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Assessment of phytobiotic supplementation to modulate pen-level infection dynamics of Swine Dysentery using a challenge model

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Assessment of phytobiotic supplementation to modulate pen-level infection dynamics of Swine Dysentery using a challenge model

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Abstract

Gut health is a key determinant of profitability and sustainability in swine production. Swine Dysentery, caused by *Brachyspira hyodysenteriae*, is a severe enteric disease affecting animal health and performance. Restrictions on antimicrobial use have increased the need for alternative strategies, including plant-derived phytobiotics, which may support intestinal health and host responses. This study evaluated the efficacy of a novel phytobiotic feed additive in an experimental Swine Dysentery challenge model.

Thirty weaned piglets were assigned to a challenged positive control group, a challenged phytobiotic-supplemented group, or a non-challenged phytobiotic-supplemented negative control group. Faecal shedding, clinical signs, growth performance, and colonic histopathology were assessed over 28 days post inoculation.

All challenged pigs shed *B. hyodysenteriae* at least once. However, phytobiotic-supplemented pigs showed fewer qPCR-positive faecal samples than positive controls, shorter shedding, and reduced proportions of diarrhoeic and dysentery-like faeces. Fewer pigs were classified as Swine Dysentery positive in the phytobiotic group, although not significantly. Histopathology showed reduced intraluminal crypt infiltrate, reaching values comparable to the negative control. Growth performance differed moderately among groups.

Overall, the additive did not prevent infection but reduced bacterial shedding and clinical outcomes, supporting its use as a complementary strategy for Swine Dysentery control under experimental challenge conditions.

Keywords

Swine Dysentery, Phytobiotics, Gut health, Essential oils, Enteric pathogens.

Background

Gut health, a concept which could be defined as normal physiological functioning of digestive organs and intestinal lining that support digestion and absorption of nutrients, immune function and balanced microbiota composition with lack of harmful disturbances, such as infections. Gut health largely determines the potential profitability of pig production and guarantees a reduced environmental impact and safe pork products (1,2). Conversely, intestinal infectious diseases directly affect animal growth, feed conversion, and increase cost associated to treatments and mortality rates (3–7). Regulations towards prudent antimicrobial use have limited the tools available and increased the complexity of control in enteric diseases over the last two decades (8–10). This situation demands novel control measures focused on improving and maintaining gut health such as probiotics (11,12) postbiotics (13), prebiotics (14) and phytobiotics-phytochemicals (15–17).

Phytobiotics were first defined as plant-derived natural bioactive compounds that can be incorporated into diets to enhance the performance and health of animals (18). The biotechnological development of novel phytobiotics has gained relevance, given their reported effects on microbiota modulation, reduction of oxidative stress, and antimicrobial properties (15,19–22). In this sense, studies conducted in nursery (19,21) and grow-finish pigs (15,23) have exhibited meaningful antibacterial activity against swine enteropathogenic bacteria, including multidrug-resistant isolates (21,24). Among the phytobiotics tested, a wide variety of plant secondary metabolites has been described with different mechanisms depending on the bioactive compounds involved, including phenolic compounds such as thymol, carvacrol and eugenol; aromatic aldehydes such as cinnamaldehyde; terpenes such as menthol and citronellol; and bioactive constituents derived from turmeric, red pepper, and rosemary (22,24–29).

Swine Dysentery (SD) is an insidious enteric disease classically observed in grower–finisher pigs (5,32,33). and produced by *Brachyspira hyodysenteriae* (onwards abbreviated as *B. hyo*), a Gram-negative, β hemolytic, anaerobic spirochete. *Brachyspira suanatina* and *Brachyspira hampsonii*, this later mostly in North America (31,32), have also been implicated in SD outbreaks (33,34). Clinically, the disease is characterized by severe mucohaemorrhagic diarrhoea, marked inflammation of the large intestine, epithelial necrosis, and alterations in mucin production and the mucus layer structure (35,36). Antimicrobial therapy halts the progression of the disease and prompts the recovery of infected animals. However, the number of molecules available to treat SD is limited to macrolides, pleuromutilins, lincosamides and aminoglycosides (37) and recent studies have demonstrated that their efficacy is limited by the development of antibiotic resistance (38). In this sense, there is an urgent need to explore alternative therapies which can help to reduce antimicrobial use or complement them in the control of SD.

Phytobiotics against SD have been poorly investigated, and the available evidence remains limited despite their potential as supportive strategies for improving intestinal health and resilience. This study evaluated the efficacy of a novel nano-emulsified phytochemical feed additive, formulated with rosemary, carvacrol, thymol, cinnamaldehyde, and turmeric, in an experimental swine dysentery challenge model using weaned pigs infected with *Brachyspira hyodysenteriae*. The infection outcome was assessed through pathogen shedding, clinical disease development, histopathological lesion scoring, and pig growth performance.

Materials and methods

Brachyspira hyodysenteriae culture

Brachyspira hyodysenteriae B204 strain (ATCC 31212) was used for the experimental challenge. The strain was cultured in Columbia agar plates with 5% defibrinated sheep

blood (Oxoid, UK) for 72 h at 37.5 °C under a strict anaerobic atmosphere (80% N_2, 10% H_2, and 10% CO_2), maintained using a Whitley A35 Anaerobic Workstation (Don Whitley Scientific, UK).

Experimental design

The study was conducted at the biosafety level 2 animal facility of the University of León and was approved by the Ethical Committee on Animal Experimentation of the University of León (OEBA-ULE-007-2025) and the regional government (Junta de Castilla y León). All experimental methods and procedures were performed in accordance with the relevant guidelines and regulations. Thirty crossbred piglets (Landrace x Large White x Pietrain x Duroc) four-weeks-old (average weight ~ 7 kg) were supplied from a commercial farm in León, Spain. All were confirmed as free of *B. hyo*, *B. pilosicoli* and *Salmonella spp.* by culture at the beginning of the study. Pigs were randomly distributed in three different groups with equal number of male and female within each group: challenged (positive control; n = 12), challenged and supplemented with the phytobiotic (phytobiotic; n = 12), and not challenged but supplemented with the phytobiotic (negative control; n = 6). Each experimental group was allocated to an individual box with controlled temperature ranging between 20-25 ºC. Pigs were individually identified using ear tags for monitoring and data collection. Pigs had *ad libitum* feed (composition of feed and phytobiotic in supplementary file 1) and water access.

