

## **BLOOD PARAMETERS, CARCASS AND MEAT QUALITY OF SLAUGHTER PIGS WITH AND WITHOUT LIVER MILK SPOTS**

**Nikola Čobanović<sup>1</sup>, Dragan Vasilev<sup>1</sup>, Mirjana Dimitrijević<sup>1</sup>, Vlado Teodorović<sup>1</sup>, Ljiljana Janković<sup>2</sup>, Nedjeljko Karabasil<sup>1</sup>**

<sup>1</sup>Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000 Belgrade, Serbia

<sup>2</sup>Department of Animal Hygiene, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000 Belgrade, Serbia

Corresponding author: Nikola Čobanović, e-mail address: [cobanovic.nikola@gmail.com](mailto:cobanovic.nikola@gmail.com)

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**Abstract:** The aim of this study was to determine the influence of liver milk spots on hematological, carcass and meat quality parameters in slaughter pigs. A total of 120 pigs with a live weight of approximately 115 kg and six months old were examined. Any signs of liver milk spots were recorded as present or absent according to *Welfare Quality® protocol (2009)*. A complete blood picture was investigated. The following carcass quality parameters were measured: live, hot and cold carcass weights, dressing percentage, backfat thickness and meatiness. pH and temperature measurements were performed 45 minutes postmortem. Pork quality classes (PSE – pale, soft and exudative, normal, DFD – dark, firm and dry meat) were determined according to *Adzitey and Nurul (2011)* using pH<sub>45</sub> value. Pigs with liver milk spots had significantly higher middle-sized cell count (monocytes, eosinophils, and basophils) and neutrophils count, but significantly lower red blood cell count, hemoglobin concentration, hematocrit and MCV than unaffected pigs. The same group of pigs had significantly lower live weight, hot carcass weight, cold carcass weight, dressing percentage and meatiness compared to the pigs free of milk spot lesions. Pigs showing liver milk spots had significantly higher pH<sub>45</sub> value and incidence of DFD meat than pigs without pathological lesions in the livers. In conclusion, assessment of liver milk spots at slaughter line has potential to serve not only as an indirect measure of pig health and welfare, but also for the carcass and pork quality.

**Key words:** DFD meat, liver milk spots, slaughter pigs, slaughterhouse

## Introduction

Although a number of studies have previously reported that ascariasis results in significant economic losses to the pig industry, the occurrence of this helminth infection both under conditions of intensive and extensive pig breeding remains high (Vlaminck et al., 2015). This can be ascribed to the facts that, in most cases, ascariasis occur in a subclinical form and that exposure of animals to this parasite cannot be unambiguously diagnosed (Vlaminck et al., 2015). Ascariasis can be identified in slaughtered pigs through the presence of milk spots – whitish healing foci which result from *Ascaris suum* larval migration through the liver stroma (Sanchez-Vazquez et al., 2012). Liver milk spots, along with pneumonia and pleurisy, are the most frequent pathological lesions in pig organs observed at the slaughter line (Sanchez-Vazquez et al., 2011, 2012; Čobanović et al., 2015, 2016a). It has been reported that housing conditions (e.g. outdoor/indoor farming, floor type, type of bedding, high stocking density), management practices (e.g. cleaning and disinfection procedures, type of feeding) and seasonal variations (e.g. temperature and relative humidity) play an important role in the development of *Ascaris suum* and in the subsequent presence of milk spots in the liver (Sanchez-Vazquez et al., 2010). The presence of *Ascaris suum* infection and liver milk spots can lead to the following negative effects: (1) farm economic losses attributed to decreased daily weight gain, anthelmintic treatment costs, depressed growth rates and feed conversion efficiency (Sanchez-Vazquez et al., 2010), (2) changes in hematological values (Makinde et al., 1996; Zanga et al., 2003; Wieczorek et al., 2006), (3) slaughterhouse operator losses due to trimmings and disposal of organs unsuitable for human consumption (Pysz-Lukasik and Prost, 1999), (4) lower carcass and pork quality (Theodoropoulos et al., 2004; Knecht et al., 2011, 2012). A number of studies revealed that pork quality is affected by many different factors, such as feeding, slaughter weight and gender, pre-slaughter and slaughter conditions (Šefer et al., 2015; Rocha et al., 2016; Đorđević et al., 2016; Čobanović et al., 2016b, 2016c). However, only one published article is available in the literature about the relationship between liver milk spots and pork quality (Theodoropoulos et al., 2004). Therefore, the aim of this study was to determine the impact of liver milk spots on hematological, carcass and meat quality parameters in slaughter pigs.

