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Influence of body mass at birth on the activity of enzymes and biocatalysts concentration in the serum of Large White piglets until weaning

Anamaria Ekert Kabalin[1]*, Tomislav Balenović[1], Marcela Šperanda[2], Velimir Sušić[1], Željko Pavičić[3], Suzana Milinković-Tur[4], Igor Štoković[1], and Sven Menčik[1]

[1]*Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Department of Animal Husbandry, Faculty of Agriculture, University of J. J. Strossmayer in Osijek, Osijek, Croatia*

[3]*Department of Animal Hygiene, Environment and Ethology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[4]*Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

EKERT KABALIN, A., T. BALENOVIĆ, M. ŠPERANDA, V. SUŠIĆ, Ž. PAVIČIĆ, S. MILINKOVIĆ-TUR, I. ŠTOKOVIĆ, S. MENČIK: Influence of body mass at birth on the activity of enzymes and biocatalysts concentration in the serum of Large White piglets until weaning. Vet. arhiv 82, 483-494, 2012.

ABSTRACT

Newborn piglets weighing less than 1000 grams at birth are more susceptible to diseases and the negative influence of stress factors, slow progress, and therefore cause greater losses than heavier ones. Also, they are physiologically immature and establish postnatal metabolical mechanisms more slowly than suckling piglets of normal body mass. The aim of our research was to determine changes in the activity of some enzymes and concentrations of biocatalysts in the blood serum of suckling piglets. In this study 48 piglets were included: 24 animals with birth weight less than 1000 g (experimental group) and 24 littermates of the same sex with body mass of 1000 g and more (control group). Blood samples were taken on the 1st, 7th, 14th and 21st days of the piglets' life from the *vena cava cranialis* (1.5 mL) in BD Vacutainer SST tubes with the gel for biochemical analysis. After centrifugation, the samples were analyzed using an automatic analyzer Olympus AU 600. In the sera of both groups of piglets the activities of creatin kinase and alkaline phosphatase as well as calcium and phosphorus concentrations were higher at weaning, while the concentrations of magnesium was below the reference value. Also, we found lower activity of creatin kinase in 1-day-old piglets with small birth mass ($P<0.05$), possibly associated with poor development of the muscle tissue. In contrast, activity of alanin

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*Corresponding author:

Assist. Prof. Anamaria Ekert Kabalin, PhD, DVM, Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: + 385 1 2390 138; E-mail: akabalin@vef.hr

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aminotransferase was significantly higher (P<0.01) in the serum of experimental animals on the 1st day of life. We assume that the observed changes in enzymes activities and biocatalyst concentrations indicate the intensive growth and development of newborn animals, especially in the control group of piglets.

Key words: Large White, suckling piglets, birth weight, enzymes, electrolytes

Introduction

Selection for the increased size of a sow's litters has resulted in the decreased rate of piglet survival until weaning and a growing number of piglets with small birth mass (MILLIGAN et al., 2002; CUTLER et al., 2006; FIX et al., 2010). Numerous studies have pointed out the significance of piglet birth mass for neonatal vitality and further growth (HOY et al., 1994 and 1997; MILLIGAN et al., 2002; QUINIOU et al., 2002; JOHANSEN et al., 2004; CUTLER et al., 2006; EKERT KABALIN et al., 2008a; WOLF et al., 2008). TUCHSCHERER et al. (2000) established the better survival of the piglets that were born among the first, with a greater body mass, who caught the sow's breast earlier, quickly sucked colostrum and had lower drop in rectal temperature after birth.

After the birth of piglets, various changes occur associated with the development and maturation of organs, sucking and taking colostrum, the breakdown of fetal erythrocytes and maternal globulins, acquiring immunocompetence, and in relation to environmental changes, influence of stress and other factors (EGELI et al., 1998; THORN, 2000; EKERT KABALIN et al., 2008a). Normal birth mass amounts to 1.3-1.4 kg (QUINIOU et al., 2002; CUTLER et al., 2006; EKERT KABALIN et al., 2008b). As PETROVIČ et al. (2009) wrote, the characteristic features of the suckling period are an extremely high growth rate and rapid development of the piglet's organism, especially bones and parenchymatous organs. CURTIS et al. (1967) stated that the vigor of the newborn animals was related to their physiological maturity at birth where development age did not correspond to chronological age, so it was achieved more rapidly by piglets who were heavier at birth and had a lower neonatal death rate than lighter animals. As WEHREND et al. (2003) and PETROVIČ et al. (2009) stated, the data required for more accurate physiological description of the first phase of life, as well as complex knowledge of some serum biochemistry parameters have been insufficient in recent publications and have not covered the entire period in detail. Furthermore, mean enzyme activity or serum biocatalyst concentrations reported by some authors represent a wide range, especially on the first day of life (EGELI et al., 1998; THORN, 2000).

