

## Quality of Beef Diaphragm Meat in Naturally Occurring *Sarcocystis* Infection in Cattle

VYTAUTAS JANUSKEVICIUS<sup>2\*</sup>, GRAZINA JANUSKEVICIENE<sup>1</sup> and GINTARE ZABORSKIENE<sup>1,3</sup>

<sup>1</sup>Department of Food Safety and Quality; <sup>2</sup>Department of Veterinary Pathobiology, Lithuanian University of Health Sciences, Veterinary Academy, Kaunas, Lithuania;

<sup>3</sup>Food Institute, Kaunas University of Technology, Kaunas, Lithuania

\*Corresponding author: [vytautas.januskevicius@lsmuni.lt](mailto:vytautas.januskevicius@lsmuni.lt)

### Abstract

Januskevicius V., Januskeviciene G., Zaborskiene G. (2018): Quality of beef diaphragm meat in naturally occurring *Sarcocystis* infection in cattle. Czech J. Food Sci., 36: 378–385.

The aim of this study was to investigate the possible harmful effect of *Sarcocystis* parasites on bovine diaphragm meat quality. Meat samples were collected from 120 bulls aged 20–24 months. Meat quality was investigated using microbiological and physico-chemical (RP-HPLC, GC) methods 48 hours after slaughter. *Sarcocystis* infection was associated with increased fat content, lightness  $L^*$  and drip loss, and decreased ash and protein percentages. Infection also had a significant effect on the amount of amino acids (AAs), which slowly decreased as the number of sarcocysts increased. The total amount of AAs correlated with glutamic acid content ( $R = 0.966$ ,  $P < 0.05$ ). Heavily infected samples contained significantly lower amounts of putrescine, histamine, spermine and spermidine ( $P < 0.05$ ) and a noticeable increase in the total count of aerobic microorganisms, but no change in the numbers of *E. coli* and coliform bacteria in comparison with no infected samples. Sarcocysts in beef diaphragms did not cause serious changes in the technological quality of the meat, but the biological quality of infected meat was reduced.

**Keywords:** amino acids; bacterial count; beef; Sarcocysts

Consumer preference for beef diaphragm dishes is not universal and varies between countries and cultures. The hanger steak from beef diaphragm has historically been more popular in Europe (GREEN 2005). In Lithuania, beef diaphragm meat is mostly used for minced meat and meat preparations, but this cut suffers from parasitic infections, which can cause disease in humans. Infection by *Sarcocystis* spp. can cause symptoms that are generally heavier for intermediate hosts. Severe infection may cause economic losses due to reduced animal meat quality and quantity. It can be assumed that *Sarcocystis* spp., including the zoonotic species, are circulating in most European food animal populations, though seemingly without major impact on public health (TAYLOR *et al.* 2010).

Modern farming systems provide favourable conditions for the spread of the disease. However,

PRICKETT *et al.* (1992) indicated that the body fat composition of 2-month-old pigs, as measured by the specific gravity of the carcass, was not affected by sarcocystis infection. A slight depression in HDL cholesterol occurred during the acute phase of infection. This study suggested that the disruption of lipid metabolism is not the primary cause of growth retardation in growing swine.

At present, six sarcosporidian (*Sarcocystis* genus) species (*S. bovifelis*, *S. bovini*, *S. cruzi*, *S. hirsuta*, *S. heydorni*, and *S. hominis*) are known to form sarcocysts in cattle muscle (GJERDE 2016; HU *et al.* 2016). Dogs are the definitive hosts of *Sarcocystis cruzi*, and *S. hirsuta* and *S. bovifelis* are found in predators in the cat family (MOULE 1988; GJERDE 2016). *S. cruzi*, *S. hirsuta* and *S. hominis* have a two-host life cycle in which canids, felids and humans are their final hosts and cattle act as the intermediate host (GHISLENI

<https://doi.org/10.17221/89/2017-CJFS>

*et al.* 2006; BUCCA *et al.* 2011; MORÉ *et al.* 2011; ORYAN *et al.* 2011; HAJIMOHAMMADI *et al.* 2014a, b).