## Material and Methods

A total of 120 slaughter pigs with an average live weight of approximately 115 kg and about six months old were examined. All the animals were of the same breed (Yorkshire x Landrace crossbreeds) and fattened on the same commercial

farm under identical conditions. They were all exposed to the same condition of pre-slaughter treatments and were killed at the same slaughterhouse

The livers of slaughtered pigs were removed from the slaughter line and visually appraised and palpated for milk spots according to the *Welfare Quality® protocol (2009)*. The complete assessment of liver milk spots scores was performed by a single trained investigator. The percentage of affected livers was calculated as the percentage of pig livers on which was detected the presence of at least one milk spot lesion.

Immediately after the onset of bleeding, blood samples were collected from each pig. They were kept refrigerated ( $4\pm 1^\circ\text{C}$ ) until processed immediately on arrival at the laboratory. The vacutainers (2 mL) coated with EDTA were used to measure hematological parameters including white blood cells, lymphocytes, middle-sized cells (monocytes, eosinophils and basophils), neutrophils, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet count. The proportions of lymphocytes, middle-sized cells and neutrophils were calculated as a percentage of leukocyte concentration on the same device. The indicators of the hematological profile were analyzed by an automatic hematological analyzer Abacus junior vet (Diatron MI PLC, Hungary).

The carcasses were weighed immediately after splitting and final washing to obtain the hot carcass weight and re-weighed 24 hours after chilling at  $4^\circ\text{C}$  to determine the weight of the cooled carcass. The dressing percentage was calculated as:  $(\text{hot carcass weight} \div \text{live weight}) \times 100$ . Carcass backfat thickness was measured with a metal ruler (accuracy of 1.0 mm) at two points (between the 13th and 15th dorsal vertebrae - fat carcass thickness on the back; and over *M. gluteus medius* - fat carcass thickness at the sacrum). Meatiness (%) was calculated according to *Official Gazette (1985)* based on hot carcass weight and the sum of carcass fat thickness on the back and at the sacrum. The pH and temperature of the *M. longissimus dorsi* were measured 45 minutes after slaughter on the left half of the carcass at the level of the 10th and 11th ribs using a pH-meter "Testo 205" (Testo AG, Lenzkirch, Germany). Pork quality classes (PSE meat, normal meat, DFD meat) were determined according to *Adzitey and Nurul (2011)* using  $\text{pH}_{45}$  value. The carcasses showing  $\text{pH}_{45}$  values lower than 6.0 were classified as PSE meat, while the carcasses showing  $\text{pH}_{45}$  values higher than 6.4 were classified as DFD meat. The carcasses with  $\text{pH}_{45}$  between 6.0 and 6.4 were classified as normal pork quality.

Statistical analysis of the results was conducted using software SPSS version 23.00 for Windows (*SPSS, 2015*). According to the presence of liver milk spots, the pigs were allocated to two groups: 1) the group of pigs with liver milk spots ( $n=69$ ) and 2) the group of pigs without liver milk spots ( $n=51$ ). Student t-test was used to examine the effect of liver milk spots on the hematological, carcass and meat quality parameters. Data were described by descriptive statistical parameters as the

mean value and standard error of the mean. The distribution of pork quality classes in relation to the liver milk spots was determined by Fisher's exact test. A value of  $P < 0.05$  was considered significant.

## Results and Discussion

From a total of 120 examined pig livers, milk spots were detected in 57.50%. The prevalence of livers affected by milk spots was higher to that found in comparable studies (Dalmau et al., 2009; Rocha et al., 2016; Dalmau et al., 2016). In addition, the results of our investigation found that the percentage of milk spots in pig livers exceeded the alarm threshold of 23% set for this health criterion by the Welfare Quality® protocol (2009). Pathological lesions detected at the slaughter line, such as liver milk spots, are often related to suboptimal production systems (Harley et al., 2012) and indicate a serious health and welfare problem on the farm of origin (Welfare Quality® protocol, 2009). According to Pyz-Lukasik and Prost (1999), in mild cases (from 1 to 7 lesions) milk spots may be removed from the liver and the remaining organ and meat may be approved for human consumption. On the other hand, in the case of more than 8 milk spots occurring, the livers should be condemned (Pyz-Lukasik and Prost, 1999). However, other researchers (Cugmas et al., 2013; Fausto et al., 2015) suggest that the livers with milk spots, regardless of their number, should be deemed unfit for human consumption. Hence, meat industry suffers indirect economic losses through increased trimmings and disposal of livers, a byproduct that adds value to the supply chain (Harley et al., 2012; Fausto et al., 2015). Even though the losses due to milk spots are very variable because of different slaughterhouse costs and the fluctuating market prices of livers, the damage can be assessed at €0.26 per kg to destroy the livers and €0.87 per liver lost (Kanora, 2009). Moreover, the financial loss to the US pig industry due to increased fees to gain ratio was estimated to US\$ 155 million annually (Stewart and Hale, 1988). In addition, Stewart (2001) estimated the financial loss of US\$ 17.5 million as a result of liver condemnation at slaughter line as well as an additional economic loss of US\$ 60.1 million for extra feed to finish pigs for slaughter.

