina, albumina, triacilglicerola, ukupnog kolesterola, glukoze, ureje, bilirubina i kreatinina. Rezultati istraživanja pokazali su da je koncentracija glukoze u serumu kastriranih mužjaka bila znakovito manja ($P < 0,05$) u usporedbi s intaktnim mužjacima. No koncentracija ureje bila je znakovito veća ($P < 0,05$) u kastriranih mužjaka u usporedbi s intaktnima. Vrijednosti ostalih istraživanih pokazatelja nisu se znakovito razlikovale među skupinama, no uočeni su obrasci različitih vrijednosti pojedinih pokazatelja u različitih skupina. Zaključno, potrebno je provesti daljnja istraživanja kako bi se preciznije definirao utjecaj spola i kastracije na vrijednosti biokemijskih pokazatelja u krvnom serumu svinja.

Ključne riječi: biokemijski pokazatelji; krvni serum; kastracija; svinje; spol