*Eosinophilic myositis* is a severe form of the disease with considerable clinical signs, and can occur in severely infected cattle (WOUDA *et al.* 2006; BUCCA *et al.* 2011). Sarcocyst infection is usually most intense in beef diaphragm. Other studies have shown a high prevalence of *Sarcocystis* in tissue samples including tongue, diaphragm, heart, oesophagus and intercostal muscle (FUKUYO *et al.* 2002; FAYER 2004; JEHLE *et al.* 2009; NOUROLLAHI *et al.* 2009; NOURANI *et al.* 2010; ORYAN *et al.* 2010; HAJIMOHAMMADI *et al.* 2014b).

Sarcocysts in buffalo meat cause serious changes that may greatly lower meat quality (MOSTAFA *et al.* 2010). However, DAUGSCHIES *et al.* (1988) found that the water-holding capacity was lower in infected pigs than in non-infected pigs, but that the water-absorbing capacity was significantly higher in the infected group, with a slight tendency towards better carcass quality and a better water-binding capacity in infected pigs. DAUGSCHIES *et al.* (2000) concluded that sarcocystosis of even moderate severity alters the performance of calves but not the meat quality.

The present work aimed to compare the microbiological and chemical indices of infected and uninfected beef diaphragm meat.

## MATERIAL AND METHODS

**Meat samples.** Meat samples were collected and examined in 2015–2016. Sarcocyst infection was evaluated in Lithuanian Black and White × Charolais crossbred bulls aged 20–24 months, grown in the same conditions and slaughtered in the same Lithuanian slaughterhouse. Meat samples from 120 animals were used for analysis. Cattle carcasses of O1 quality were selected (classification refers to the conformation and fat cover of carcasses of beef referred to in Article 3(1) of the Commission Delegated Regulation (EU) 2017/1182 of 20 April 2017) and divided into four groups ( $n = 30$  in each group) according to sarcocyst infection intensity. Sarcocysts were diagnosed by means of the compressor-microscopic method. According to MALAKAUSKAS *et al.* (2001), the samples were divided into four groups based on the number of sarcocysts in 28 oat-sized muscle sections, i.e. 0 – no infection; 1–10 – low infection; 11–40 – moderate infection; > 40 – intense infection.

**Meat quality investigation.** Meat quality was assessed 48 h after slaughter. The amount of dry matter was measured by drying the samples at 105°C

until steady weight. The amount of dry matter was measured using the automatic scale for humidity assessment SMO – 01 (Scaltec Instruments GmbH, Germany). Protein content in samples was determined according to the Kjeldahl method (AOAC 2000) using a FOSS Tecator Kjelttec system consisting of a 1001 digestion unit and 6002 distilling unit (Foss Analytical AB, Sweden). Protein content was calculated by converting the nitrogen content (N%) obtained by digestion ( $6.25 \times N\%$ ). To measure intramuscular and total fat content, samples were dried in the oven at 100°C for 4 h, and then fat was extracted using the method of Soxhlet with *n*-Hexane (AOAC 2000). The amount of ashes was determined by burning organic matter at 700°C in a muffle oven. Cooking loss was calculated by boiling vacuum-packed meat at 70°C in a circulating bath for 30 min and calculating the weight change after cooking. Meat tenderness was determined using the Warner-Bratzler method and an analyser equipped with a Warner Bratzler shear cell (INSTRON 5542, Canada). The cooked samples were kept overnight at 4°C and a 10-mm (diameter) sample of each of them was sheared with blades at a right angle to the fibres using a 50-kg load cell and crosshead speed of 200 mm/minute. The shear force values are reported as kilograms of shear per cm<sup>2</sup> of sample. To determine the coherence of meat water we used the press method (Grau and Hamm) and measured the damp areas formed on the filter paper. Meat lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) were measured by the CIE–LAB method using a Minolta Chromameter (CR-410; Konica Minolta, Japan). The pH was measured 24 and 48 h after slaughter ( $pH_{24}$  and  $pH_{48}$ ) at 1 cm depth with a portable pH/mV/- meter (model IQ150; Dual Technology, UK) and a stainless steel pH probe according to ISO 2917:1999. The pH meter was standardised by a 2-point method against standard buffers of pH 4 and 7.

