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Research article

THE EFFICACY OF TWO PHYTOGENIC FEED ADDITIVES IN THE CONTROL OF SWINE DYSENTERY

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Control of swine dysentery with antibiotics is often ineffective due to the resistance of *Brachyspira hyodysenteriae*. The potential of some herbal-based components against *B. hyodysenteriae* was previously studied *in vitro*. This study aims at the evaluation of *in vivo* efficacy of phytogenic feed additives in the control of swine dysentery

The study involved 64 seven-week old weaned pigs allotted to 4 groups: two were fed on feed supplemented with either Patente Herba® or Patente Herba® Plus, the third received tiamulin (positive control), while the negative control was not given antibiotics or additives. Fecal consistency was recorded daily. The presence of *B. hyodysenteriae* in the feces was investigated weekly using microbiological assays and the PCR test. Weight gain and feed conversion ratio were calculated for each week, and for the whole experiment.

B. hyodysenteriae was detected in all samples by both methods. The additives showed efficacy in the prevention and control of swine dysentery as only normal and soft stool was observed in the treated groups. By contrast, in the negative control all feces categories were detected. Frequencies of feces categories significantly differed ($p<0.001$) between feed-supplemented groups and the negative control. Efficacy of both additives in the prevention of SD is comparable to tiamulin, based on insignificant differences in the frequency of the various feces categories.

Beneficial effects of both additives resulted in significantly ($p\leq0.05$) higher weight gain and lower feed conversion ratio in comparison to the negative control. The average weight gains between additive-fed groups and tiamulin-treated group did not differ significantly.

Key words: *Brachyspira hyodysenteriae*, piglets, diarrhea, performance, phytogenic compounds

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INTRODUCTION

Swine dysentery (SD) is a severe form of mucohaemorrhagic colitis of pigs, and one of the most serious and damaging pig diseases in many swine-rearing countries. The etiological agent of SD is the anaerobic intestinal spirochaete *Brachyspira hyodysenteriae* [1-6].

For the treatment of SD, three groups od antibiotics are used: pleuromutilins (tiamulin, valnemulin), macrolides (tilozin, tylvalosin) and lincosamides (lincomycin), despite numerous side effects reported following their use, such as lethargy, depression, erythema, edema, diarrhea, pruritus, prolapse pyrexia, ataxia, anorexia and even death [7].

Pleuromutilins were considered most effective in SD treatment although their increased use caused reduced susceptibility of *B. hyodysenteriae* [8-12]. Antimicrobial resistance against macrolides and lincosamides is widespread, and recent investigations have shown that almost 100% of *B. hyodysenteriae* isolates originating from EU countries are resistent to tylosine and lincomycin [9, 13-15]. In addition, low susceptibility to the same antibiotics has been recently proved for four *Brachyspira* species originating from U.S. swine herds [16]. Moreover, multiresistant isolates of *B. hyodysenteriae* were recorded [14, 17-19]. These facts suggest serious problems in SD treatment and a great need for alternative control of SD.

In intensive animal production, different alternatives to in-feed antibiotics have been increasingly considered due to comparable growth and health promoting effects [20-22]. In recent years, plant-derived compounds (such as herbal extracts, spices and essential oils), known as phytogenic feed additives, have attracted attention because of their multiple favorable properties such as antimicrobial, antioxidant and anti-inflammatory effects that lead to the improvement of gut function, growth performance and carcass quality [23-27].

There is no scientific clinical evidence of the effect of phytogenic feed additives on the prevention and control of SD. Verlinden et al. [28] demonstrated *in vitro* antibacterial potential of several EO components against poultry *Brachyspira* isolates. Besides, feed supplemented with coated *trans*-cinnamaldehyde reduced *Brachyspira* colonization in *in vivo* experiments with young pullets [28]. Recently, the efficacy of herbal components against *B. hyodysenteriae* was tested in several separate studies [29-31], but they were all conducted *in vitro*. However, in order to verify the efficiency of the extract, all the above mentioned authors made a note that its effects should be tested in field conditions. Thus, no phytogenic compound or product has been tested *in vivo* to evaluate their effect against SD.

The objective of our study was to investigate the *in vivo* efficacy of two phytogenic feed additives (Patente–Herba® and Patente–Herba® Plus) in the prevention and control of outbreaks of SD.