Thus, the aim of this research was to investigate the activities of some serum enzymes and biocatalysts concentrations at intervals of a week during the preweaning period and related to the piglets' birth mass. We assume that these biochemical parameters play an important role in metabolism during the growth and development of newborn animals.

Materials and methods

Forty-eight suckling Large White piglets from 17 litters were included in this study. According to their birth body mass, the suckling piglets were distributed into two groups: the experimental group comprised 24 animals with birth mass less than 1000 grams (705-995 g), while in the control group there were 24 littermates of 1000 g or more (1050-2150 g). To lessen the possible impact of external factors, the manner of keeping and feeding, and genetic influences and the milk of sows as well as the piglets' sex, offspring of the same sex were taken as a control from each litter in which a lightweight piglet was observed. Furthermore, during the observation period, the piglets were kept in the farrowing pen under the same conditions (automatic control of microclimate conditions: temperature 22 °C with space heating for piglets at 28-34 °C; relative humidity 55-65%; air circulation up to 0.3 m/s; illumination of 80-100 lux; concentration of harmful gases within tolerable limits). Besides sucking the sow's milk, piglets were offered water and from the 7th day prestarter mixture (with 22% crude protein) to become accustomed to solid food (*ad libitum*). Weaning was undertaken between 21 and 24 days.

Blood samples were taken from the vena cava cranialis (1.5 mL) into BD Vacutainer SST tubes (Vacutainer Systems, Belliver Industrial Estate, Plymouth, UK) containing a gel for biochemical analysis. The blood samples were centrifuged (4000 RPM / 10 minutes) and sera were stored at +4 °C until analysed, within 24 hours after collection. The samples were taken at the same time of the day (between 9.00 and 11.30 in the morning) so that the obtained results would be as relevant as possible since the suckling piglets were held on the tit. Analysis of the samples was performed on the 1st, 7th, 14th and 21st days of the piglet's life, which encompassed the suckling period. Activities of enzymes in blood serum: creatin kinase (CK, EC 2.7.3.2.), alkaline phosphatase (AP, EC 3.1.3.1.), γ-glutamiltransferase (GGT, EC 2.3.2.2.), aspartat aminotransferase (AST, EC 2.6.1.1.), alanin aminotransferase (ALT, EC 2.6.1.2.), as well as biocatalyst concentrations (calcium, phosphorus, magnesium) were analyzed with the automatic analyst Olympus AU 600 (Diagnostica GmbH, Wendenstrasse 14-18, Hamburg, Deutschland, EU) using commercial kits (catalog numbers: OSR 6179 (CK), OSR 6104 (AP), OSR 6120 (GGT), OSR 6109 (AST), OSR 6107 (ALT), OSR 60117 (calcium), OSR 6122 (phosphorus), OSR 6189 (magnesium)).

Statistical analysis was performed using Statistica v.9 software (StatSoft Inc.). Basic data processing was performed with standard procedures of descriptive statistics. Significance of differences between the experimental and control group was determined with Student *t*-test or Mann-Whitney U test (depending on normality of values distribution). For determining the significance of differences between sequential samplings within groups, we used ANOVA Repeated Measures (with Unequal n HSD test for post-hoc analysis) or Friedman ANOVA.

Results

Average birth body mass in the experimental group was 854.92 ± 85.75 g, while piglets in the control group were on average 1516.67 ± 332.98 g. During the observation period, losses were lower in the control group compared to the experimental and the numbers of piglets on the day of sampling are shown in the tables.

Average enzyme activities in the blood serum of piglets are presented in Table 1. Taking into account the wide range of enzyme activities in the serum of piglets, and the fact that some parameters did not follow a normal distribution, as well as the mean and standard deviation, the central value (median), with minimum and maximum are also presented.