After 7-day acclimation period to ensure proper adjustment to the housing conditions, handling procedures, and environment, pigs belonging to groups positive control and phytobiotic, were challenged over three consecutive days (days 0, 1, and 2 of the experiment) with a Columbia agar plate with 5% defibrinated sheep blood (Oxoid, UK) grown with the *B. hyo* B204 strain (ATCC 31212). Only plates exhibiting a growth over

90% of the plate surface, containing approximately 1×10^8 spirochetes, were used for the challenge.

Faecal monitoring was performed daily by digital rectal stimulation. Faeces were scored as described previously (39) with values ranging from 0 to 4 (0 = normal faeces; 1 = soft faeces; 2 = liquid diarrhoea; 3 = diarrhoea with mucus; 4 = bloody diarrhoea). All faecal samples were stored at −80 ºC for later analysis (DNA extraction and *B. hyo* qPCR quantification). Weight was recorded periodically, starting with an initial measurement at the pigs arrival (day -7; W1), just before the challenge (day 0, W2), during the challenge monitoring period (day 14 and day 21 post inoculation hereafter P.I., W3, W4), and at the end of the study (day 28, W5). The average daily gain (ADG) was subsequently determined for each individual pig and for each experimental group respectively whereas average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated at the group level based on total feed intake and total body weight gain for each treatment group. Severely affected pigs (faecal score 3 to 4 for two consecutive days) received a rescue treatment with a three-day regimen of lincomycin (Linalten®, Spain) administered intramuscularly at a dose of 1 mL per 10 kg of body weight.

Euthanasia was performed on days 27 and 28 P.I. Pigs were first sedated with midazolam (0.5 mg/kg) and ketamine (10 mg/kg), then euthanized via intravenous administration of pentobarbital (140 mg/kg). During necropsy, colon contents were collected through an incision at the apex of the spiral colon and stored at - 20 °C until processing. A section of the apex sections (at least 5 cm in length, together with the associated lymph nodes, were washed with PBS as and fixed in 10% buffered formalin for subsequent staining and microscopic examination.

DNA extraction

Faecal and intestinal content DNA was extracted using a commercial stool DNA extraction kit following the manufacturer's instructions (Stool DNA Purification Kit, EurX, Poland). Briefly, extraction was performed from 200 mg of faecal or intestinal content, and DNA was finally eluted in 100 µl.

Detection and quantification *of B. hyo* by quantitative real-time PCR (qPCR)

The quantification of *B. hyo* in faecal and intestinal content samples was estimated by quantitative PCR (qPCR) in the QuantStudio 1 Flex System (Applied Biosystems, USA) using specific *B. hyo* primers targeting the NOX gene, BRACHY-F (5′-TTG CTA CTG GTT CTT GGC CTG-3′) and BRACHY-R (5′-GAA TGC TTC TAT AAG TTC AAC ACC TAT-3′), with a TaqMan-specific probe (Thermo Scientific, USA), BRACHY-PROBE (FAM-5'-CGA AGG CTT AAA ACA AGA AGG A-3'-BHQ) (40). The reaction was carried out in a 20 µL volume containing 10 µL of maxima probe qPCR Master Mix (Thermo Scientific, USA), 0.2 µL of BRACHY-Probe at 10 µM (Applied Biosystems, USA), 0.3 µL of each primer, 7.08 µL of nuclease-free water (Thermo Fisher Scientific, USA), 0.12 µL of ROX 5 µM (Thermo Fisher Scientific, USA), and 2 µL of template DNA. An in-house standard curve was used to convert Ct values into bacterial counts per gram of faeces (bacteria/g of faeces). The curve was generated from ten-fold serial dilutions of *B. hyo* B204 (initial titre 10^8 bacteria/g of faeces; range 10^8 to 10^3 bacteria/g of faeces). The detection limit was established at 10^3 bacteria/g of faeces. A *B. hyo* shedder was defined as an animal with at least one positive faecal sample to *B. hyo* qPCR with a load ≥ 3.0 $\log_{10}$ (10^3 bacteria/g of faeces). A pig was defined as SD positive when it had qPCR-positive faecal samples with a *B. hyo* concentrations above 10^3 bacteria/g of faeces and, among those positive samples, met at least one of the following faecal-score criteria: three or more days with a faecal score of 2 or higher, two or more days with a faecal score of 3 or higher, or one day with a faecal score of 4.

Histopathological evaluation of colon lesions

Tissue samples from the apex of the spiral colon were fixed in 10% buffered formalin for 48 h, processed using conventional histological techniques, and stained with haematoxylin–eosin (H&E) for microscopic examination. Histopathological assessment was performed independently by two blinded veterinary pathologists following a methodology developed by the Department of Anatomy and Comparative Pathology and Toxicology of the University of Córdoba, Spain.

Lesions were evaluated using a semiquantitative scoring system based on three main parameters:

1. Mucosal epithelial status (0–5): 0 = normal, unaltered epithelium (simple columnar); 1 = areas with flattened or detaching epithelium; 2 = focally extended flattened epithelium; 3 = focal erosion (loss of epithelium); 4 = focally extended erosions; and 5 = ulceration reaching the lamina propria.
2. Inflammatory infiltrate in the lamina propria (0–4): evaluated by intensity and distribution as 0 = absent; 1 = infiltrate without crypt separation; 2 = infiltrate with focal crypt separation; 3 = multifocal crypt separation; and 4 = diffuse crypt separation.
3. Intraluminal crypt infiltrate (0–4): determined by counting crypts with infiltrate foci in five fields using a 10× objective, where 0 = absent; 1 = 1–5 crypts with infiltrate foci; 2 = 6–10 crypts; 3 = >10 crypts; and 4 = presence of giant cells within crypts.