The effects of liver milk spots on hematological parameters in slaughter pigs are shown in Table 1. Pigs with liver milk spots showed increased values of middle-sized cells (monocytes, eosinophils and basophils), neutrophils and the percentage of middle-sized cells compared to the pigs without pathological lesions in the livers ( $P < 0.05$ ). These results correspond to the findings of Wieczorek et al. (2006), who observed leukocytosis and eosinophilia in fattening pigs caused by the presence of *Ascaris suum* infection. Moreover, Kalai et al. (2012) point out that parasitic infections lead to necrosis and subsequently to neutrophilia and eosinophilia. In addition, pigs with liver milk spots had a significantly lower number of red blood

cells, hemoglobin concentration, hematocrit and MCV ( $P<0.05$ , Table 1). Similar results were reported by *Makinde et al. (1996)*, who found that liver milk spots, alone or in combination with pneumonia, induced changes in hematological values, such as reduced red blood cell count, hemoglobin concentration, hematocrit and MCV. Furthermore, *Zanga et al. (2003)* reported that the presence of *Ascaris suum* infection in fattening pigs decreased the red blood cell count, hemoglobin concentrations, hematocrit, MCV, MCH and MCHC. Therefore, hematological alterations in slaughtered pigs associated with liver milk spots are presumably connected with a recent uptake of infective *Ascaris suum* eggs.

**Table 1. Mean values ( $\pm$ standard error of the mean) of hematological parameters according to liver milk spots (n=120).**

Parameter	Milk spots	No milk spots	Significance
Number of pigs	69	51	-
White blood cells ( $10^9/L$ )	21.94 $\pm$ 0.74	20.4 $\pm$ 0.71	ns
Lymphocytes ( $10^9/L$ )	13.92 $\pm$ 0.49	14.16 $\pm$ 0.69	ns
Middle-sized cells ( $10^9/L$ )	0.27 $\pm$ 0.04	0.12 $\pm$ 0.01	*
Neutrophils ( $10^9/L$ )	7.77 $\pm$ 0.58	6.10 $\pm$ 0.38	*
Lymphocytes (%)	64.90 $\pm$ 1.75	67.95 $\pm$ 2.03	ns
Middle-sized cells (%)	1.31 $\pm$ 0.22	0.59 $\pm$ 0.01	*
Neutrophils (%)	33.80 $\pm$ 1.71	30.09 $\pm$ 1.65	ns
Red blood cells ( $10^{12}/L$ )	7.22 $\pm$ 0.14	7.95 $\pm$ 0.13	*
Hemoglobin (g/L)	132.80 $\pm$ 2.07	146.40 $\pm$ 2.12	*
Hematocrit (%)	39.37 $\pm$ 0.43	40.76 $\pm$ 0.57	*
MCV (fl)	49.28 $\pm$ 0.38	51.10 $\pm$ 0.49	*
MCH (pg)	17.96 $\pm$ 0.14	18.04 $\pm$ 0.12	ns
MCHC (g/L)	359.60 $\pm$ 1.10	360.50 $\pm$ 1.33	ns
Platelet count ( $10^9/L$ )	244.00 $\pm$ 15.05	248.40 $\pm$ 15.81	ns

**Legend:** MCV – Mean corpuscular volume; MCH – Mean corpuscular hemoglobin, MCHC – Mean corpuscular hemoglobin concentration.

\* –  $P<0.05$ ; ns – no significance ( $P>0.05$ ).