MATERIAL AND METHODS

Tested additives and positive control

Commercially available phytogenic feed additives Patente Herba® and Patente Herba® Plus (Patent Co. DOO, Mišićevo, Serbia) were tested. Patente Herba® is a mixture of an essential oil blend (mostly from *Thymus vulgaris*, *Origanum vulgare* and *Coriandrum* sp.), and a plant extract of *Castanea sativa*. Apart from the same ingredients as Patente Herba®, Patente Herba® Plus (Patent Co. DOO, Mišićevo, Serbia) contains lysozyme and nicotinamide. The details of products' recipe are proprietary. Both phytogenic additives were added to the feed in concentrations recommended by the producer: the former in a concentration of 2 kg/t feed and the latter in a concentration of 1 kg/t. Tiamulin - P - 10% (Vetmedic d.o.o., Vršac, Serbia) was used as the positive control in a concentration of 1 kg/t feed.

Experimental design

The study involved 64 seven-week-old weaned pigs originating from the piggery belonging to the Institute of Animal Husbandry, Belgrade, Serbia. All procedures on animals were approved by the Ethical Committee of the Institute of Animal Husbandry, Belgrade-Zemun, Serbia, Ministry of Agriculture and Environmental Protection Republic of Serbia (No 323-07-03476/2017-05, dated 09.05.2017), according to the Serbian Animal Welfare Protection Law, and Directive 2010/63/EU. All animals were kept under the same accommodation and hygiene conditions throughout the experiment. Clinically healthy seven-week-old pigs from 16 litters were tagged, measured, and homogenously allotted to 4 groups, each comprising 16 pigs (one pig from every litter).

The experimental groups of pigs were placed in pens separated by an empty one between any of the two. Group 1 received the feed supplemented with Patente Herba® (PH), and group 2 the feed supplemented with Patente Herba® Plus (PHP). In the third group SD was treated with tiamulin (TC+) and it was the positive control. The fourth group was fed with feed without antibiotics or additives and was the negative control (C-).

Piglets were observed three times a day for feed and water consumption, and symptoms of illness. Fecal consistency was scored as 0 = well-formed, normal feces; 1 = soft (the consistency of wet cement); 2 = runny and/or watery feces; 3 = diarrheic feces containing mucus; 4 = diarrheic feces containing blood.

The presence of *B. hyodysenteriae* in the feces was determined on day 0, one day before the administration of the additives began, and on days 7, 14 and 21. The fecal samples were collected from individual pigs by rectal swabbing for PCR analyses and pooled stool samples obtained from each pen were used for isolation of *B. hyodysenteriae*. A total of 256 individual and 16 pooled stool samples were collected.

Detection of *B. hyodysenteriae*

B. hyodysenteriae was isolated from the samples by the BAM-SR method described by Calderaro et al. [32] including a BHI-SR pre-treatment step. Isolates were further identified by PCR method described by La et al. (2006), after isolation of DNA by boiling. Primers H1 (5´-ACTAAAGATCCTGATGTATTTG-3´) and H2 (5´-CTAATAAACGTCTGCTGC- 3´) designed by La et al. [33] were used also for identification of *B. hyodysenteriae* individual fecal samples after isolation of DNA by QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. PCR was performed in Multigene Gradient Thermal Cycler (Labnet International, Inc) according to conditions given by La et al. [33].

Performance recording

Piglets were weighed on day 0, 7, 14 and 21 to calculate weekly weight gain for each week. Weight gain in the three-week period (from day 0 to 21) was also calculated. Feed intake and feed conversion ratio were recorded weekly, and also for the three-week period (from 0 to 21st day).

Statistical analysis

Statistical analysis of the results was carried out using STATISTICA v6.0 (StatSoft, Inc, Tulsa, USA) software. A chi-squared test was used to evaluate the differences in the frequency of feces categories between groups. Given that the data about pig weight gain for the negative control in the 2nd and 3rd week, as well as in the experiment as a whole were heterogeneous (coefficient of variation >30%), the transformation y=x+1 was applied to all data. The averages were compared in the one-way analysis of variance for completely randomized block design and with Tukey's post hoc test. In the analysis of feed intake and FCR relative numbers were used.

RESULTS

In the 21-day period, in the negative control pigs (C-) all feces categories were detected, and in pigs in other experimental groups (TC+, PH and PHP) only normal and soft feces was seen (Figure 1). χ^2-test revealed a significant difference in the frequency of feces categories between the negative control (C-) group and the TC+ group (χ^2=20.70; p<0.001), between C- and PH group (χ^2=20.74; p<0.001), as well between C- and PHP group (χ^2=22.54; p<0.001). The frequency of normal and soft feces did not differ significantly between groups TC+ and PH (χ^2=0.33; p=0.565), and between TC+ and PHP group (χ^2=2.63; p=0.105). In addition, there was no significant difference in the structure of feces category between groups PH and PHP (χ^2=0.93; p=0.33).

