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Original article

The influence of the newly isolated *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244 strains on blood and faeces parameters in endurance horses

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Abstract

The aim of this study was to evaluate the influence of the newly isolated *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244 strains grown in potato juice (with a cell count of 8.0-9.0 $\log_{10}$ CFU/ml) on the blood and faeces parameters of exercising horses. The horses were classified into four different groups: a control group (which received no probiotics); the first group (which received 200 ml of *L. plantarum* culture in potato juice); the second group (which received 200 ml of *L. paracasei* culture in potato juice); and the third group (which received an *L. plantarum* and *L. paracasei* mix (with the mix consisting of 100 ml of each). Indices for the blood and faeces microflora were obtained before and after treatment of horses (on days zero and thirty). It was observed that the count for lactic acid bacteria (LAB) in the faeces was significantly higher on day thirty, whereas it was lower when it came to the total enterobacteria count (TCE). Despite the ambiguous influence of any treatment on blood parameters, the *L. plantarum* × *L. paracasei* mixture increased the concentration of HGB and O_2 saturation in blood samples which were taken from the horses. *L. paracasei* significantly decreased the lactate concentration levels in horse blood samples. As a result of the present study, it can clearly be seen that the strains being used revealed their potential application as probiotics; however, further studies are required to prove the survival and action mechanisms of the newly isolated strains.

Key words: horses, *Lactobacillus plantarum*, *Lactobacillus paracasei*, blood parameters, faeces microbiological parameters

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Introduction

The attention of the sporting community towards probiotic supplementation as a way of promoting exercise and training performance, as well as good health, has increased in recent years (West et al. 2011). Despite widespread use, too little is known about the use of commercial probiotic formulations in horses (Schoster et al. 2014). Research about probiotics as a way of improving health status and, in turn, performance has also focused on horses with promising results (Garcia et al. 2015). Unfortunately, the ambiguity of the results seems also to affect the choice of the formulations that are to be employed in horses, mainly due to the loosely regulated quality of commercial over-the-counter products, together with incoherence in the selection of strains and dosages (Schoster et al. 2014). It was reported that the colonisation of the adult equine gastrointestinal tract with *L. rhamnosus* LGG of human origin was shown to be poor and colonisation can only be detected if the animal is shedding the bacterium at the time at which sampling takes place (Costa and Weese. 2012). Furthermore, the relationship between probiotics and low pH conditions in the intestines has previously been reviewed. Poppi et al. (2015) clearly demonstrated that probiotic bacteria release carboxylic acids such as lactic and acetic acid, and have inhibitory effects on the growth and invasivibility of *E. coli* under low pH conditions. At the same time, Ishizaka et al. (2014) stated that the administration of probiotics had no adverse effects on equine clinical conditions and served to improve the intestinal condition of adult horses without any adverse effects being shown.

Lactobacillus spp are well-known constituents of the equine microbiome and common ingredients in probiotics. They have many benefits, such as improving insulin sensitivity, acting as an anti-inflammatory, antioxidant, or antimicrobial agent, and even inducing cancer cell death (Chen et al. 2016). It was clearly demonstrated that an increase in horse performance can be ensured with the supplementation of the probiotic bacterium *Lactobacillus plantarum* (Chen et al. 2016). The adverse effects of antibiotics on faeces bacteria in horses can be prohibited with probiotic administration (Pyles et al. 2017). Indeed, antibiotic treatment has been shown to induce changes in the faecalbacteria of horses, which may increase the risk of antibiotics--associated diarrhoea. The normal microbial flora of faecal bacteria during antibiotic therapy can be stabilised with probiotic administration, preventing the proliferation of pathogenic bacteria. As is already known, gastric ulcers are common in horses. Andrews et al. (2016) reported that disturbances of the gastric and hindgut ecosystems can lead to damage of the mucosa in the stomach, and also to intestinal colic (Jullianda et al. 2017). *L. plantarum* was considered by the EFSA to be suitable for the 'Qualified Presumption of Safety' approach to safety assessment (EFSA 2012). Probiotic products which are applied to horses generally comprise live bacterial cultures and are intended to be used to prevent the undesirable consequences of stress due to a failure to ingest the colostrum, weaning, switching feeds, transportation, adverse weather, disease recurrence, nutritional debilitation and prolonged antibiotic therapy (Silva et al. 2017).