On the first day of life the average activity of creatin kinase in the serum of lighter piglets was significantly lower ($P<0.05$) compared to the control group, while it did not differ significantly in later periods. In the group of normal birth weight piglets, after a decline during the first week, we observed a tendency of continuous increase in CK activity, while in small piglets an increase during the first two weeks and a slight drop in the third week was observed. Table 1 shows that no significant differences were observed in alkaline phosphatase activity between the two groups of piglets. During the entire preweaning period AP activity decreased in both groups and was significantly lower on the 7th and 21st days ($P<0.01$) compared to the first day of life. Similar changes were observed for γ-glutamiltransferase activity and values determined at weaning were significant lower than at birth ($P<0.01$ and $P<0.05$ in experimental and control animals, respectively) due to the continuous decrease. Also, a decline in activities of aspartat aminotransferase up to weaning was observed in both groups ($P<0.01$ for the control group). During the first two weeks of life higher serum alanin aminotransferase activities were observed in piglets with low birth weight ($P<0.01$ on the first day of life). A significant decline in ALT activity ($P<0.01$) in both groups of piglets was recorded during the first week, which is similar to the kinetics of AST. In the experimental group of piglets this significantly lower ALT activity ($P<0.01$) remained until weaning. It was not recorded in the control group of piglets due to the increase in enzyme activity during the second week.

Table 2 shows the determined statistical parameters of investigated biocatalysts during suckling.

Due to the continuous recorded decrease in calcium concentration in our research (especially during the first week; $P<0.01$), values determined at weaning were significantly lower than after birth ($P<0.01$ in the experimental group and $P<0.05$ in control animals), and within the reference range in piglets with normal birth weight. Increase in average phosphorus concentrations during the first week ($P<0.01$ in both groups) led to the fact that the mean values obtained at weaning in the blood sera of the animals were higher than on the 1st day ($P<0.05$ in lightweight piglets, and $P<0.01$ in piglets with normal birth

Table 1. Serum enzyme activities in two groups of Large White suckling piglets

Enzyme (U/L)	Group	Statistical parameter	Piglets' age (N = number of animals in experimental / control group) Day 1 (N=24/24)	Day 7 (N=20/22)	Day 14 (N=18/22)	Day 21 (N=17/22)
CK	Experimental	Mean ± SE	284.29** ± 45.51	400.25 ± 90.52	601.72 ± 144.88	480.77 ± 114.75
		Median (Min-Max)	210 (83-1053)	341.5 (115-1988)	426.5 (146-2875)	366 (88-2085)
	Control	Mean ± SE	504.29 ± 95.93	386.96 ± 57.15	515.46 ± 65.36	672.59 ± 139.74
		Median (Min-Max)	400.5 (80-2118)	302.5 (107-1056)	473 (138-1331)	370.5 (189-2763)
AP	Experimental	Mean ± SE	2853.21 ± 349.82	1149.20[a] ± 115.98	788.11 ± 112.87	532.65[a] ± 63.95
		Median (Min-Max)	2261 (962-7150)	1007 (500-2374)	726.5 (88-1892)	504 (168-1298)
	Control	Mean ± SE	2830.75 ± 329.47	1107.82[a] ± 106.87	739.91 ± 66.17	462.78[a] ± 46.78
		Median (Min-Max)	2483 (698-7416)	1042 (416-2697)	719.5 (299-1434)	462 (179-1059)
GGT	Experimental	Mean ± SE	56.38 ± 4.68	44.10[aa] ± 3.42	38.78 ± 3.36	36.24[a] ± 3.01
		Median (Min-Max)	52.5 (21-108)	44.5 (16-70)	39.5 (14-65)	40 (12-58)
	Control	Mean ± SE	52.04 ± 3.02	45.14 ± 4.74	41.05 ± 2.95	39.18[aa] ± 3.07
		Median (Min-Max)	51 (30-78)	42 (17-121)	39.5 (23-72)	37.5 (18-70)
AST	Experimental	Mean ± SE	73.83 ± 7.95	55.65 ± 5.17	56.39 ± 5.92	45.47 ± 5.53
		Median (Min-Max)	62.5 (36-195)	50 (33-129)	44 (30-116)	36 (21-102)
	Control	Mean ± SE	88.33 ± 9.38	48.59[a] ± 3.11	64.64 ± 12.90	48.41[a] ± 4.66
		Median (Min-Max)	76 (39-193)	44.5 (24-95)	44.5 (28-318)	41.5 (22-96)
ALT	Experimental	Mean ± SE	53.38* ± 2.62	37.25[a] ± 2.89	36.61 ± 3.22	35.00[a] ± 2.72
		Median (Min-Max)	52 (30-80)	35 (18-74)	34 (24-73)	32 (17-64)
	Control	Mean ± SE	43.42 ± 2.56	31.59[a] ± 1.69	37.91 ± 2.84	36.86 ± 2.14
		Median (Min-Max)	40.5 (30-88)	27.5 (23-52)	34 (21-71)	37 (19-59)