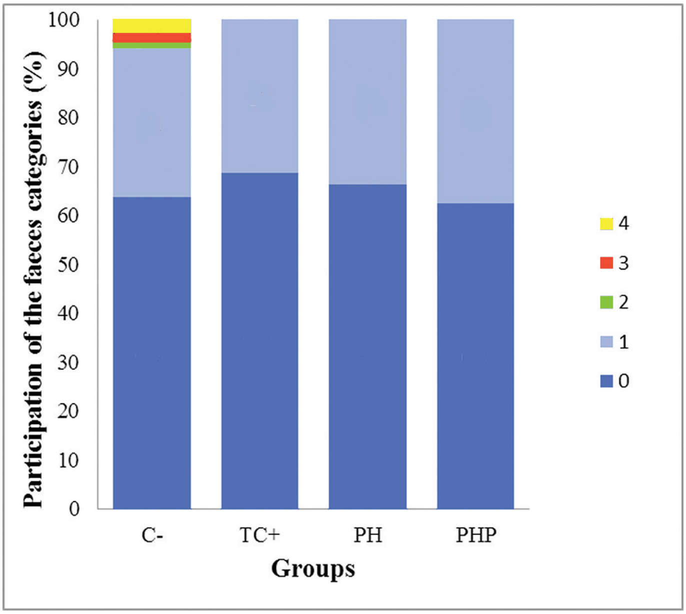

Figure 1. Distribution of faeces categories per groups. The faecal consistency was classified as: 0 = well-formed, normal faeces; 1 = soft yellow to gray faeces; 2 = watery yellow diarrhea; 3 = faces containing large amounts of mucus and flecks of blood; 4 = watery stools containing blood, mucus, and shreds of white mucofibrinous exudates.

In all samples examined with microbiological tests, isolates of spirochaetes were identified as *B. hyodysenteriae*. PCR confirmed the same bacterial pathogen in all samples analyzed.

At the beginning of the experiment (day 0) there were no significant differences in the average weight between the experimental groups of pigs ($F=0.068$, $p=0.376$). The least average weight gain in all of the periods observed (first, second and third week and three-week experimental period as a whole) was detected in group C-. The highest average weight gain in the first week was in groups TC+, PH and PHP, in the second week in groups PH and PHP, in the third week in groups TC+, PH and PHP and throughout the three-week long experimental period in groups PH and PHP. Given the coefficient of variation in the four observation periods, it can be concluded that groups TC+, PH and PHP were homogeneous, unlike group C- (Table 1).

One-way ANOVA showed that there were significant differences in the average weight gain between groups in all of the periods monitored (in the first week $F=10.983$, $p<0.001$, in the second $F=14.868$, $p<0.001$, in the third $F=5.368$, $p=0.003$, and in the whole three-week experimental period $F=17.315$, $p<0.001$). Tukey test revealed significantly ($p\leq0.05$) lower weight gain in group C- than in pigs treated with the antibiotic (TC+), or with phytogenic additives (PH and PHP) in all of the four periods. The average weight gains between groups TC+, PH and PHP did not differ significantly in any of the observed periods.

Table 1. Basic statistical parameters of pig weight gain (per observed periods and per group)

Period (days)	Group	Average	Minimum	Maximum	Coefficient of variation (Cv)
First week (0-7)	C-	0.37	0.26	0.46	16.41
	TC+	0.43	0.37	0.49	7.31
	PH	0.43	0.40	0.46	4.47
	PHP	0.43	0.40	0.46	4.73
Second week (8-14)	C-	0.35	0.04	0.51	43.86
	TC+	0.47	0.37	0.56	8.45
	PH	0.52	0.47	0.59	6.50
	PHP	0.52	0.47	0.59	6.05
Third week (15-21)	C-	0.37	-0.34	0.63	78.21
	TC+	0.54	0.49	0.59	5.82
	PH	0.54	0.46	0.63	8.40
	PHP	0.54	0.41	0.63	8.63
Three-week period (0-21)	C-	0.36	0.10	0.51	32.90
	TC+	0.48	0.44	0.51	3.68
	PH	0.50	0.47	0.52	2.73
	PHP	0.50	0.46	0.51	3.33