The main objective of this study was to evaluate the influence of the newly isolated *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244 strains on the blood and faeces parameters in horses.

Materials and Methods

Animals and feeding

Thirty-four male Arabian horses were used in this study, aged between eight and ten years and weight between 404.0kg and 469.5kg, all of which were involved in endurance riding. All of the horses were engaged in the same activity fields before the start of the trials. The horses were classified into four different levels of treatment: the control group (which would receive no probiotics), the first group (which received 200 ml of *L. plantarum* culture in potato juice), the second group (which received 200 ml of *L. paracasei* culture in potato juice), and the third group (which received an *L. plantarum* and *L. paracasei* mixture (a mix of 100 ml of each).

Each horse was stabled in an individual box, bedded with wood shavings, and subjected to a standardised diet which was composed of hay (a mix of ryegrass and wild oats) and complementary feed (containing a mix of oats, barley, wheat, and alfalfa pellets) given twice a day (2.5% and 1% of live weight respectively, divided into two rations). Water and mineral salt were provided *ad libitum*. All animals had been de-wormed at least three months before the start of the study using a commercial product which contained a mix of ivermectine (200ng/kg body weight) and praziquantel (1.5mg/kg body weight) and, according to Lithuanian rules, were also vaccinated against influenza and tetanus.

All of the animals were ridden five times a week for ninety minutes at an average speed of 15km/h on a mechanical circular exerciser. The horses were already accustomed to such exercise.

A total of 200 ml of potato juice with a high content of selected LAB (8.0-9.0 $\log_{10}$ CFU/ml) was added to the standard feeding. All clinical evaluations were

carried out at rest in the morning, one hour after the end of feeding.

Isolation, purification, and the identification of LUHS135 and LUHS244

Lactobacillus plantarum LUHS135 *and Lactobacillus paracasei* LUHS244 were isolated from spontaneously fermented rye (Bartkienė et al. 2017). *L. plantarum* and *L. paracasei* strains which showed a broad spectrum of carbohydrate metabolism (Table 1) were tolerant to a low pH, served to inhibit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Corynebacter* spp, *Klebsiella pneomoniae*, *Enterococcus faecalis*, *Bacillus cereus*, *Proteus mirabilis*, *Clostridium* spp, and *Streptococcus* spp, and were considered to be non-resistant to all EFSA recommended antibiotics (Collado et al. 2008).Venous blood samples were collected at rest from the jugular vein into sterile tubes with and without EDTA before and after treatment (on days zero and thirty). Physiological indices for the blood and faeces microflora were investigated before and after treatment (on days zero and thirty).

All blood samples, prior to analysis, were kept at a temperature of 18°C. Haematological testing of the blood samples was carried out within the five hours from being collected. Haematological parameters were analysed using an automatic cell counter, an Abacus Junior Vet Haematology Analyser (Diatron Messtechnik GmbH, Austria, 2006). pH, PCO_2, PO_2, Na, K, Ca, glucose (Glu), lactates, haematocrit (Hct), HCO_3, TCO_2, O_2, saturation and haemoglobin (Hb) were all analysed using a blood gas analyser (EPOC, Canada).

Activity-related concentrations of serum alanine aminotransferase (AST), albumins (Alb), total proteins (TP), urea (UREA), glucose (Glu), and magnesium (Mg) in the blood of the horses were measured using an automated analyser, an Hitachi 705 (Hitachi, Japan).

The faeces samples were collected from the horses immediately after they had defecated, both before and after treatment, and were stored in vials (at +4°C) with a transport medium (Faecal TM enteric Plus, Oxoid, Basingstoke, England), being analysed on the same day. The De Man, Rogosa, and Sharpe (MRS) agar (Oxoid Ltd, Basingstoke, England), the Violet Red Bile Glucose (VRBG) agar (Oxoid Ltd, Basingstoke, England), and the Plate Count Agar (Bio life Italian aSrl, Milan, Italy) were all used for determination of the LAB count in the faeces the total count of enterobacteria (TCE), and the total aerobic bacteria and facultative anaerobics count (TCM) in the faeces respectively. At the same time, Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Liofilchem, Milan, Italy) was used for the yeast and mould count (Y/F) determination in the faeces. The results were expressed as a $\log_{10}$ of the CFU/g of a sample.