The baseline histopathological score was calculated as the sum of these three main parameters (maximum 13 points). Where observed, additional findings including haemorrhage (+1) and the presence of *Balantidium coli* (+1) were added to this baseline, allowing for a maximum cumulative score of 15 points.

198

199 **Statistical analysis**

200 All statistical analyses and graphs for comparing among treatments (phytobiotic, positive
201 control and negative control) were performed using R software (v. 4.5.2) (41) within R-
202 studio environment (v. 2026.01.1) (42). Differences in the proportion of qPCR-positive
203 samples were tested using Fisher's exact test while differences in the proportion of
204 diarrhoea (faecal score ≥ 2 vs < 2) and dysentery-like faeces (faecal score ≥ 3 vs < 3)
205 were assessed using Pearson's chi-square tests. The proportion of SD-positive pigs
206 between groups was also assessed using a chi-square test.

207 The risk of disease onset (SD-positive) was analysed using Kaplan–Meier curves, and
208 potential differences were tested using the log-rank test (43). Shedding duration (days)
209 and number of medication events per pig were compared using the Wilcoxon rank-sum
210 test (Mann–Whitney U) while comparison of *B. hyo* concentration among qPCR-positive
211 samples was performed using the Kruskal–Wallis test (two-group non-parametric
212 comparison) on the $\log_{10}$-transformed scale.

213 For growth performance, linear models were fitted with ADG as the outcome and
214 cumulative faecal score (sum), group, and their interaction (group × faecal score) as
215 predictors. Models were fitted for the full period (0–27 P.I. days) and separately for each
216 interval (0–14 days, 14–21 days, and 21–27 days). The same modelling approach was
217 repeated after stratifying the dataset by health status at the time of scoring (healthy vs SD-
218 positive). Evidence for differential ADG–faecal score associations between groups was
219 assessed via the interaction term. All analyses were performed in R using dplyr/tidyr
220 package (v. 1.2.1 and v. 1.3.2) for data handling (44,45), lme4 for mixed-effects modelling
221 (v. 2.0-6) (46) and emmeans (v. 2.0.4) to estimate group-specific slopes and test between-

group differences via pairwise contrasts of marginal trends (positive control vs phytobiotic; two-sided *p*-values, no multiplicity adjustment) (47).

Group differences in the histopathology scores (total score, mucosal epithelial damage, inflammatory lamina propria infiltrate/crypt separation, and intraluminal crypt infiltrate) were tested using one-way ANOVA, followed by pairwise between-group comparisons.

Results

Brachyspira hyodysenteriae shedding outcome and faecal score results

During the follow-up period (day 27-28 P.I.) the 12 pigs in each challenged group shed *B. hyo* in faeces in at least one point while no samples in the negative control group tested positive for *B. hyo.* The number of positive samples in the positive control group was 111 out 276 samples (40.2%) in the phytobiotic group compared to 76 of 276 (27.5%) in the phytobiotic group ($p = 0.002$).

A mean excretion period of 9.25 days per animal was observed in the positive control group, which was significantly longer than the 6.33 days observed in the phytobiotic group ($p = 0.03$) (Fig. 1). The analysis of the mean concentration of *B. hyo* in positive faeces (Fig. 2) revealed no significant differences between the positive control group (5.91 $\log_{10}$ bacteria/g of faeces) and the phytobiotic group (6.23 $\log_{10}$ bacteria/g of faeces).

The study examining the evolution of faecal scores in shedding pigs was conducted starting on day 5 P.I. which coincided with the first *B. hyo* qPCR positive sample. The positive control group attained an average faecal score of 1.52, the phytobiotic group recorded a mean value of 0.93, and the negative control arrange a mean value of 0.33. Additionally, a cumulative measurement was performed by aggregating the faecal scores recorded from days 5 to 27 P.I., resulting in values of 29.67 and 20.17 for the positive

control and phytobiotic groups, respectively. The statistical analysis primarily aimed to distinguish between normal faeces (scores of 0–1) and diarrhoea (scores ≥2) (Fig. 3). The positive control exhibited diarrhoea in 91 out 260 faecal samples (37.0%), compared to 58 out 262 (22.1%) in the phytobiotic group (p = 0.001). A subsequent analysis was conducted to compare the proportion of dysenteric faeces (scores 3–4), recording 53 out 260 samples in the positive control group (20.4%) and 31 out 262 samples in the phytobiotic group (11.8%) (p = 0.01).

Swine Dysentery outcome results

A total of eight pigs were recorded as SD positive in the positive control group, compared with five pigs in the phytobiotic group, with no statistically significant differences between groups. Disease outcome was further assessed using a Kaplan–Meier curve to compare the time to disease onset between groups (Fig. 4). The log-rank test did not reveal significant differences between the survival curves. Similarly, no significant differences were observed in the proportion of qPCR-positive samples or in the shedding period (Figs. 1 and 2).

Faecal scores, diarrhoea incidence, dysenteric faeces, bacterial excretion levels, and the number of medication administrations were comparable in SD positive animals between positive control and phytobiotic groups. Although the positive control group showed slightly higher mean faecal scores, cumulative faecal scores, and medication requirements, none of these differences reached statistical significance. Overall, the phytobiotic group showed a lower number of SD positive animals and medication events, but the clinical outcomes among SD positive animals did not differ significantly from those observed in the positive control group.

Histopathological analyses

The histopathological evaluation of the colonic mucosa revealed differences in the overall mean lesion score among experimental groups (Figs. 5 and 6).

The negative control group exhibited the lowest mean score (3.50 ± 1.26), representing the baseline structural integrity of the tissue. In contrast, the positive control group recorded the highest degree of tissue damage (5.60 ± 1.75), showing a statistically significant increase compared to the negative control. The phytobiotic group presented an intermediate overall score (4.58 ± 1.48), positioning between the healthy baseline and the severe tissue damage observed in the untreated infected animals.