In the first week feed conversion ratio (FCR) in the TC+, PH and PHP groups (1.84, 1.80 and 1.81, respectively) was lower by 6.60%, 8.63% and 8.12%, respectively, compared to C- group (1.97). In the second week the ratio between feed input and weight gain was even more favorable: in comparison with the FCR of C- group (2.07), FCR in the TC+, PH and PHP groups (1.85, 1.82 and 1.82, respectively) decreased 10.63%, 12.08% and 12.08%, respectively). At the end of the experiment, in its third week, FCR was 1.95, 1.97, 1.96 and 2.38 in the TC+, PH, PHP and C- groups, respectively; so for each kilogram of weight gain 18.06%, 17.23% and 17.65% less feed was consumed in the TC+, PH and PHP groups, respectively, than in the C- group. In addition, in the first two weeks the FCR was 2.16% lower in PH and PHP groups than in the TC+ group. In the experiment as a whole (days 0-21), FCR was 1.89, 1.87, 1.87 and 2.13 in the TC+, PH, PHP and C- groups, respectively. Thus, FCR decreased by 11.27%, 12.21% and 12.21% in the TC+, PH and PHP groups in comparison with the C- group.

The lowest feed intake was recorded in the C- group. In comparison with the average feed intake in the negative control, in the 1st week the average feed intake in group TC+ increased by 7.71%, in group PH by 5.75% and by 6.12% in group PHP. In the week which followed, these differences increased: 21.25% (TC+), 30.34% (PH) and 30.83% (PHP). In the 3rd week the increase in average feed consumption reached 25.10% (TC+), 23.84% (PH) and 25.00% (PHP). In the experiment as a whole (days

0-21), the average feed intake was higher than in the C- group by 18.39% in the TC+ group, 20.17% in group PH and 20.87% in group PHP.

DISCUSSION

To the authors' knowledge this is the first research into the efficacy of phytogenic products in SD prevention and control evaluated in a clinical trial. The need for testing the efficacy of such products has increased in the recent years due to the abundance of the reports about decreased susceptibility of *B. hyodysenteriae* to antibiotics most commonly used in the prevention and treatment of SD. Besides, inefficacy of drugs in the treatment of SD in endemically infected herds or preventing disease outbreak in SD-free herds, and decreased susceptibility of *B. hyodysentariae* isolates present an additional risk for the emerging of resistant *B. hyodysenteriae* clones to some or even all antibiotics that are commonly used to treat SD, and which then can be spreading on the farm or between the farms through common sources [11, 19]. Consequently, alternative measures for the control of SD are more than welcome.

The potential of phytogenic compounds in the prevention and control of enteric diseases in pigs is worth to be studied because of their beneficial impact on gut microbiota, digestive function and growth performance of weaning pigs [25, 34-36]. Besides, herbal-based products are generally recognized as safe (GRAS), and do not cause resistance in bacteria or adverse effects on animal health and consumer safety [21, 26]. However, only in a few *in vitro* studies, the activity of herbal-derived compounds against *B. hyodysenteriae* was tested [29-31]. In our study, the efficacy of phytogenic products in the prevention and control of outbreaks of SD was monitored *in vivo*, by evaluating the effects of two phytogenic feed additives, Patente–Herba® and Patente–Herba® Plus.

The results of this study suggest that both additives tested exert efficacy in prevention and control of outbreaks of SD. *B. hyodysenteriae*, the causative agent of SD, was confirmed in all samples microbiologically and with PCR. This conclusion is based on the results of fecal analysis which detected normal or soft stool in groups supplemented with either additive (Patente–Herba® or Patente–Herba® Plus), while all feces categories were recorded in the negative control. It is important to emphasize that the frequencies of feces categories significantly differed ($p<0.001$) between feed-supplemented groups and the negative control group.

The results of weight gain measurements also suggest beneficial effect of both additives in this study, as significant differences between groups in all of the periods monitored resulted from significantly ($p \leq 0.05$) lower weight gain in the negative control compared to groups treated with either additive. Higher feed intake in groups that consumed feed with phytogenic additives can be associated to essential oils stimulative effects on appetite due to enhanced flavor and odor of feed [24]. Lower FCR results observed in the whole three-week experimental period (days 0-21) in PH

(1.89), PHP (1.87) and CT+ (1.87) groups compared to the negative control (2.13), can contribute to the cost efficacy of the tested phytogenic additives. In the same line, it is worth highlighting that PHP additive in a dose of 1 kg/t exerts the same effect as PH in the dose of 2 kg/t.