The effects of liver milk spots on carcass and meat quality parameters and pork quality classes are depicted in Table 2. Pigs showing liver milk spots had significantly lower slaughter, hot and cold carcass weights, and dressing percentage compared to the pigs free of milk spot lesions ( $P<0.05$ ), which is consistent with the findings of *Hale et al. (1985)* and *Theodoropoulos et al. (2004)*. It has been reported that the occurrence of a subclinical form of *Ascaris suum* infection in fattening pigs leads to a decrease in daily weight gain of about 80 g and increase in feed consumption of 230 g on 1 kg of body weight gain (*Knecht et al., 2012*), resulting in later date of slaughter weight attainment of 10–15 days (*Knecht et al., 2011*). Considering that milk spots can appear as early as 3 days post-infection, start to resolve after about 2–3 weeks (*Vlaminck et al., 2015*), and disappear in the course of 3–6 weeks post-infection (*Boes et al., 2010*), it can be assumed that the

reduction in slaughter weight occurred during the last few weeks of fattening. In addition, pigs showing liver milk spots had a significantly higher fat thickness, but had a lower meatiness than pigs without pathological lesions in the livers ( $P < 0.05$ , Table 2). *Knecht et al. (2011)* examined the influence of gastrointestinal parasites, including *Ascaris suum*, on the percentage of meat, and demonstrated that pigs free from parasites had a significantly higher meatiness than infected animals (53.68% vs. 52.12%). Moreover, the same researchers reported that meat obtained from fattening pigs with gastrointestinal parasites, such as *Ascaris suum*, was of a lower class compared to the pigs free from parasites. Parasitic infection, even in subclinical form (i.e. no apparent clinical signs), decreases feed intake and assimilation (*Jankowska-Mąkosza and Knecht, 2015*), and negatively affects digestion and the intestinal absorption of nutrients (*Hale et al., 1985; Kanora, 2009*). Hence, when animals are infected with parasites, they ingest fewer nutrients than what is necessary for the maximum expression of their genetic potential for protein deposition (*Kipper et al., 2011*). In addition, instead of utilizing nutrients to increase in body mass, parasitic infection leads to a reduction in the muscle and fat tissue synthesis and increases their degradation rate (*Kipper et al., 2011*). This results in a repartition of nutrients from the productive processes, like muscle deposition and bone formation, for the processes that have a greater need - the plasmatic protein synthesis, gastrointestinal repair and mucus replacement, which induces a reduction in body weight and significantly downgrades carcass quality (*Kipper et al., 2011; Knecht et al., 2011, 2012*).

**Table 2. Mean values ( $\pm$ standard error of the mean) of carcass and meat quality parameters according to liver milk spots (n=120).**

Parameter	Milk spots	No milk spots	Significance
Number of pigs	69	51	-
<i>Carcass quality</i>			
Slaughter weight (kg)	113.50 $\pm$ 0.92	115.90 $\pm$ 0.45	*
Hot carcass weight (kg)	92.55 $\pm$ 0.78	95.23 $\pm$ 0.47	*
Cold carcass weight (kg)	89.48 $\pm$ 0.77	92.95 $\pm$ 0.46	*
Dressing percentage (%)	81.55 $\pm$ 0.18	82.15 $\pm$ 0.24	*
FTB (mm)	22.03 $\pm$ 1.02	13.90 $\pm$ 0.56	*
FTS (mm)	49.70 $\pm$ 2.53	21.84 $\pm$ 1.05	*
Meatiness (%)	36.58 $\pm$ 0.69	44.10 $\pm$ 0.30	*
<i>Meat quality parameters</i>			
pH <sub>45</sub>	6.32 $\pm$ 0.02	6.19 $\pm$ 0.02	*
T <sub>45</sub> (°C)	39.48 $\pm$ 0.12	39.49 $\pm$ 0.13	ns
<i>Pork quality classes (%)</i>			
PSE	18.84	15.69	ns
Normal	55.07	76.47	*
DFD	26.09	7.84	*

**Legend:** FTB – fat carcass thickness on the back; FTS – fat carcass thickness at the sacrum; pH<sub>45</sub> – meat pH values measured 45 minutes postmortem; T<sub>45</sub> – Meat temperature measured 45 minutes postmortem. **DFD meat** – pH<sub>45</sub> > 6.4; **Normal meat** – pH<sub>45</sub> between 6.0 and 6.4; **PSE meat** – pH<sub>45</sub> < 6.

\* –  $P < 0.05$ ; ns – no significance ( $P > 0.05$ ).