**Amino acid content.** Was determined using an AccQ-Fluor™ Reagent Kit (Waters, USA), Waters AccQ Tag Fluor Reagent Diluent, AccQTag™ eluent A concentrate (Waters, USA) and amino acid standard solutions (Sigma-Aldrich, USA). The concentration of amino acids in the standard solutions was 2.5 µmoles per ml except for cysteine, which was 1.25 µmoles per ml. L-2-aminobutyric acid was purchased from FlukaChemie (Germany) acetonitrile was from Merck (Germany), HPLC grade Ultrapure water was supplied by the Purelab Ultra purification system (Elga, UK), hydrochloric acid was from Riedel de Haen (Germany), 0.22 mm syringe

filters were from Restek (USA), and sample tubes were from Waters (USA).

**Sample preparation.** Hydrolysis of samples was performed as described in Commission regulation No. 152/2009 (EC).

**HPLC system.** A Shimadzu (Shimadzu, Japan) low pressure gradient HPLC system consisting of solvent delivery module LC-10AT<sub>VP</sub>, auto injector SIL-10AD<sub>VP</sub>, column oven CTO-10AC<sub>VP</sub>, spectrofluorometric detector RF-10A<sub>XL</sub>, system controller SCL-10A<sub>VP</sub>, and on-line degasser DGU-14A was used for amino acid analyses in samples. Workstation LC Solution (Shimadzu, Japan) was used for HPLC system control and data collection. Amino acid derivatives were separated on a Nova-Pak C18, 4 mm, 150 × 3.9 mm (Waters, USA) chromatography column at 37°C.

**Microbiological analysis.** Samples of 10 g were taken at random and aseptically weighed into a sterile stomacher bag with 90 ml of sterile buffered peptone water 0.1% (w/v) (REF 611014, Italy) and homogenized for 1 min in a model 400 Stomacher (Seward Medical, UK). Serial decimal dilutions were made and the total count of aerobic microorganisms (PCA medium, 30°C, 72 h) was determined. The coliform count was performed by plating on Plate Count Agar (REF 610040, Italy) at 30°C for 72 h; *Escherichia coli* were enumerated by plating on Tryptone Bile X-Glucuronide Medium Agar (REF 4021562, Italy) at 37°C for 24 hours.

**Determination of biogenic amines.** A reversed-phase high-performance liquid chromatography method was used for the quantitative analysis of the biogenic amines putrescine, cadaverine, histamine, tyramine, spermidine, and spermine. Biogenic amines were extracted from a homogenized sample with 0.4 mol/l perchloric acid. The derivatization of samples was carried out using the modified methodology of BEN-GIGIREY *et al.* (2000). The extract was derivatized for 45 min by dansyl chloride (5-dimethylaminonaphthalene-1-sulfonylchloride) solution in acetone at 40°C. The samples were filtered through a 0.45 µm membrane filter (Millipore, JAV) and 10 µl was injected into the chromatographic system (Aligent 1200 Series, Germany). Analysis was performed using a LiChro column CART® 95 125-4. The carrier phase comprised the following eluents: B – acetonitrile, A – ammonium acetate 0.1 mol/l. Analysis lasted 28 minutes. The eluents were changed during the first 19 min from 50% B to 90% B (from 50% A to 10% A, respectively), and then left constant for 1 min at 90% B. Later, to ensure isolation of ma-

terials for another analysis eluent comprising 50% B and 50% A was added to the chamber for 8 minutes. The flow rate was 0.9 ml/min; this did not change throughout the analysis; the column temperature was 40°C. UV detection was carried out at 254 nm. Biogenic amines were identified by comparing the retention time of each amine in the chamber with the retention time of the respective reference material. An internal standard method of calculating the peak area for the defined amount of reference material was used to perform the quantitative analysis. The limit of detection is between 0.02 and 0.1 µg/ml for different biogenic amines.