In vivo experiment ethical guidelines

The horses were hosted indoors, being individually tethered and cared for in accordance with the Lithuanian State Food and Veterinary Service Requirements. Research was carried out in accordance with the 6 November 1997 Republic of Lithuania act covering animal care, maintenance, and the use of the appropriate legal act, 8-500 (Valstybės Žinios, (Official Gazette) No 130-6595: 2012).

Statistical analysis

The data were analysed using *SPSS* (Statistical Package for the Social Sciences, 13.0). The statistical analyses were carried out by means of the one-way and multivariate analysis of variance (ANOVA).

Results

No adverse reaction was observed during the trial. All horses maintained their previous weight and body condition score during the entire duration of the experiment.

The influence of strains on the microbiological parameters of horse faeces

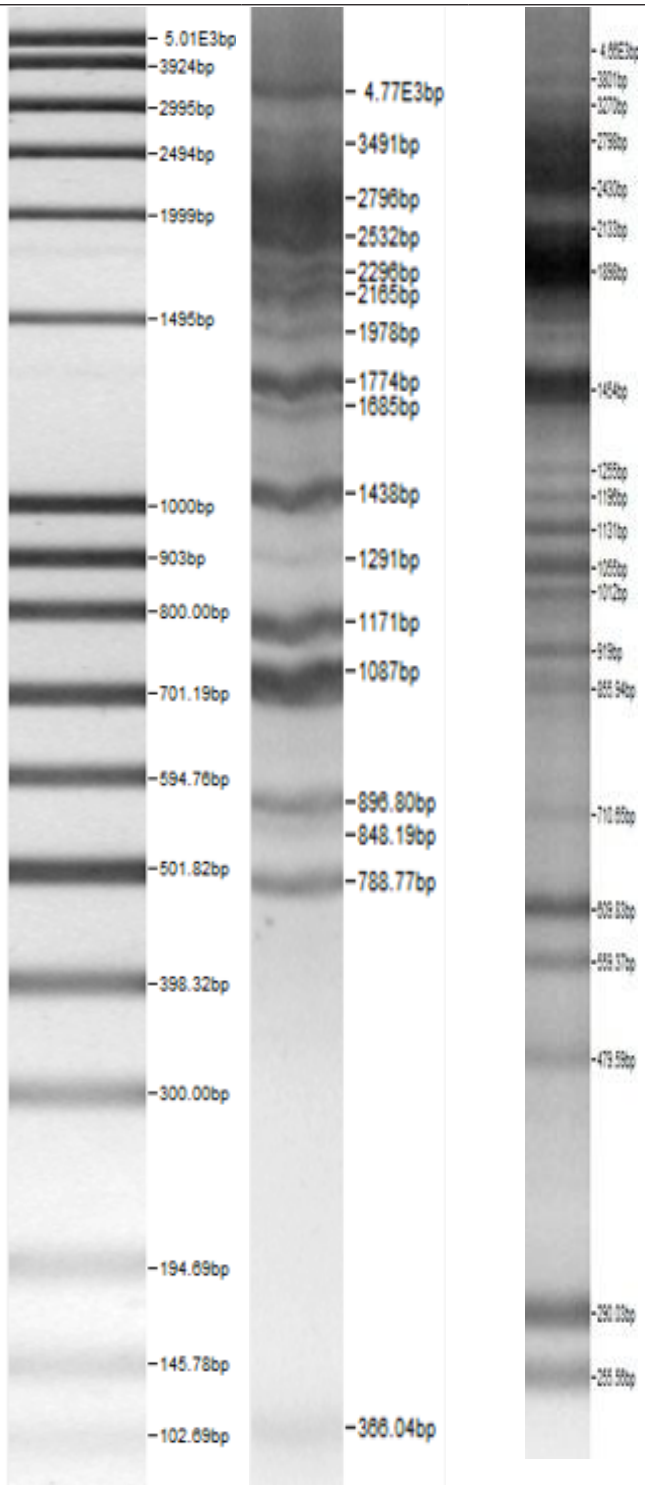
Those horses that received *Lactobacillus plantarum, Lactobacillus paracasei*, and their mixtures had a significantly higher LAB count ($p<0.001$) and lower TCE count ($p<0.001$) in their faeces on day thirty when compared to the control group (Table 2). At the same time, we observed that there was a decrease in the TCM and Y/F count in the faeces of all treatment groups. The TCM count in the faeces of the first and third groups was significantly reduced ($p<0.05$-$p<0.01$). By contrast, the TCM count in the second group was significantly increased with treatment ($p<0.001$).