Pigs with liver milk spots had significantly higher pH<sub>45</sub> value and incidence of DFD meat than unaffected pigs ( $P < 0.05$ ). This can be attributed to the fact that pigs during period of sickness need a higher amount of energy which leads to a reduction in glycogen and adenosine-triphosphate reservoirs in muscles after slaughter, resulting in a lower production of lactic acid and higher pH value of meat which increasing tendency towards DFD meat (*Dailidavičienė et al., 2008*). Furthermore, pigs without liver milk spots had a significantly higher percentage of normal meat quality ( $P < 0.05$ ), while there were no differences between two groups of pigs for the incidence of PSE meat ( $P > 0.05$ ) (Table 2). The impact of *Ascaris suum* infection and liver milk spots on pork quality has not been well studied. Only one published article is available in the literature about the association between *Ascaris suum* infection and meat quality parameters in pigs (*Theodoropoulos et al., 2004*). However, *Theodoropoulos et al. (2004)* did not examine the effect of *Ascaris suum* infection on the incidence of PSE and DFD meat. Based on *Theodoropoulos et al. (2004)* results, the meat obtained from slaughtered pigs with liver milk spots had increased moisture values and was more red and yellow than from pigs free of milk spot lesions. Therefore, it may be argued that pigs with liver milk spots produce lower meat quality.

## Conclusion

The study showed a high prevalence of liver milk spots in slaughtered pigs, indicating a serious health and welfare problem on the farm of origin. Liver milk spots caused significant changes in several hematological parameters, including middle-sized cells (monocytes, eosinophils and basophils), neutrophils, red blood cell count, hemoglobin concentration, hematocrit and MCV. Furthermore, the presence of milk spots in pig livers significantly downgraded carcass quality, so that slaughter weight, hot and cold carcass weights, dressing percentage as well as the percentage of meat became significantly reduced. In addition, the occurrence of milk spots in pig livers caused a significant deterioration in meat quality. It can, therefore, be concluded that scoring of liver milk spots at the slaughter line has potential to serve not only as an indirect measure of pig health and welfare, but also for the carcass and pork quality.

## Hematološki parametri, kvalitet trupa i mesa zaklanih svinja sa i bez mlečnih pega na jetri

*Nikola Čobanović, Dragan Vasilev, Mirjana Dimitrijević, Vlado Teodorović, Ljiljana Janković, Nedjeljko Karabasil*

## Rezime

Cilj istraživanja bio je da se utvrdi uticaj prisustva mlečnih pega na hematološke parametre, kvalitet trupa i mesa svinja. Ispitivanja su obavljena na 120 svinja, starosti šest meseci, žive mase oko 115 kg. Prisustvo mlečnih pega na jetri svinja je ocenjivano na liniji klanja na osnovu *Welfare Quality*® (2009) protokola. Pored toga, analizirana je i kompletna krvna slika. U cilju utvrđivanja kvaliteta trupa, analizirani su sledeći parametri: živa masa, masa toplog i hladnog trupa, randman, debljina leđne slanine i mesnatost. Od parametara kvaliteta mesa određivani su pH vrednost i temperatura 45 minuta *post-mortem*. Meso svinja je razvrstavano u klase kvaliteta (bledo meko vodenasto-BMV; meso normalnog kvaliteta; tamno, čvrsto i suvo meso-TČS) na osnovu pH vrednosti merene 45 minuta *post-mortem* (Adzitey i Nurul, 2011). Analizom hematoloških parametara utvrđeno je da su svinje sa mlečnim pegama na jetri imale statistički značajno veće vrednosti monocita, eozinofila, bazofila i neutrofila, dok su vrednosti za broj eritrocita, koncentraciju hemoglobina, hematokrit i MCV bile statistički značajno niže u odnosu na svinje bez promena na jetri. Svinje sa mlečnim pegama na jetri imale su statistički značajno nižu živu masu, masu toplog i hladnog trupa, randman i mesnatost u odnosu na svinje bez mlečnih pega na jetri. Ista grupa svinja je imala statistički značajno višu pH vrednost kao i učestalost pojave TČS mesa. Stoga se može zaključiti da ispitivanje prisustva mlečnih pega na jetri na liniji klanja može da bude značajan pokazatelj ne samo dobrobiti svinja na farmi, već i kvaliteta trupa i mesa svinja.

**Ključne reči:** TČS meso, mlečne pege, svinje, klanica

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