**Fatty acids (FA) content.** The FA content was determined by gas chromatography using a flame ionization detector. The samples were prepared according to the standard LST EN ISO 12966-2:2011. Chromatographic analysis of FA methyl esters was performed using a Shimadzu GC – 17A gas chromatograph equipped with a BPX – 70, 120 m column following the methodology established in LST EN ISO 15304:2003/AC: 20052. Analytical conditions were as follows: column temperature 60°C for 2 min, increased by 20°C/min to 230°C, and maintaining that temperature for 45 min; evaporator temperature 250°C, flame ionization detector temperature 270°C, gas-carrier: nitrogen.

**Statistical analysis.** SPSS 20.0 software (SPSS, USA) was used to calculate the average values of indicators, standard deviations and linear correlations. A correlation was considered reliable when  $P < 0.05$ . The differences between the data were evaluated by analysis of variance (one-way ANOVA) with a significance level of  $P \leq 0.05$  (DRAPER & SMITH 1998). Multiple comparisons were estimated by Fisher's Least Significant Difference method, and the Dunnett test was applied when a control group was present.

## RESULTS AND DISCUSSION

The most noticeable effects of sarcocyst infection were on the dry matter, fat, ash, protein content, lightness  $L^*$ , drip loss, water holding capacity and tenderness of the diaphragm meat (Table 1). The data are consistent with the results of ELSASSER *et al.* (2011) who found that fibres rich in mitochondria that possess more inherent oxidative energy capacity generate more nitrated proteins than glycolytic fibres and as such are more affected by the proinflammatory response to infections like

<https://doi.org/10.17221/89/2017-CJFS>

sarcocystosis. In the case of non-infected meat, the percentage of the ash, protein, and tenderness were significantly higher than in the moderately infected and intensely infected meat samples. This could be explained by a decrease in the appetite of sick animals and the absorption of nutrients. On the other hand, sick animals move less and rest more than healthy animals, and perhaps therefore, the fat content in the non-infected meat was lower than in the infected meat. Since all carcasses were from the same breed and category of bulls, the intramuscular fat content in all tested samples was similar and high, ranging from  $4.5 \pm 0.658\%$  to  $4.62 \pm 1.091\%$ . These values are consistent with good culinary properties. MOSTAFA and YASEIN (2010) found contrasting results, as the total fat percentage was significantly lower but the amount of ash was, as in our study, higher in infected samples than in non-infected samples. These results may be attributed to weakness and loss of appetite in infected animals, which lead to excessive utilization of body fat and consequently the lowering of fat in their meat (DUBEY *et al.* 1982). On the other hand, sick animals move less and rest more than healthy animals.

The total amount of amino acids (AAs) decreased with higher infection intensity (Table 2). The total amount of AAs was correlated with glutamic acid content in the meat samples ( $R = 0.966$ ,  $P < 0.05$ ). The most decrease was fixed on amount

of glutamic acid, a little bit smaller decrease was fixed on amounts of aspartic acid, glycine and lysine in the intensely infected samples. The amount of total amino acids in non-infected samples was  $826.12 \pm 12.222$  g/kg and did not differ significantly from that in meat with low levels of infection. The lowest amino acid content ( $715.40 \pm 21.854$  g/kg) was found in moderately infected samples but it did not differ significantly from that in intensely infected samples ( $732.27 \pm 0.259$  g/kg).