The influence of strains on the parameters of horse blood

The effect of strains on blood biochemical parameters, morphological parameters, and concentrations of blood gases are presented in Tables 3, 4, and 5, respectively. In the first group'which additionally received the *L. plantarum* strain, significantly higher concentrations of Alb and Mg (at 5.2% and 10.8% respectively) were foundcompared to blood samples from the same group before feeding with

Table 1. Characteristics of *Lactobacillus plantarum* and *Lactobacillus paracasei* strains used in the experiment.

		Lacto-bacillus plantarum LUHS135	*Lacto-bacillus paracasei* LUHS244
Glicerol		-	-
Erythritol		-	-
D-arabinose		-	-
L-arabinose		+++	-
D-ribose		+++	+++
D-xylose		-	-
L-xylose		-	+++
D-adonitol		-	+
Methyl-ßd-xYlopiranoside		-	-
D-galactose		+++	+++
D-glucose		+++	+++
D-fructose		+++	+++
D-mannose		+++	+++
L-sorbose		-	-
L-rhamnose		+	+++
Dulcitol		-	+++
Inositol		-	-
D-mannitol		+++	+++
D-sorbitol		+++	+++
Methyl-αD-mannopyranoside		+++	-
Methyl-αD-glucopyranoside		+	+++
N-acetylglucosamine		+++	+++
Amigdalin		+++	+++
Arbutin		+++	+++
Esculin		+++	+++
Salicin		+++	+++
D-cellobiose		+++	+++
D-maltose		+++	+++
D-lactose		+++	+++
D-melibiose		+++	-
D-sucrose		+++	+++
D-trehalose		+++	+++
Inulin		-	+++
D-melezitose		+++	+++
D-raffinose		-	-
Amidon		-	-
Glycogen		-	-
Xylitol		-	-
Gentiobiose		++	+++
D-turanose		+++	+++
D-lyxose		-	-
D-tagatose		+++	+++
D-fucose		-	-
L-fucose		-	-
D-arabitol		-	-
L-arabitol		-	-
Potassium gluconate		++	++
Potassium 2-ketogluconate		-	-
Potassium 5-ketogluconate		-	-
Gas production (+/-)		-	-
Tolerance to temperature	10 °C	-	-
	30 °C	+++	+++
	37 °C	++	++
	45 °C	-	-
pH	0 h $\log_{10}$ CFU mL^{-1}	8.08±0.2	9.03±0.2
	2 h $\log_{10}$ CFU mL^{-1}	7.69±0.1	7.55±0.1



Interpretation of LAB growth in API 50 CH system +++= high growth (yellow); ++= quite growth (green); += little growth (dark green); -= not growth (blue).

Table 2. The influence of the *L. plantarum* and *L. paracasei* supplements on the microbiological parameters of the horse faeces.

Microbiological parameters of horses faeces								
	Control		First Group		Second Group		Third Group	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
TCM $\log_{10}$ CFU/g	7.01±0.09	6.97±0.07	8.36±0.08^{a}	6.69±0.04^{b}	6.90±0.03^{a}	7.09±0.06^{b}	7.10±0.07^{a}	6.60±0.09^{b}
LAB $\log_{10}$ CFU/g	6.15±0.03	6.18±0.07	4.70±0.05^{a}	6.16±0.04^{b}	5.77±0.05^{a}	6.43±0.04^{b}	5.83±0.03^{a}	7.03±0.07^{b}
TCE $\log_{10}$ CFU/g	3.99±0.04^{a}	4.34±0.05^{b}	4.75±0.01^{a}	4.05±0.02^{b}	3.57±0.04^{a}	3.49±0.03^{b}	3.25±0.05^{a}	3.18±0.02^{b}
Y/F $\log_{10}$ CFU/g	3.77±0.0^{a}	4.77±0.06^{b}	5.31±0.05^{a}	4.60±0.07^{b}	5.77±0.05^{a}	4.28±0.07^{b}	4.71±0.04^{a}	4.18±0.05^{b}

Control: before and after treatment (received no probiotics); first group: fed with *L. plantarum* before and after treatment; second group: fed with *L. paracasei* before and after treatment; third group: fed with an *L. plantarum* and *L. paracasei* mix before and after treatment. ^{a,b} The differences between means in each group were significant ($p<0.05$-$p<0.01$).