Our results indicate that both tested additives have an efficacy in the prevention of SD comparable to tiamulin, since the noticed frequency of the different feces categories did not significantly differ between additives-fed groups and antibiotic-treated group. In addition, the effects of tested additives are comparable, as there were no significant differences in the structure of feces between additive-fed groups. Accordingly, neither of the tested additives showed any advantage over the other. The same conclusions were drawn from the results of weight gain measurements, as this parameter did not differ significantly between additive-fed groups, and between any of additive-fed groups and the tiamulin-treated group in all of the periods observed.

The efficacy of thymol and carvacrol against *B. hyodysenteriae,* as well as their synergistic effect has been confirmed *in vitro* [30]. Since both essential oils are present in the tested additives we could speculate that the *in vivo* antimicrobial activity of these is certainly partly responsible for the efficacy of Patente–Herba® and Patente–Herba® Plus in the control of SD. In a recent research conducted by Draskovic et al. [27] Patente–Herba® Plus has shown efficacy in the control of proliferative enteropathy (caused by *Lawsonia intracellularis*) and improved feed efficiency in pigs.

In summary, to our best knowledge, this is the first *in vivo* study in which the effect of phytogenic products in the control of SD was assessed. The tested additives Patente–Herba® and Patente–Herba® Plus proved effective in the treatment of this devastating pig illness caused by *B. hyodysenteriae.* Although the effects of the products in the prevention and control of SD were comparable with those of tiamulin, the use of herbal-based products should be stimulated to reduce the risk for the emergence of resistant *B. hyodysenteriae* isolates and avoid problems associated with the use of antibiotics.

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Authors' contributions

DN carried out the experiment, made substantial contribution to acquisition, analysis and interpretation of data and participated in manuscript writing. SZ and SJ conceived and designed the study. They made substantial contributions to the writing of manuscript, critically revised the manuscript and approved its submission. DV coordinated experiment performance and has been involved in manuscript writing. SB na NJB made substantial contributions to interpretation of data and writing the manuscript. LN performed the statitistical analysis and made substantial contributions to interpretation of data. All authors read and approved the final manuscript.

Declaration of conflicting interests

One co-author (Jasna Bosnjak-Neumuller) is employed in Patent Co. DOO None of the other authors has any other financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of the paper.

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EFIKASNOST DVA FITOGENA ADITIVA U KONTROLI DIZENTERIJE SVINJA

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Suzbijanje dizenterije svinja antibioticima često je neefikasno zbog rezistencije uzročnika *Brachyspira hyodysenteriae*. Dejstvo nekih biljnih sastojaka protiv *B. hyodysenteriae* već je bilo izučavano u uslovima *in vitro*. Cilj ovog rada je procena *in vivo* efikasnosti fitogenih aditiva u kontroli dizenterije svinja.

Istražiavanje je obavljeno na 64 odlučena praseta starosti sedam nedelja podeljenih u 4 grupe: dve su dobijale hranu u koju je dodat aditiv Patente Herba®, odnosno Patente Herba® Plus, treća je dobijala tiamulin (pozitivna kontrola), a četvrta je predstavljala negativnu kontrolu jer nije dobijala ni aditiv, niti antibiotik. Konzistencija fecesa je beležena svakog dana. Prisustvo *B. hyodysenteriae* u fecesu ispitivano je jednom nedeljno primenom mikrobioloških metoda i PCR testa. Prirast i konverzije hrane izračunati su za svaku nedelju posebno, kao i za ceo eksperimentalni period.

B. hyodysenteriae je dokazana u svim uzorcima primenom obe metode. Aditivi su pokazali efikasnost u prevenciji i suzbijanju dizenterije svinja s obzirom da je u svim treti-

ranim grupama zabeležen samo normalan i mek feces. Nasuprot tome, u negativnoj kontroli ustanovljene su sve kategorije fecesa. Učestalost kategorija fecesa značajno ($p<0,001$) se razlikovala između tretiranih grupa i negativne kontrole. Efikasnost oba aditiva u prevenciji dizenterije svinja može se uporediti sa efikasnošću tiamulina, s obzirom da između tih grupa nije bilo značajnih razlika u učestalosti kategorija fecesa.

Korisni efekti oba ispitivana aditiva doveli su do značajno većeg ($p\leq0,05$) prirasta i značajno niže ($p\leq0,05$) konverzije hrane u poređenju sa negativnom kontrolom. Prosečan prirast nije se značajno razlikovao između grupa koje su dobijale aditive i grupe tretirane antibiotikom.