Regarding the individual parameters, a detailed assessment showed that the negative control group displayed a well-preserved colonic mucosa, characterized only by isolated areas of flattened epithelium (1.58 ± 0.66). Conversely, both the phytobiotic and positive control groups showed a greater extent of focal epithelial flattening and greater interindividual variability, yielding mean scores of 1.87 ± 1.44 and 1.81 ± 1.25, respectively. Evaluation of the lamina propria demonstrated a mild presence of immune cells with minimal crypt separation in the negative control group (1.75 ± 0.61). In contrast, the positive control group exhibited a severe inflammatory response, predominantly characterized by a dense neutrophilic infiltrate that resulted in a marked separation between crypts (2.31 ± 0.51). The phytobiotic group displayed an inflammatory response in this layer (2.29 ± 0.45) comparable to that of the positive control group, with crypt separation predominantly occurring in a focal and occasionally multifocal distribution.

Notably, the assessment of intraluminal infiltrate within the crypts revealed the most marked histological difference between the challenged groups; while the positive control group exhibited a prominent accumulation of inflammatory cells within the crypts (1.45

± 0.93), the phytobiotic group displayed a minimal intraluminal infiltrate (0.41 ± 0.46). Consequently, for this parameter, the phytobiotic-treated animals where similar to the negative control group (0.16 ± 0.40), effectively differentiating their lesional pattern from that of the positive control group.

Production parameters

Assessment of growth performance based on ADG, FCR, and ADFI revealed a consistent ranking among treatments (Fig. 8, Supplementary file 2). Overall, the negative control showed the highest ADG and the most favourable FCR (1.376), whereas the positive control exhibited the poorest feed efficiency (FCR, 1.487). The phytobiotic group consistently displayed intermediate performance, with an overall FCR of 1.465.

Interval-based analyses of ADG (W1–W2, W2–W3, W3–W4, and W4–W5) showed that growth performance varied over time, with the negative control consistently outperforming the other groups. This difference became more evident during the final interval (W4–W5), when the negative control reached an ADG of 0.687 kg/day. The phytobiotic group followed a similar but less pronounced pattern, reaching 0.531 kg/day, whereas the positive control showed a more limited improvement, with an ADG of 0.468 kg/day. Feed efficiency was generally better in the negative control, while the positive control and phytobiotic groups displayed greater fluctuations across intervals.

Interval-based analyses of ADFI showed that feed intake varied over time, decreasing in all groups during W2–W3 before recovering in W3–W4 and increasing further during the final interval (W4–W5). In this final interval, ADFI reached 0.990 kg/day in the negative control and 0.862 kg/day in the phytobiotic group, compared with 0.675 kg/day in the positive control. This late increase in feed intake coincided with the improvement in ADG

observed in the negative control and, to a lesser extent, in the phytobiotic group, whereas the positive control maintained both lower feed intake and lower growth performance. Further analysis of growth dynamics (ADG) and clinical burden (cumulative faecal score) were tested for between-group differences by the group × faecal score interaction. Analyses included each interval time period (W2–W3, W3–W4, and W4–W5) and were additionally stratified including all animals and by health status at the time of scoring (sheeder vs SD–positive).

At the overall level (D0–27 PI; W2–W5), no significant between-group differences were observed in the ADG–faecal score relationship. Likewise, when these models were fitted using data from all animals, no significant differences in slopes were detected during the W2–W3 and W3–W4 intervals. In contrast, during the final interval (W4–W5), the negative association between faecal score and ADG was significantly attenuated in the phytobiotic group compared with the positive control (interaction $p = 0.030$). Stratified analyses showed that, among healthy pigs, slope differences were better for the phytobiotic group in the W3–W4 ($p = 0.009$) and the W4–W5 intervals ($p < 0.001$) compared to the positive control. Among SD positive animals in both groups, a significant between-group difference was detected in the W3–W4 interval ($p < 0.001$), driven by a markedly stronger negative slope in the positive control and an essentially flat relationship in the phytobiotic group.

Discussion

The present study evaluates a novel commercial phytobiotic, formulated with plant extracts and essential oils, in an experimental model of SD induced by *B. hyo*. Challenge models offer an opportunity to evaluate control strategies under controlled conditions,

341 removing potential external factors which may bias field studies (36). On the other hand,
342 the high dose usually used to guarantee the success of the infection may overcome the
343 protective action of the active compounds. The findings of the study indicate that the
344 tested product may contribute to the prevention and alleviation of SD caused by *B. hyo*,
345 as reflected by a reduction in the prevalence of qPCR-positive samples, the number of
346 shedding days per group, the overall mean faecal score, and the severity of
347 histopathological lesions in the colon.

348 Overall, the *in vivo* results suggest that the phytobiotic intervention did not completely
349 prevent the occurrence of *B. hyo* shedding, as the same number of shedding pigs was
350 observed in both challenged groups. However, when the total shedding was considered,
351 the phytobiotic decreased the proportion of qPCR-positive samples, leading to a 31%
352 relative reduction in positivity among shedding pigs. Furthermore, the duration of
353 shedding was significantly reduced in the phytobiotic group by 32%, equivalent to
354 approximately three less days of shedding. From a disease management perspective, these
355 findings are particularly relevant, since reducing both the percentage of positive samples
356 and the duration of bacterial excretion may decrease environmental contamination and
357 infection pressure during SD outbreaks, which is a key strategy to limit disease spread
358 not only within a batch but also to subsequent batches of pigs. Infection success depends
359 strongly on the quantity and persistence of bacteria shed into the environment; therefore,
360 greater shedding increases faecal–oral transmission and intensifies the exposure of pen-
361 mates through environmental contamination (48–50). This reduction in shedding was
362 accompanied by improved faecal outcomes, particularly regarding diarrhoea and
363 dysentery-like diarrhoea, suggesting a protective effect in the phytobiotic group, where
364 the clinical disease process appeared to be limited, as reflected by a significantly better
365 overall faecal score and relative reduction of both diarrhoea and dysentery-like diarrhoea.