The content of biogenic amines in raw meat depends on the initial meat quality. The differences in polyamine levels could reflect peculiarities in the metabolism of ruminant compared to non-ruminant species (KRAUSOVÁ *et al.* 2006). The production of biogenic amines in meat products has been attributed to the action of several microorganisms: *Pseudomonas* spp., *Enterobacteriaceae*, *Enterococci* and *Lactobacilli* (HALÁSZ *et al.* 1994). In our case, the total amount of biogenic amines was higher in non-infected meat samples than in infected samples (Table 3). The amount of putrescine, histamine, spermidine, and spermine after 48 h *post-mortem* was significantly higher in non-infected and low-infected meat samples, compared with intensely infected ones ( $P < 0.05$ ).

Secondary amines (spermine and spermidine) do not react with *o*-phthaldialdehyde and thus determination of their liquid chromatography is only

Table 1. The effect of sarcocyst infection on the nutrient content and technological quality of meat samples 48 h after slaughtering

Indicators	No infection	Low infection	Moderate infection	Intense infection
Dry matter (%)	$23.59 \pm 1.010^a$	$22.98 \pm 0.620$	$22.16 \pm 0.870$	$21.97 \pm 0.807^b$
Fat (%)	$4.16 \pm 0.451^a$	$13.55 \pm 0.152^b$	$19.97 \pm 0.345^c$	$14.51 \pm 0.253^b$
Intramuscular fat (%)	$4.62 \pm 1.091$	$4.50 \pm 0.658$	$4.61 \pm 0.996$	$4.54 \pm 1.025$
Ash (%)	$1.16 \pm 0.070^a$	$1.09 \pm 0.047$	$1.03 \pm 0.086^b$	$1.02 \pm 0.136^b$
Protein (%)	$18.09 \pm 0.563^a$	$17.00 \pm 0.424^b$	$16.09 \pm 0.629^c$	$15.47 \pm 0.638^d$
pH after 24 h	$5.90 \pm 0.040$	$5.91 \pm 0.058$	$5.88 \pm 0.057$	$5.90 \pm 0.051$
pH after 48 h	$5.95 \pm 0.030$	$5.98 \pm 0.035$	$5.99 \pm 0.105$	$5.97 \pm 0.034$
lightness ( $L^*$ )	$37.16 \pm 2.352^a$	$35.38 \pm 5.112^b$	$40.52 \pm 2.691^c$	$39.40 \pm 2.430^d$
Colour				
redness ( $a^*$ )	$19.24 \pm 1.445$	$19.42 \pm 1.738$	$19.12 \pm 2.332$	$19.65 \pm 1.408$
yellowness ( $b^*$ )	$6.96 \pm 1.716$	$6.52 \pm 1.904$	$5.27 \pm 1.664$	$5.08 \pm 1.550$
Drip loss (%)	$0.82 \pm 0.342^a$	$1.04 \pm 0.426^b$	$1.68 \pm 0.434^c$	$1.80 \pm 1.127^c$
Water holding capacity (%)	$63.17 \pm 1.710^a$	$62.91 \pm 1.742$	$64.81 \pm 3.217^b$	$64.11 \pm 2.617^b$
Cooking loss (%)	$20.63 \pm 1.454$	$19.85 \pm 1.588$	$21.71 \pm 3.198$	$21.48 \pm 3.328$
Tenderness (kg/cm <sup>2</sup> )	$3.79 \pm 0.520^a$	$3.45 \pm 0.339^b$	$3.31 \pm 0.398^b$	$3.47 \pm 0.409^b$

Results are presented as mean  $\pm$  SD; means with different letters in the same line differ significantly ( $P < 0.05$ );  $n = 30$

Table 2. The effect of sarcocyst infection on the amino acids content in samples 48 h after slaughtering