Table 3. The influence of the *L. plantarum* and *L. paracasei* supplements on the blood parameters of the horses.

Blood parameters								
	Control		First Group		Second Group		Third Group	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Alb	35.63±0.1	36.15±0.5	34.72±0.4^{a}	36.63±0.2^{b}	33.65±0.4	33.77±0.4	33.77±0.7^{a}	39.87±0.6^{b}
TP	61.73±0.9	61.68±1.2	63.95±1.0	69.88±2.3	62.82±1.2	65.30±0.2	65.35±0.1	66.15±0.8
Urea	7.48±0.4	7.48±0.4	6.35±0.3	5.68±0.3	4.40±0.4	4.64±0.2	5.64±0.2^{a}	6.28±0.3^{b}
AST	255.25±5.0	239.50±4.2	231.25±4.9	233.25±3.2	292.25±5.3^{a}	243.00±4.00^{b}	294.00±6.2	271.50±5.0
Glu	5.50±0.3	5.50±0.5	5.03±0.3	5.58±0.3	5.58±0.7^{a}	5.70±0.5^{b}	5.60±0.2^{a}	5.90±0.3^{b}
Mg	0.86±0.01	0.94±0.03	0.83±0.01a	0.93±0.01b	0.79±0.02a	0.87±0.01b	0.77±0.02a	0.93±0.01b

Control: before and after treatment (received no probiotics); first group: fed with *L. plantarum* before and after treatment; second group: fed with *L. paracasei* before and after treatment; third group: fed with an *L. plantarum* and *L. paracasei* mix before and after treatment. ^{a,b} The differences between means in each group were significant ($p<0.05$-$p<0.01$).

Table 4. The influence of the *L. plantarum* and *L. paracasei* supplements on the morphological blood parameters of the horses.

Morphological blood parameters								
	Control		First Group		Second Group		Third Group	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
WBC	8.57±0.2	8.61±0.3	8.50±0.1^{a}	9.93±0.4^{b}	11.79±0.6	11.46±0.4	8.46±0.4^{a}	9.38±0.3^{b}
LYM	3.78±0.02	3.82±0.01	3.45±0.01^{a}	4.27±0.02^{b}	3.25±0.03^{a}	3.64±0.02^{b}	3.24±0.04^{a}	3.83±0.03^{b}
MID	0.37±0.01	0.38±0.01	0.40±0.01	0.39±0.01	0.53±0.02^{a}	0.17±0.01^{b}	0.24±0.01^{a}	0.41±0.01^{b}
GRA	5.31±0.2	5.32±0.1	5.15±0.1^{a}	4.61±0.1^{b}	8.01±0.1^{a}	7.65±0.2^{b}	4.65±0.31^{a}	5.10±0.2^{b}
LY	36.23±1.2	36.25±2.4	37.65±2.2	44.08±2.1	30.00±3.00	33.73±1.2	35.73±3.2^{a}	44.90±2.3^{b}
MI	5.68±0.1^{a}	5.50±0.14^{b}	5.08±0.1	4.93±0.2	4.45±0.2^{a}	4.88±0.1^{b}	1.48±0.01^{a}	4.88±0.2^{b}
GR	53.43±1.2	53.53±2.1	57.30±2.3^{a}	53.50±3.6^{b}	65.55±3.2	65.80±2.13	64.80±3.3	63.43±2.3
RBC	9.71±0.1	9.90±0.7	9.46±0.5	10.95±0.5	9.81±0.8	9.99±0.6	9.29±0.6	9.42±0.5
HGB	145.75±2.6^{a}	138.75±2.7^{b}	140.00±2.4^{a}	155.25±2.4^{b}	140.50±2.6	143.50±2.7	141.50±2.9^{a}	164.75±2.8^{b}
HCT	37.25 ±1.2	30.00 ±1.1	29.50 ±1.2	30.50±1.3	41.00±1.00	26.50±0.9	32.50±1.0	31.00±1.0
MCV	44.75±1.2	43.00±1.3	45.75±2.3	44.00±2.5	46.50±1.4	45.50±3.6	49.50±1.5	48.25±2.2