To the best of the authors' knowledge, only a limited number of studies have evaluated the specific mitigation of SD using essential-oil-based phytobiotic blends. Previous *in vivo* studies have likewise supported the potential of essential-oil-based phytobiotic supplementation for controlling SD with a formulations containing essential oils derived from thyme, oregano, and coriander, together with chestnut extract. This supplementation prevented the development of severe diarrhoea, with supplemented pigs showing only normal or soft faeces and an improved clinical response. However, *B. hyo* remained detectable by culture and PCR throughout the 21-days trial, indicating control of clinical disease rather than bacterial clearance (51). In a subsequent field study, a phytobiotic composed of essential oils from thyme, oregano, and coriander, chestnut extract, and clinoptilolite progressively reduced diarrhoea, with no clinical cases observed from week 3 onwards. In this case, supplementation was associated with a reduction in the faecal load of *B. hyo* which fell below the detection limit only at the end of the seven-week study (52).

Notably, the effect of the phytobiotic was not statistically evident among SD–positive pigs, which showed similar mean *B. hyo* shedding during the acute phase and a similar shedding duration to non-supplemented challenged pigs. Although the differences did not reach statistical significance, the mean faecal score among clinically affected pigs was 0.48 points lower in the phytobiotic group than in the positive control group. A similar non-significant trend was observed for therapeutic requirements, with pigs receiving the phytobiotic requiring, on average, 2.2 fewer days of medication than untreated challenged pigs. Only one pig in the phytobiotic group required a second treatment cycle, whereas four pigs in the positive control group required a second and, in some cases, a third cycle. The comparable positive correlation between *B. hyo* shedding and faecal score in both

challenged groups suggests that the phytobiotic did not alter the relationship between bacterial excretion and clinical severity. Overall, these findings indicate that the phytobiotic did not prevent infection or substantially alter shedding dynamics once clinical disease was established. However, its association with lower bacterial excretion and disease expression at the group level may help limit relapse and reduce transmission pressure within the pen. Consistently the lower therapeutic requirements may be related to the reduced clinical severity rather than to an additional protective effect during the acute phase.

Multiple mechanisms of action have been described for essential oil compounds, depending on the nature and composition of the product tested. Some of them can support beneficial bacterial populations, preserve microbial balance, reduce intestinal pH, reinforce epithelial barrier integrity or modulate oxidative and inflammatory responses (15,19,20,53–55). Some of these mechanisms are particularly relevant in *B. hyo* pathogenesis, as neutrophil-driven inflammation which can promote the internalization of tight-junction proteins, removing them from the apical membrane and weakening the paracellular seal. In addition, similar formulations may help counteract the oxidative stress and mitochondrial dysfunction associated with SD which may contribute to disease development (56), by activating pathways that upregulate antioxidant enzymes and other protective proteins involved in cellular stress and inflammatory responses (15,17,19). Overall, these combined effects on the microbiota, epithelial barrier, and redox balance provide a coherent mechanistic framework for interpreting the reduced expression of acute disease observed in the present study and the subsequent containment of infection dynamics at the pen level.

Although no significant differences were detected in the mucosal epithelial status or lamina propria inflammatory infiltrate between the challenged groups, the intraluminal crypt infiltrate was significantly reduced in the phytobiotic group, reaching values comparable to the negative control. This parameter quantifies intraluminal inflammatory foci within colonic crypts. In acute SD, clinically affected pigs develop microscopic haemorrhagic colitis with abundant neutrophil infiltration in the lamina propria and lumen, which leads to the formation of *intraluminal foci* (57). Therefore, the reduction observed in the phytobiotic group indicates an attenuation of the active and exudative components of colitis at the crypt level. This aligns with experimental SD scoring systems that incorporate crypt lesions and abscesses as key indicators of severity (58,59). Finally, the lack of significant differences in mucosal damage or lamina propria infiltrate between the challenged groups may reflect the spatially heterogeneous and stage-dependent nature of SD lesions.

Growth performance outcomes (ADG and FCR) should be interpreted as supportive endpoints in enteric challenge models, because reductions in growth are often driven by time-varying decreases in feed intake and increased maintenance costs during infection (60). Consistent with this framework, experimental *B. hyo* infection has been shown to markedly impact ADG and FCR negatively with the largest performance penalties matching the onset and peak of clinical disease (60). Previous challenges evaluating phytobiotic supplementation, reported an improved feed efficiency in supplemented pigs compared with infected controls (51,61,62). In our study, the phytobiotic group showed an intermediate performance profile between the non-challenged negative control and the challenged positive control. However, the distance from the positive control was smaller when summarised across the full period. Notably, the phytobiotic-related effect was most evident in the ADG–faecal score relationship in the late phase of the study (the last two

440 measured intervals), where the phytobiotic attenuated the growth penalty associated with
441 increasing clinical burden compared with the positive control. This phase-specific
442 protection in phytobiotic animals prevents a decline in ADG during the acute phase of
443 SD, facilitates recovery, and establishes the basis for restoration from a higher level than
444 the positive control.
445
446 **Conclusions**

447 This *B. hyo* experimental challenge provides a proof-of-concept evidencing that the tested
448 phytobiotic can support SD control. The most consistent benefits were observed at the
449 group level, with reduced qPCR positivity and a shorter shedding period, together with
450 an improved faecal-score profile and a reduction of the severity of histopathological
451 lesions in the colon, which is consistent with lower infection pressure and reduced within-
452 pen re-exposure. Effects within SD positive animals were less evident; however, trends
453 toward fewer medication days and retreatment cycles suggest a potential contribution to
454 relapse control. Growth performance outcomes were not markedly separated from the
455 positive control when summarized across the full period, but the treatment attenuated the
456 growth penalty associated with acute disease. Histopathology showed a selective effect,
457 with reduced inflammatory intraluminal crypt infiltrate reaching levels comparable to the
458 negative control despite similar mucosal epithelial and lamina propria scores between the
459 challenged groups. Future studies should evaluate and clarify underlying mechanisms,
460 particularly microbiota modulation, epithelial barrier function, and redox/inflammatory
461 pathways. Phytobiotic strategies, such as the one evaluated here, represent a useful
462 complementary approach to reduce reliance on antibiotics within integrated SD control
463 programs.

Declarations

Ethics approval and consent to participate

The study was conducted at the biosafety level 2 animal facility of the University of León and was approved by the Ethical Committee on Animal Experimentation of the University of León (OEBA-ULE-007-2025) and the regional government (Junta de Castilla y León).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

This study was funded by Animal Health Concept. The funder participated in the study design and reviewed the manuscript before submission but had no role in data collection, data analysis, interpretation of the results, or the decision to publish. The authors declare no other competing interests.