* statistically significant difference according to the control group ($P<0.01$), ** statistically significant difference according to the control group ($P<0.05$), [a] statistically significant difference according to the value at the 1st day of life within the group ($P<0.01$), [aa] statistically significant difference according to the value at the 1st day of life within the group ($P<0.05$)

weight) and above the reference range. In both groups, after an initial rise in the average concentrations of magnesium, there was a decrease until weaning, which was pronounced in the experimental group ($P<0.01$ during the third week).

Table 2. Changes in biocatalyst concentrations in two groups of Large White suckling piglets

Biocatalysts (mmol/L)	Group	Piglets' age (N = number of animals in experimental / control group)			
		Day 1 (N=24/24)	Day 7 (N=20/22)	Day 14 (N=18/22)	Day 21 (N=17/22)
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Ca	Experimental	3.31 ± 0.12	3.23[a] ± 0.13	3.08 ± 0.06	2.98[a] ± 0.06
	Control	3.19 ± 0.07	3.04[a] ± 0.04	2.97 ± 0.04	2.85[aa] ± 0.06
PO_4	Experimental	2.66 ± 0.15	3.45[a] ± 0.16	3.67 ± 0.13	3.33[a] ± 0.11
	Control	2.93 ± 0.13	3.74[a] ± 0.10	3.67 ± 0.06	3.40[aa] ± 0.09
Mg	Experimental	1.10 ± 0.04	1.27[a] ± 0.04	1.30 ± 0.06	1.04[b] ± 0.04
	Control	1.07 ± 0.05	1.35 ± 0.05	1.21 ± 0.03	1.12 ± 0.03

[a] statistically significant difference according to the value at the 1st day of life within the group ($P<0.01$), [aa] statistically significant difference according to the value at the 1st day of life within the group ($P<0.05$), [b] statistically significant difference according to the previous value within the group ($P<0.01$)

Discussion

A greater variability of total enzyme activity and concentrations of biocatalysts was observed in the serum of suckling piglets than those within the reference ranges for pigs (KANEKO et al., 1997). The raised mean values of individual enzymes (Table 1) with regard to the reference ranges are in line with the study by EGELI et al. (1998), which stated that enzyme activity had a high mean values and wide reference ranges on the 1st day of piglets life.

The activity of creatin kinase (Table 1) showed the greatest variability throughout the period of suckling and was significantly higher in comparison to reference values (KANEKO et al., 1997). This fact is consistent with the study of KRAMER and HOFFMANN (1997) which stated that CK catalyzed an enzymatic reaction, which released an additional amount of ATP from creatine-phosphate when it was necessary. As newborn piglets have extreme muscle activity (due to tremor and maintaining body heat, among other things), and ATP reserves are less than are needed, the additional amount of energy is provided through increased CK activity. EGELI et al. (1998) reported that activity of CK was in a very wide range in younger pig categories, and also mentioned muscle tissue damage during sample taking as one of the possible causes. They recorded even higher mean activity and standard deviation for CK than us. Raised activity of CK was also reported by FRIENDSHIP et al. (1984).

Mean values of alkaline phosphatase were much higher than reference values (KANEKO et al., 1997) during the whole preweaning period, and especially in the first week (Table 1). That is in line with the findings of other authors (FRIENDSHIP et al., 1984; EGELI et al., 1998). Bone tissue has the strongest growth impulse before birth, so the growth of AP activity in the blood plasma may be a sign of intensified osteoblastic activity and is related to bone construction (POND et al., 1997; EGELI et al., 1998; PETROVIČ et al., 2009). The extremely increased activity of AP immediately after birth is probably due to its significant role in metabolic processes in the organism that are very intensive after birth. As KRAMER and HOFFMANN (1997) stated, in young, growing animals osseus AP was the predominant form of serum AP, which decreased as maturation progressed and the epiphysis closed. In line with this statement, mean AP values determined in our research showed that osteoblastic activity in the control and experimental groups was the most intense after birth and decreased with ageing ($P<0.01$). The extremely increased activity in one-day-old piglets was in line with findings provided by EGELI et al. (1998). The mean AP activity determined by FRIENDSHIP et al. (1984) were similar to our findings and they also stated that it decreased with ageing. LONG et al. (1965) also found that activity of AP generally, but not always, decreased after birth, whereby it was negatively correlated with body weight, so lighter piglets also had greater activity of AP, as was found in our study. Similar changes and a decline in AP activity from eight hours to three weeks of age ($P<0.01$) were found by TUMBLESON and KALISH (1971).