Control: before and after treatment (received no probiotics); first group: fed with *L. plantarum* before and after treatment; second group: fed with *L. paracasei* before and after treatment; third group: fed with an *L. plantarum* and *L. paracasei* mix before and after treatment. ^{a,b} The differences between means in each group were significant ($p<0.05$-$p<0.01$).

Table 5. The influence of the *L. plantarum* and *L. paracasei* supplements on the blood gas parameters of the horses.

	Blood gas parameters							
	Control		First Group		Second Group		Third Group	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
pH	7.40±0.2	7.48±0.2	7.39±0.3	7.39±0.01	7.39±0.2	7.41±0.1	7.40±0.3	7.39±0.1
PCO_2. mmHg	38.70±2.5	48.10±2.4	51.88±2.3^a	49.38±2.2^b	49.28±2.3^a	48.68±2.2^b	47.68±3.1	50.20±2.1
Lact. mmol/L	1.20±0.01	1.25±0.02	0.73±0.01	0.94±0.01	0.78±0.01^a	0.72±0.01^b	0.75±0.03^a	0.89±0.01^b
PO_2. mmHg	53.45±2.1^a	56.20±2.3^b	42.90±3.2	42.60±2.1	42.88±1.9^a	41.38±2.1^b	40.38±2.2^a	42.50±2.1^b
Na. mmol/L	139.50±4.1^a	138.00±4.2^b	137.75±4.0^a	135.25±3.8^b	135.75±3.9	134.00±2.1	138.00±4.2	136.00±3.1
K. mmol/L	3.95±0.1^a	4.50±0.3^b	3.65±0.2	3.70±0.2	2.88±0.01	3.07±0.1	3.08±0.1	3.75±0.2
iCa. mmol/L	1.60±0.01	1.59±0.01	1.65±0.02	1.61±0.03	1.56±0.02^a	1.64±0.01^b	1.60±0.01	1.61±0.01
Glu. mmol/L	4.60±0.1	4.90±0.1	5.10±0.2	5.18±0.2	5.90±0.1^a	6.50±0.2^b	6.55±0.1^a	5.55±0.4^b
HCO_3.mmol/L	30.95±2.3	31.80±1.2	31.45±1.4	29.50±2.3	29.90±1.1	29.47±1.3	29.23±1.2	30.43±2.2
TCO_2.mmol/L	32.53±1.2	33.20±1.2	33.05±2.3	31.03±1.00	31.00±2.0	34.80±2.1	34.85±2.1	37.10±2.1
O_2 saturation	75.95±4.2	78.68±3.6	59.30±3.9^a	75.58±2.5^b	84.95±2.7^a	89.10±4.1^b	71.68±4.2^a	84.28±2.5^b
Hb. g/L	10.18±0.2^a	8.95±0.4^b	12.68±0.1	13.10±0.2	9.99±0.1^a	10.50±0.2^b	10.03±0.1^a	10.62±0.2^b

Control: before and after treatment (received no probiotics); first group: fed with *L. plantarum* before and after treatment; second group: fed with *L. paracasei* before and after treatment; third group: fed with an *L. plantarum* and *L. paracasei* mix before and after treatment. ^{a,b} The differences between means in each group were significant ($p<0.05$-$p<0.01$).

the *L. plantarum* strain. In the second group', which additionally received the *L. paracasei* strain, significantly lower concentrations of AST (by 16.9%) were observed, as well as significantly higher concentrations of Glu and Mg (2.2% and 9.2%, respectively), compared with blood samples from the same group before feeding with a probiotic strain. It was found that in the third group's with horses being fed a probiotics mixture, after thirty days a higher Alb, urea, glucose, and Mg contents were found, by 15.3%, 10.2%, 5.1%, and 17.2%, respectively). Additionally, most of the blood parameters that are included in Table 4 have been influenced by feeding the horses in question with separate *Lactobacillus plantarum* and *Lactobacillus paracasei*, as well as a mixture of the two.