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Authors' contributions

A.C. and H.A. conceived and supervised the study. S.G.M, A.U.C, A.L.G, L.P.P, and C.G. participated in the experimental challenge, animal handling and sampling, and laboratory work. H.P. and C.D.A. performed the histopathological processing and evaluation. S.G. analysed the data, prepared the figures, and S.G.M, A.C., H.P and H.A. wrote the manuscript. All authors have read and approved the final manuscript.

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Figures

Figure 1. Shedding period, expressed in days, by treatment group and health status. Violin plots with embedded boxplots show the distribution of shedding time for the phytobiotic (Phytob) and the positive control groups, shown separately for shedder animals and Swine Dysentery (SD) positive animals. Points represent individual animals, diamonds indicate group means, and labels indicate the mean shedding time and the number of animals included in each group

Figure 2. *B. hyodysenteriae* shedding load by treatment group and animal status. Boxplots show the distribution of *B. hyodysenteriae* load, expressed as log10 bacteria/g of faeces, in the phytobiotic group (Phytob) and positive control group, shown separately for shedder animals and Swine Dysentery (SD)-positive animals. Points represent individual qPCR-positive samples, boxes indicate the interquartile range, horizontal lines

indicate medians, and whiskers show the data range. Labels indicate group means. Between-group comparisons were performed using the Wilcoxon rank-sum test, with *p*-values shown above each panel.

Figure 3. Faecal score and infection-status matrices in pigs during the experimental period. On the top, colour matrices show the temporal evolution of individual faecal scores by sampling day, stratified by treatment group: phytobiotic group (Phytob) at the top and positive control group at the bottom. Faecal consistency was scored as follows: 0, normal faeces; 1, semi-solid faeces; 2, liquid diarrhoea; 3, diarrhoea with mucus; and 4, haemorrhagic diarrhoea. Diarrhoeic samples were defined as faecal score ≥ 2, and dysenteric diarrhoea as faecal score ≥ 3. White-highlighted animal IDs indicate animals not classified as Swine Dysentery (SD) positive, whereas non-highlighted IDs indicate SD positive animals. On the bottom, heatmaps show the temporal evolution of *B. hyo* shedding load and faecal score for each pig during the experimental period, stratified by treatment group: Phytob at the top and positive control at the bottom. Numerical values within cells indicate *B. hyo* concentration, expressed as log10 bacteria/g of faeces, for qPCR-positive samples, defined as Quantity_log ≥ 3.0. White-highlighted animal IDs indicate animals not classified as SD-positive, whereas non-highlighted IDs indicate SD-positive animals. Black cells represent non-sampled timepoints or animal death. Black-bordered squares indicate medicated animals.

Figure 4. Kaplan–Meier curves showing the probability of remaining free from diarrhoea during the experimental period by treatment group. Survival probability represents the proportion of pigs that had not yet developed diarrhoea at each sampling day. Diarrhoea was defined as a faecal score ≥ 2. Curves are shown for the phytobiotic group (Phytob) and the positive control group. Shaded areas represent 95% confidence

intervals. The number of animals at risk at each sampling day is shown below the plot. Differences between-group were assessed using the log-rank test ($\chi^2 = 2.06$, $p = 0.151$).

Figure 5. Colon lesion score by treatment group. Bar plot showing the total colon lesion score in the phytobiotic group (Phytob), positive control group, and Negative Control group. The total colon lesion score was calculated as the sum of the mucosal epithelium score, lamina propria score, and crypt damage score. Bars represent group means, error bars indicate variability around the mean, and black points represent individual animals. Pairwise statistical comparisons are shown above the plot; ns indicates non-significant differences, and * indicates a statistically significant difference between groups ($p < 0.05$).

Figure 6. Representative colon tissue sections by treatment group and histological compartment. Representative haematoxylin and eosin-stained colon sections are shown for the Negative Control, phytobiotic group (Phytob), and Positive Control group. Columns correspond to the evaluated histological compartments: mucosal epithelium, inflammatory infiltrate in the lamina propria, and intraluminal crypt infiltrate. Images were selected as representative sections based on the average score for each analysed compartment within each treatment group. Scale bars are shown in each image.

Figure 7. Performance metrics by treatment group across the experimental period. Line plots on the left show feed conversion ratio (FCR), average daily feed intake (ADFI), and average daily gain (ADG) by experimental week interval (W1–W2, W2–W3, W3–W4, and W4–W5). Points represent observed group means, with numerical values displayed above each point. Treatment groups are shown as phytobiotic group (Phytob) in green, Positive Control pigs in blue, and Negative Control pigs in black. Heatmaps on the right show the percentage difference for the Positive Control and Phytob groups

relative to the Negative Control group for each week interval and for the overall experimental period. Red indicates lower values than the Negative Control group, whereas blue indicates higher values.

Figure 8. Relationship between average daily gain and faecal score by animal status and time interval. Scatterplots show average daily gain (ADG; kg/day per pig) as a function of cumulative faecal score within each interval, displayed separately for D0–14, D14–21, and D21–27, and stratified by all animals, Swine Dysentery (SD) positive animals, and non SD positive animals. Points represent individual pigs, with labels indicating animal ID, and are coloured by treatment group: Positive Control in blue and Phytobiotic-treated pigs (Phytob) in green. Solid lines represent group-specific linear fits from models including the Group × faecal score interaction within each interval and animal-status category. Annotation boxes report the between-group difference in slope between Positive Control and Phytob ($\Delta\beta = \beta\text{Positive Control} - \beta\text{Phytob}$) and the interaction *p*-value testing whether the association between ADG and cumulative faecal score differs between treatment groups.

References

1. Finatto, A. N. Swine dysentery: a review of the factors involved in disease expression. Anim. Health Res. Rev. 1-47; 10.1017/S1466252326100073 (2026).

2. Alarcón, L. V., Allepuz, A. & Mateu, E. Biosecurity in pig farms: a review. Porcine Health Manag. 7, 5; 10.1186/s40813-020-00181-z (2021).