Indicators/dry matter (g/kg)	No infection	Low infection	Moderate infection	Intense infection
Dry residue (%)	100	100	100	100
Aspartic acid	75.18 ± 0.251	72.42 ± 0.738	65.12 ± 0.781	67.42 ± 0.345
Threonine	39.48 ± 0.854	42.85 ± 0.542	34.97 ± 0.561	35.55 ± 0.899
Serine	31.50 ± 0.322	34.00 ± 0.364	28.40 ± 0.657	29.04 ± 0.254
Glutamic acid	133.95 ± 2.159 <sup>a</sup>	124.42 ± 1.098 <sup>a</sup>	115.26 ± 1.151 <sup>b</sup>	118.02 ± 1.259 <sup>b</sup>
Proline	40.91 ± 0.425	38.05 ± 0.283	34.13 ± 0.467	35.37 ± 0.478
Glycine	41.53 ± 0.478	36.11 ± 0.368	34.29 ± 0.332	33.97 ± 0.224
Alanine	55.45 ± 0.568	57.78 ± 0.574	49.60 ± 0.625	49.02 ± 0.511
Valine	42.84 ± 0.435	41.07 ± 0.422	36.07 ± 0.893	37.06 ± 0.178
Methionine	27.90 ± 0.278	25.07 ± 0.258	23.32 ± 0.454	24.43 ± 0.241
Isoleucine	37.12 ± 0.238	35.54 ± 0.366	32.28 ± 0.259	32.88 ± 0.366
Leucine	66.94 ± 0.662	65.05 ± 0.653	58.68 ± 0.892	59.83 ± 0.594
Tyrosine	27.55 ± 0.310	26.48 ± 0.241	23.78 ± 0.453	24.96 ± 0.352
Phenylalanine	35.03 ± 0.350	33.14 ± 0.331	30.18 ± 0.204	31.11 ± 0.318
Histidine	38.87 ± 0.391	43.10 ± 0.431	35.54 ± 0.843	34.19 ± 0.345
Lysine	70.01 ± 0.704	62.33 ± 0.623	59.43 ± 0.504	63.05 ± 0.685
Arginine	61.86 ± 0.622	59.50 ± 0.512	54.34 ± 0.235	56.37 ± 0.782
Total amino acids amount	826.12 ± 12.222 <sup>a</sup>	796.93 ± 10.244 <sup>a</sup>	715.40 ± 21.854 <sup>b</sup>	732.27 ± 0.259 <sup>b</sup>

The results are presented as mean ± SD; means with different letters on the same line differ significantly ( $P < 0.05$ );  $n = 30$

possible after derivatization with dansylchloride. Their level during fermentation and/or long-term storage is relatively constant or slightly decreased compared with the initial one (SMĚLÁ *et al.* 2003). Therefore, in our experiment at 48 h *post-mortem* they had no influence on the level of polyamines.

The observed changes in total amounts of biogenic amines and amino acids cannot be explained only by protein depletion. Levels may have been influenced by poor absorption of proteins or decreasing activity of proteases, peptidases and decarboxylases in the digestive tract or possibly in the infected muscle. More research is needed on this topic. MITCHELL

(2003) noted that the amount of polyamines in normal tissues is controlled by biosynthetic and catabolic enzymes. The latter enzymes include spermidine and spermine acetyltransferase, flavin-containing polyamine oxidase, copper-containing diamine oxidase, and possibly other amino oxidases. Multiple abnormalities in the control of polyamine metabolism and uptake might be responsible for increased levels of polyamines. Putrescine, cadaverine, spermine and spermidine in living tissues stimulate cell growth and division. The significant decrease in amounts of putrescine and spermine in moderately and intensely infected meat samples and low amounts of cadaverine and

Table 3. The effect of sarcocyst infection on the amount of biogenic amines in samples 48 h after slaughtering