Determined mean γ - glutamyltransferase values were within the reference values for pigs according to KANEKO et al. (1997), although higher enzyme activity was recorded in individual animals (Table 1). Similar values and dinamics of enzyme activity were determined by EGELI et al. (1998) in the blood sera of 1 and 21 days old piglets.

The research results of aspartat aminotransferase activity were within the reference range (KANEKO et al., 1997) throughout the study period and decreased up to weaning (Table 1). A similar decline was determined by EGELI et al. (1998), but their research reported values were in an even wider range. The range and average values found by FRIENDSHIP et al. (1984) were similar to our results. Also, TUMBELSON and KALISH (1971) noted similar trends in the activities of AST until the third week. According to TENNANT (1997), AST activity is very high in erythrocytes and when cells are damaged, its activity in the serum may also be expected to increase. In that way, higher AST activity in the sera of newborn animals may be partially caused by the increased degradation of fetal erythrocytes.

In both groups the mean activity of alanin aminotransferase was within the reference range according to KANEKO et al. (1997), although some animals had higher values (Table 1). The reason for the significantly higher ALT activity in the one-day piglets of the experimental group ($P<0.01$) could, among other things, be related to the fact that perinatal asphyxia occurs more frequently in light piglets (especially if the duration of farrowing was long or the interval before the birth of light piglets was greater). The occurrence of asphyxia due to lack of oxygen could lead to further complications, such as secondary hypoxic-ischemic encephalopathy, but may affect other organs and systems (lungs, heart, liver) as well. This could lead to additional aggravation of the regulation of body temperature, followed by consequent metabolic acidosis (due to an insufficient supply of oxygen to tissues) which could affect pulmonary hypertension and lead to a lower capacity for oxygenation. This further increases acidosis and ischemia. Due to ischemia, ischemic hepatitis occurs and liver damage is detected by the increased ALT activity (ALONSO-SPILSBURY et al., 2005). A similar decrease in ALT activity as in our research was determined by EGELI et al. (1998). In the study performed by FRIENDSHIP et al. (1984) mean ALT activity in the serum of piglets at weaning was something lower than ours and below reference values.

Concentrations of calcium and phosphorus in our research were somewhat increased compared to the reference values (KANEKO et al., 1997), while the concentration of magnesium was lower. A difference in Ca : PO_4 ratio was observed in both groups. In newborn animals a higher concentration of calcium was recorded and the Ca : PO_4 ratio was 1.09 to 1.24 : 1 (in the control and experimental group, respectively), while the concentration of phosphorus was to some extent higher in the later period, so the Ca : PO_4 ratio was 0.84 to 0.90 : 1. These findings are in agreement with KUPSKI et al. (1984), TUCHSCHERER et al. (2000) and WEHREND et al. (2003), who emphasized the fact that some losses could be explained by a deviation in electrolyte homeostasis and the higher calcium and phosphorus concentration determined in the plasma of animals that died during the first 10 days of life. A similar decline in Ca : PO_4 ratio was mentioned by TUMBLESON and KALISH (1971). Due to changes in biocatalysts concentrations, we found a slight decline in the Ca : Mg ratio. In the sera of one-day old piglets it was 2.98 to 3 : 1 (in the control and experimental group, respectively) while at the age of 21 days it amounted to 2.54 and 2.87 : 1. Similar Ca : PO_4 ratios as we determined at weaning were found by PRIKOSZOVITS and SCHUH (1995) in the sera of fattening pigs, while the Ca : Mg ratio was slightly lower in their research.