3. Panah, F. M., Lauridsen, C., Højberg, O. & Nielsen, T. S. Etiology of colitis-complex diarrhea in growing pigs: a review. Animals 11, 2151; 10.3390/ani11072151 (2021).

4. Turlewicz-Podbielska, H. & Pomorska-Mól, M. Porcine coronaviruses: overview of the state of the art. Virol. Sin. 36, 833-851; 10.1007/s12250-021-00364-0 (2021).

5. Alvarez-Ordóñez, A., Martínez-Lobo, F. J., Arguello, H., Carvajal, A. & Rubio, P. Swine dysentery: aetiology, pathogenicity, determinants of transmission and the

fight against the disease. Int. J. Environ. Res. Public Health 10, 1927-1947; 10.3390/ijerph10051927 (2013).

6. Luppi, A., D'Annunzio, G., Torreggiani, C. & Martelli, P. Diagnostic approach to enteric disorders in pigs. Animals 13, 338; 10.3390/ani13030338 (2023).

7. Vidal, A. et al. Prevalence of enteric pathogens in diarrheic and non-diarrheic samples from pig farms with neonatal diarrhea in the North East of Spain. Vet. Microbiol. 237, 108419; 10.1016/j.vetmic.2019.108419 (2019).

8. European Parliament & Council of the European Union. Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. Off. J. Eur. Union L 4, 43-167 (2019).

9. European Parliament & Council of the European Union. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Off. J. Eur. Union L 268, 29-43 (2003).

10. Xu, C., Kong, L., Gao, H., Cheng, X. & Wang, X. A review of current bacterial resistance to antibiotics in food animals. Front. Microbiol. 13, 822689; 10.3389/fmicb.2022.822689 (2022).

11. Jiang, Z. et al. Probiotics in piglet: from gut health to pathogen defense mechanisms. Front. Immunol. 15, 1468873; 10.3389/fimmu.2024.1468873 (2024).

12. Barba-Vidal, E., Martín-Orué, S. M. & Castillejos, L. Review: are we using probiotics correctly in post-weaning piglets? Animal 12, 2489-2498; 10.1017/S1751731118000873 (2018).

13. Polimeni, B., Correa, F., Trevisi, P. & Luise, D. Review: a systematic review on the use and effects of postbiotics on the general health, gut health, and performance of sows and piglets. Animal 20, 101749; 10.1016/j.animal.2025.101749 (2026).

14. Kiernan, D. P., O'Doherty, J. V. & Sweeney, T. The effect of prebiotic supplements on the gastrointestinal microbiota and associated health parameters in pigs. Animals 13, 3012; 10.3390/ani13193012 (2023).

15. Sung, J. Y., Parnsen, W., Garavito-Duarte, Y. & Kim, S. W. Efficacy of an herb and essential oil-based phytobiotic blend and its interaction with an antibiotic on intestinal health, growth performance, and carcass characteristics in growing pigs. J. Anim. Sci. 103, skaf370; 10.1093/jas/skaf370 (2025).

16. Ferdous, M. F. et al. Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. J. Adv. Vet. Anim. Res. 6, 409-415; 10.5455/javar.2019.f361 (2019).

17. Pandey, S. et al. Cutting-edge knowledge on the roles of phytobiotics and their proposed modes of action in swine. Front. Vet. Sci. 10, 1265689; 10.3389/fvets.2023.1265689 (2023).

18. Vidanarachchi, J. K., Mikkelsen, L. L., Sims, I., Iji, P. A. & Choct, M. Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds. Recent Adv. Anim. Nutr. Aust. 15, 131-144 (2005).

19. Garavito-Duarte, Y., Deng, Z. & Kim, S. W. Literature review: opportunities with phytobiotics for health and growth of pigs. Ann. Anim. Sci. 25, 1237-1247; 10.2478/aoas-2024-0119 (2025).

20. Garavito-Duarte, Y. et al. Investigation of the nutritional and functional roles of a microencapsulated blend of botanicals on intestinal health and growth of nursery pigs challenged with F18+ Escherichia coli. J. Anim. Sci. 103, skaf047; 10.1093/jas/skaf047 (2025).

21. Keum, G. B. et al. Effects of phytobiotics on intestinal barrier function and gut microbiome in weaned piglets challenged with enterotoxigenic Escherichia coli. J. Anim. Sci. Technol. 68, 181-196; 10.5187/jast.2500128 (2026).

22. Kaschubek, T. et al. Effects of phytogenic feed additives on cellular oxidative stress and inflammatory reactions in intestinal porcine epithelial cells. J. Anim. Sci. 96, 3657-3669; 10.1093/jas/sky263 (2018).

23. Juhász, Á., Molnár-Nagy, V., Bata, Z., Tso, K. H. & Posta, K. Phytobiotic-prebiotic feed additive containing a combination of carob pulp, chicory, and fenugreek improve growth performance, carcass traits, and fecal microbiota of fattening pigs. Animals 13, 3621; 10.3390/ani13233621 (2023).

24. Liu, H. Y. et al. Alternatives to antibiotics in pig production: looking through the lens of immunophysiology. Stress Biol. 4, 1; 10.1007/s44154-023-00134-w (2024).

25. Zhao, B. C. et al. Effects of dietary supplementation with a carvacrol-cinnamaldehyde-thymol blend on growth performance and intestinal health of nursery pigs. Porcine Health Manag. 9, 24; 10.1186/s40813-023-00317-x (2023).

26. Gómez-García, M. et al. In-depth in vitro evaluation of the activity and mechanisms of action of organic acids and essential oils against swine enteropathogenic bacteria. Front. Vet. Sci. 7, 572947; 10.3389/fvets.2020.572947 (2020).

27. Gómez-García, M. et al. Combined in-vitro and on-farm evaluation of commercial disinfectants used against *Brachyspira hyodysenteriae*. Porcine Health Manag. 8, 3; 10.1186/s40813-021-00244-9 (2022).