Biogenic amines	No infection	Low infection	Moderate infection	Intense infection
Putrescine	21.01 ± 2.362 <sup>a</sup>	16.92 ± 5.347 <sup>b</sup>	13.92 ± 2.143 <sup>c</sup>	11.79 ± 2.961 <sup>c</sup>
Cadaverine	6.72 ± 3.022	3.91 ± 0.235	3.32 ± 1.196	3.36 ± 0.958
Histamine	4.78 ± 0.842 <sup>a</sup>	4.64 ± 1.654 <sup>a</sup>	3.92 ± 1.841 <sup>b</sup>	1.91 ± 0.911 <sup>c</sup>
Tiramine	0.0 ± 0.00	0.0 ± 0.00	0.92 ± 0.342	0.89 ± 0.154
Spermidine	4.28 ± 5.341 <sup>a</sup>	6.81 ± 1.048 <sup>b</sup>	4.92 ± 0.247 <sup>a</sup>	3.24 ± 1.803 <sup>c</sup>
Spermine	13.43 ± 2.211 <sup>a</sup>	12.63 ± 3.672 <sup>a</sup>	10.92 ± 0.554 <sup>b</sup>	3.05 ± 0.546 <sup>c</sup>
Total amount	50.22 ± 13.778 <sup>a</sup>	44.9 ± 11.956 <sup>b</sup>	37.92 ± 6.323 <sup>b</sup>	24.24 ± 7.433 <sup>c</sup>

The results are presented as mean ± SD; means with different letters on the same line differ significantly ( $P < 0.05$ );  $n = 30$

<https://doi.org/10.17221/89/2017-CJFS>

Table 4. The effect of sarcocyst infection on the total count of aerobic microorganisms, number of *E. coli* and coliform bacteria

Microorganisms (CFU/g)	No infection	Low infection	Moderate infection	Intense infection
Total count of aerobic microorganisms	$3.40 \times 10^2 \pm 5.57 \times 10^{2a}$	$3.50 \times 10^2 \pm 2.58 \times 10^{2a}$	$3.80 \times 10^4 \pm 2.58 \times 10^{3b}$	$8.10 \times 10^4 \pm 1.11 \times 10^{4c}$
<i>E. coli</i>	$0.00 \times 10 \pm 0.00 \times 10$	$1.00 \times 10 \pm 1.08 \times 10$	$1.20 \times 10 \pm 0.02 \times 10$	$1.10 \times 10 \pm 0.11 \times 10$
Coliform bacteria (CFU/g)	$0.40 \times 10 \pm 0.02 \times 10$	$1.50 \times 10 \pm 0.58 \times 10$	$1.80 \times 10 \pm 0.48 \times 10$	$2.10 \times 10 \pm 1.11 \times 10$

The results are presented as mean  $\pm$  SD; means with different letters in lines differ significantly ( $P < 0.05$ );  $n = 30$

spermidine levels in intensely infected samples may indicate low metabolically active tissue (THOMAS & THOMAS 2003).

Table 4 shows the effect of sarcocyst infection on the total aerobic bacterial count, coliform bacteria and *E. coli* numbers in the meat samples. The total aerobic bacterial count after 48 h *post-mortem* was  $3.40 \times 10^2 \pm 5.57 \times 10^2$  CFU/g in non-infected samples,  $3.50 \times 10^2 \pm 2.58 \times 10^2$  CFU/g in the case of weak infection,  $80 \times 10^4 \pm 2.58 \times 10^3$  CFU/g in the samples with moderate infection and  $8.10 \times 10^4 \pm 1.11 \times 10^4$  CFU/g with heavy infection.

There was a significant increase in the total aerobic microorganisms count in the moderately and intensely infected meat samples compared with low-infected and uninfected meat samples ( $P < 0.05$  in all cases). The results show a positive relationship between the number of sarcocysts and total aerobic microorganisms count. These data may reflect the high stress resulting from parasitic infection, which leads to immune depression and thus the opportunity for bacterial multiplication (COLLEE *et al.* 1989).