Most of the blood parameters that were analysed, which are presented in Table 4, were influenced by a *Lactobacillus plantarum* and *Lactobacillus paracasei* mix. A probiotics mix significantly increased Mg, WBC, LYM, MID, GRA, LY, MI, and HBG concentrations in blood samples (7.20%, 9.81%, 15.40%, 41.46%, 8.82%, 20.42%, 69.67%, and 14.11%, respectively). In the first group, which was fed with *L plantarum*, significantly higher Mg, WBC, LYM, LY, and HGB concentrations were found in blood samples (10.75, 14.40%, 19.20%, 14.59%, and 9.82%, respectively), and these tendencies were similar in the third group. However, in the first group a significant lower GRA count and GR was detected (10.49% and 6.63%, respectively). Five of the twelve results shown in Table 4 presented blood parameters for the horses that were significantly influenced by feeding them with *L paracasei*. In the second group a significant increase in Mg, LYM, and MI could be found (9.20%, 10.71%, and 8.81%, respectively), and in opposition to the first and third groups, MID and GRA concentrations in the blood samples of these horses were found to be significantly lower (67.92% and 4.49%, respectively). The blood gas concentrations for particular groups of horses are presented in Table 5. It was found that an *L plantarum* supplement significantly decreased PCO_2 levels (4.82%) and Na concentrations (1.81%) in blood samples that were taken from horses in the first group. Also in the first group a significant increase in O_2 saturation levels was found (51.54%). A significant decrease of PCO_2, lactate concentrations, and PO_2 in the second group was also found (2.21%, 7.69%, and 3.50%, respectively). In opposition to those readings, iCa, Glu, and O_2 saturation levels, and also Hb concentrations, were found to be significantly increased (4.89%, 9.23%, 4.66%, and 4.86%, respectively). The same tendencies for O_2 saturation levels and Hb concentrations as for the second group could also be observed in the third group (significantly increased by 14.95% and 5.56%, respectively). However, Glu content in the third group's blood samples was significantly decreased (15.27%). Also, in opposition to the findings for the second group, the third group demonstrated lactate and PO_2 concentrations that were significantly increased (15.73% and 4.99%, respectively). However, a significant increase of PO_2 concentration levels in the control group was also found (4.89%).

Discussion

Nowadays, probiotics have become a highly attractive feed ingredient thanks to their positive influence on many parameters in the animals to which they are given. The characteristics of the particular probiotics to be used should be selected on the basis of the animal species that will be receiving them. Our group hypothesis is that antimicrobial properties of such a type of supplement is very important because the ability to maintain a balanced microflora in the digestive tract what plays a key role in the animal's health. What is more, balanced microflora leads to better nutrient absorption,and these parameters can have a relation to the blood formation processes and, further, to the good physiological function of all organs.

The effects of probiotics can be explained in various ways and the auto-aggregation capacities of the probiotics in question play an important role (Collado et al. 2008). Ishizaka et al. (2014) reported that the oral administration of probiotics for a period of 28 days increased the LAB count and reduced *E. coli* and *C. perfringens* counts in the gut, resulting in improved intestinal health. Botes et al. (2008) indicated that *L. plantarum* 423 also had a high auto-aggregation capacity. It was thought that these effects could be associated with the stronger competition from pathogenic microbes to the epithelia cells. In addition, after the seventh day of treatment to Wistar rats with the *L. plantarum* strain, a significant decrease (in 3 $\log_{10}$ units) in *E. coli* populations could be observed (Zavisic et al. 2012). In our study, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and a mixture of the two in horses significantly increased the LAB count and significantly reduced the enterobacteria count in the faeces of those horses after thirty days of treatment.