In the first two weeks, recorded calcium values in both groups were higher that the reference range for pigs (KANEKO et al., 1997) but, due to the continuous slight decline, average concentrations at the age of 21 days were within this range (Table 2). WEHREND et al. (2003) determined that the mean calcium concentration slightly increased during the first two weeks of the piglets' life. In accordance with them, similar changes in the sera of 1 and 21 day old animals and somewhat lower values than in our study were observed by EGELI et al. (1998). TUMBLESON and KALISH (1972), in contrast, stated that after a peak on the 7th day of the piglets' life following the decline in Ca concentration, while ULLREY et al. (1967) found slight fluctuations up to three months of age. All of them recorded slightly lower mean values compared to our results, but they were also at the upper value of the reference range.

Concentrations of serum phosphorus after birth were within the reference range (KANEKO et al., 1997), although in some animals, higher values were recorded (Table 2). Similar to our results, WEHREND et al. (2003) recorded the greatest variations in phosphorus concentrations during the first 24 hours of life, although average values in their research were somewhat higher. The changes determined in PO_4 concentration in our research were similar to those found by ULLREY et al. (1967), TUMBLESON and KALISH (1972), as well as EGELI et al. (1998). Something lower values were determined by FRIENDSHIP et al. (1984) for weaned piglets. EGELI et al. (1998) stated that some authors considered that the higher phosphorus levels in young animals could be explained by intensive bone growth.

Due to the recorded changes, concentrations of magnesium on the first day after birth and at the end of the suckling period were below the reference range (KANEKO et al., 1997) (Table 2). As ROSOL and CAPEN (1997) concluded, lower concentrations of magnesium could be related to lipolysis which occurs at times of stress, cold or starvation. WEHREND et al. (2003) reported that the greatest variability for individual animals was observed in the first 24 hours. Values recorded in their research were similar to ours during the first two weeks of life. In contrast, values recorded by EGELI et al. (1998) were somewhat lower than ours and even below the reference range during suckling.

Conclusion

From the results obtained we may conclude that the age of piglets, more than their body mass during the suckling period, had a significant impact on the majority of the observed serum indices. The extremely increased activity of CK in the piglets could be the result of an increased need for ATP in younger animals. Also, in the piglets from both groups, after 21 days of life, there was still intensive AP activity reflecting very intensive metabolic processes and bone growth. Due to the intensive growth of the animals before weaning, concentrations of calcium and phosphorus were raised, while magnesium concentration was somewhat lower. The obtained results may represent a contribution to a better understanding of physiological status as well as metabolic changes during the development of newborn animals.

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SAŽETAK

Odojci tjelesne mase pri porođaju manje od 1000 grama osjetljiviji su na bolesti i negativne utjecaje stresnih čimbenika, sporije napreduju te su uzrokom većih gubitaka u usporedbi s težim jedinkama. Nadalje, fiziološki su manje zreli te sporije uspostavljaju postnatalne metaboličke mehanizme u usporedbi s odojcima normalne porođajne mase. Cilj ovoga istraživanja bio je utvrditi kretanje aktivnosti određenih enzima, kao i koncentracije biokatalizatora u krvnom serumu odojaka do odbića. Istraživanjem je obuhvaćeno 48 odojaka: 24 životinja tjelesne mase pri rođenju manje od 1000 g (pokusna skupina) i 24 istospolnih potomaka tjelesne mase veće od 1000 grama (kontrolna skupina). Uzorci krvi prikupljeni su 1., 7., 14. i 21. dana života iz v. cave cranialis (1,5 mL) u BD Vacutainer SST epruvete s gelom za biokemijsku analizu. Nakon centrifugiranja, uzorci seruma analizirani su pomoću automatskog analizatora Olympus AU 600. U serumu obje skupine odojaka u dobi od tri tjedna utvrđene su povišene aktivnosti kreatin-kinaze i alkalne fosfataze te koncentracije kalcija i fosfora, dok je koncentracija magnezija bila ispod referentnih vrijednosti za svinje. Također, utvrđena je značajno manja aktivnost kreatin-kinaze u jednodnevnih odojaka male porođajne mase ($P<0,05$) u usporedbi s kontrolnom skupinom, a vjerojatno povezana sa slabije razvijenim mišićnim tkivom. Nasuprot tome, aktivnost alanin-aminotransferaze je bila značajno veća ($P<0,01$) u serumu eksperimentalnih životinja prvog dana života. Pretpostavili smo da utvrđene promjene u aktivnosti enzima i koncentraciji biokatalizatora upućuju na intenzivan rast i razvoj novorođenih životinja, osobito u kontrolnoj skupini odojaka.

Ključne riječi: veliki jorkšir, sisajuća prasad, porođajna masa, enzimi, elektroliti