It can be assumed that a low level of *Sarcocystis* infection has no significant impact on coliform bacteria contamination. In contrast to the results of MOSTAFA

and YASEIN (2010), who reported differences in total enterobacteriaceae count, in our experiment the differences in the *E. coli* and coliform bacteria counts between the infected and non-infected samples were not significant ( $P > 0.05$ ).

The average amounts of saturated fatty acids (FA) in the moderately and intensely infected samples of cattle diaphragms were higher than in the non-infected and low-infected cattle meat samples ( $P < 0.05$ ) (Table 5) and the average amounts of monounsaturated FA were significantly lower in moderately and intensely infected meat samples than in low-infected and non-infected samples ( $P < 0.05$ ). The amounts of polyunsaturated FA and trans-isomers in the samples did not differ significantly ( $P > 0.05$ ), nor did the ratio of omega 6 and omega 3 FA in the samples ( $P > 0.05$ ).

## CONCLUSIONS

Sarcocyst infection may significantly decrease dry matter, protein, amino acids (AAs) and biogenic amines with increasing numbers of sarcocysts. These results may be attributed to the weakness and loss of appetite of infected animals. On the other hand,

Table 5. The effect of sarcocyst infection on the amounts of fatty acids, percent total fatty acids content, and omega 6/3 ratio

Fatty acids	No infection	Low infection	Moderate infection	Intense infection
Saturated	$41.75 \pm 0.450^a$	$41.79 \pm 0.511^a$	$44.55 \pm 0.100^b$	$45.88 \pm 0.422^c$
Monounsaturated	$49.91 \pm 0.311^a$	$49.72 \pm 0.366^a$	$47.36 \pm 0.423^b$	$46.76 \pm 0.386^c$
Polyunsaturated	$6.10 \pm 0.052$	$5.46 \pm 0.028$	$4.31 \pm 0.352$	$4.38 \pm 0.040$
Trans isomers	$2.17 \pm 0.554$	$3.96 \pm 0.039$	$4.22 \pm 0.295$	$2.98 \pm 0.052$
Omega-3	$0.20 \pm 0.046$	$0.03 \pm 0.012$	$0.42 \pm 0.445$	$0.00 \pm 0.00$
Omega-6	$5.90 \pm 0.053$	$5.44 \pm 0.044$	$3.90 \pm 0.105$	$4.38 \pm 0.004$
Ratio omega 6/3	$9.14 \pm 0.095$	$8.04 \pm 0.084$	$5.71 \pm 0.015$	$6.93 \pm 0.012$

The results are presented as mean  $\pm$  SD; means with different letters on the same line differ significantly ( $P < 0.05$ );  $n = 30$

sick animals move less and rest more than healthy animals and the sarcocyst infection increased the fat and saturated fatty acid content. Glutamic acid showed the greatest decrease of all AAs. Glutamic acid (L-GA) physiologically exists as glutamate, and plays a major role in amino acid metabolism and thus in maintaining nitrogen balance in the body. Glutamate is an important immunomodulator, and therefore a reduction of glutamic acid in the meat affects the body's resistance to infection. Microbiological analyses showed a noticeable increase in the total aerobic count, but the numbers of coliform bacteria and *E. coli* were not significantly altered by infection of diaphragm meat.

In conclusion, sarcocysts in beef diaphragms did not cause serious changes in the technological quality of the meat. However the biological quality of infected diaphragm meat was lower. Co-infections with more than one *Sarcocystis* spp. in cattle are frequent (MORÉ *et al.* 2013, 2014). As cattle can be simultaneously infected with more than one type of *Sarcocystis*, it can be assumed that the impact on the quality of meat depends not only on pathogenicity, but also on the type of *Sarcocystis*, so further research should consider different *Sarcocystis* types in infected cattle.

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Received: 2017–02–28

Accepted after corrections: 2018–09–10

Published online: 2018–10–23