In addition, treatment with probiotics increased the lymphocyte count and acted as an haemogenic agent by increasing the haemoglobin concentration and total erythrocyte count, which may be related to an improvement in performance during exercise by means of increased oxygen transportation (O'Neill et al. 2002). Resting venous (Hb) is often used as a screening tool for health, but this is not a reliable measurement tool for carrying out a fitness and performance assessment in horses (McGowan 2008). Higher RBC levels, plus increased haemoglobin, glucose, and lactate concentration levels which were initially seen during this experiment may, therefore, be linked to those horses that were part of the experiment undergoing exercise during this time. Nervous horses will have elevated heart rates, a factor that also needs to be taken into consideration. High blood lactate concentration levels can lead to acidosis (Jassim et al. 2005). During the experimental period, lactate concentration levels decreased gradually because the experiment used LAB which did not cause an increase in blood lactate concentration levels. However, lactate concentrations significantly increased in the third group. Several mechanisms have been described that could account for this finding, mainly focusing on the possibility of the muscles using short-chain fatty acids instead of carbohydrates as an energy source. Lactobacilli supplementation can modify hindgut pH and induce a proliferation of other genus such as *Veillonella* spp, the most abundant lactate-utilising bacteria in the horse gut (Biddle et al. 2013), one which modifies the energy source during exercise. It was reported that unconditioned horses which had been supplemented with yeast exhibited lower plasma lactate concentrations than those that remained unsupplemented (Glade et al. 1990). The authors speculated that the administration of probiotics can modify gut fermentation and increase the amount of circulating short-chain fatty acids, which are efficiently used as an energy source during exercise. These findings are supported by the results that were obtained by Garcia et al. (2015), who found that horses which received probiotics were more able to digest hemicelluloses. In a previous work, Medina et al. (2002) showed that probiotics such as *Saccharomyces cerevisiae* were able to modify the production and proportion of SCFAs in the large intestine. This could aid the horses in using a lower amount of carbohydrates, which would account for the lower production of muscular lactic acid observed in the present study. Other parameters were statistically influenced by the administration of probiotics, although this was not clinically relevant, such as calcium, magnesium, and glucose, with trends that could be linked to the production of short-chain fatty acids which can modulate both their release and absorption (Skrypnik et al. 2017).

According to Botha et al. (2012), the administration of *L. equigenerosi* Le1 (1 × 109CFU per 50kg of body weight) to healthy horses did not increase the white blood cell count or the differential white blood cell count, whereas AST levels remained constant. Similarly, glucose, lactate, cholesterol, and urea levels remained constant during the administration of prebiotics. In that study it was reported that the gradual decrease in blood glucose and lactate levels in animals that had been administered *L. equigenerosi* Le1 is viewed as being a positive change, since high levels of urea in the blood may lead to encephalopathy (Botha et al. 2012). The lower activity of aspartate aminotransferase in the blood of those horses that were given probiotics suggests their beneficial effect on muscular cell preservation, as it would seem to control intracellular acidosis

thankt to the mechanisms described previously (Filippis et al. 2016).

Salminen et al. (1993) demonstrated that *L acidophilus* leads to lower blood ammonia levels, and similar findings were reported for *Enterococcus faecium* SF68 (Loguercio et al. 1987) and *L. equigenerosi* Le1 (Botha et al. 2012). It was observed that there were no differences in their blood measurements in treatments with *S. cerevisiae* and their evolution over time in horses (Faubladier et al. 2013). However, very few studies have been published about the influence of probiotics on the blood parameters of horses. It was demonstrated that probiotic treatment (*Lactobacillus plantarum* and *Bacillus coagulans*) generated marked reductions in the levels of creatinine, urea, bilirubin and AST, indicating the positive influence of the probiotics on the adverse effects of Hg in rats (Majlesi et al. 2017).

Conclusions

The results of the present study suggest that supplementation with *L. plantarum* LUHS135 and *L. paracasei* LUHS244 strains and mixtures containing both may be beneficial for horses. The results showed that there were significantly higher LAB counts and lower TCE counts in the faeces of horses on day thirty. Probiotic supplementation could reduce blood lactate concentrations in endurance horses that are undergoing athletic activities. According to the results from studying the haematological and biochemical parameters of the blood, it can be seen that the mechanism of this positive effect is connected to a switchover of energy source in muscle - from carbohydrates to short-chain fatty acids. Finally, the preliminary findings suggest that the strains used revealed a potential application as probiotics; however further studies are required to be able to prove survival and the action mechanisms of the newly isolated strains.

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