28. Verlinden, M. et al. In vitro sensitivity of poultry *Brachyspira intermedia* isolates to essential oil components and in vivo reduction of *Brachyspira intermedia* in

rearing pullets with cinnamaldehyde feed supplementation. Poult. Sci. 92, 1202-1207; 10.3382/ps.2012-02690 (2013).

29. Toso, F. et al. Antimicrobial activity of Origanum vulgare L. and Salvia rosmarinus Spenn. (syn. *Rosmarinus officinalis* L.) essential oil combinations against *Escherichia coli* and *Salmonella Typhimurium* isolated from poultry. Processes 13, 2856; 10.3390/pr13092856 (2025).

30. Pérez-Pérez, L. et al. From predisposition to recovery: field evidence of interactions between the gut microbiota and *Brachyspira hyodysenteriae* infection. Vet. Res. 57, 25; 10.1186/s13567-025-01646-1 (2026).

31. Puentes, R. et al. A curcumin derivative metalloprotease inhibitor (CMC2.24) mitigates *Brachyspira spp*.-induced swine dysentery. Int. Immunopharmacol. 163, 115312; 10.1016/j.intimp.2025.115312 (2025).

32. Hill, J. E. et al. Retrospective detection of *Brachyspira hampsonii* in archived colitis cases from western Canadian swine. Transbound. Emerg. Dis. 66, 381-388; 10.1111/tbed.13032 (2019).

33. Mushtaq, M., Zubair, S., Råsbäck, T., Bongcam-Rudloff, E. & Jansson, D. S. Brachyspira suanatina sp. nov., an enteropathogenic intestinal spirochaete isolated from pigs and mallards: genomic and phenotypic characteristics. BMC Microbiol. 15, 208; 10.1186/s12866-015-0537-y (2015).

34. Mirajkar, N. S., Phillips, N. D., La, T., Hampson, D. J. & Gebhart, C. J. Characterization and recognition of *Brachyspira hampsonii* sp. nov., a novel intestinal spirochete that is pathogenic to pigs. J. Clin. Microbiol. 54, 2942-2949; 10.1128/JCM.01717-16 (2016).

35. Alvarez-Ordóñez, A., Martínez-Lobo, F. J., Arguello, H., Carvajal, A. & Rubio, P. Swine dysentery: aetiology, pathogenicity, determinants of transmission and the fight against the disease. Int. J. Environ. Res. Public Health 10, 1927-1947; 10.3390/ijerph10051927 (2013).

36. Pérez-Pérez, L. et al. New insights into swine dysentery: faecal shedding, macro and microscopic lesions and biomarkers in early and acute stages of *Brachyspira hyodysenteriae* infection. Porcine Health Manag. 10, 24; 10.1186/s40813-024-00375-9 (2024).

37. Vega, C. et al. In vitro evaluation of gentamicin activity against Spanish field isolates of *Brachyspira hyodysenteriae*. Porcine Health Manag. 8, 48; 10.1186/s40813-022-00291-w (2022).

38. Hampson, D. J. et al. Antimicrobial resistance in *Brachyspira*—an increasing problem for disease control. Vet. Microbiol. 229, 59-71; 10.1016/j.vetmic.2018.12.019 (2019).

39. Vangroenweghe, F. & Hammer, J. M. Adapted faecal clinical scoring systems for post-weaning diarrhea due to enterotoxigenic Escherichia coli and swine

dysentery due to *Brachyspira hyodysenteriae*. *Med. Res. Arch.* **12**, 5875; 10.18103/mra.v12i10.5875 (2024).

40. Song, Y. & Hampson, D. J. Development of a multiplex qPCR for detection and quantitation of pathogenic intestinal spirochaetes in the faeces of pigs and chickens. Vet. Microbiol. 137, 129-136; 10.1016/j.vetmic.2008.12.020 (2009).

41. R Core Team. R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, 2025).

42. Posit Software, PBC. RStudio: Integrated Development Environment for R (Posit Software, PBC, Boston, 2025).

43. Kaplan, E. L. & Meier, P. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53, 457-481; 10.1080/01621459.1958.10501452 (1958).

44. Wickham, H., François, R., Henry, L., Müller, K. & Vaughan, D. dplyr: A grammar of data manipulation. https://CRAN.R-project.org/package=dplyr; 10.32614/CRAN.package.dplyr (2026).

45. Wickham, H., Vaughan, D. & Girlich, M. tidyr: Tidy messy data. https://CRAN.R-project.org/package=tidyr; 10.32614/CRAN.package.tidyr (2025).

46. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1-48; 10.18637/jss.v067.i01 (2015).

47. Lenth, R. V. & Piaskowski, J. emmeans: Estimated marginal means, aka least-squares means. https://CRAN.R-project.org/package=emmeans; 10.32614/CRAN.package.emmeans (2026).

48. Mahu, M. et al. An avirulent *Brachyspira hyodysenteriae* strain elicits intestinal IgA and slows down spread of swine dysentery. Vet. Res. 48, 55; 10.1186/s13567-017-0465-y (2017).

49. Vangroenweghe, F. et al. Evaluation of a zinc chelate on clinical swine dysentery under field conditions. Porcine Health Manag. 6, 1; 10.1186/s40813-019-0140-y (2020).

50. Parra-Aguirre, J. et al. Experimental natural transmission (seeder pig) models for reproduction of swine dysentery. PLoS ONE 17, e0275173; 10.1371/journal.pone.0275173 (2022).

51. Delić, N. et al. The efficacy of two phytogenic feed additives in the control of swine dysentery. Acta Vet. Beograd 68, 178-189; 10.2478/acve-2018-0016 (2018).

52. Wu, C. F. et al. The effect of a phytogenic-based feed additive on concurrent Lawsonia intracellularis and *Brachyspira hyodysenteriae* infections in pigs. S. Afr. J. Anim. Sci. 55, 85-97; 10.4314/sajas.v55i2.04 (2025).

53. Juhász, Á., Molnár-Nagy, V., Bata, Z., Tso, K. H. & Posta, K. Phytobiotic-prebiotic feed additive containing a combination of carob pulp, chicory, and fenugreek improve growth performance, carcass traits, and fecal microbiota of fattening pigs. Animals 13, 3621; 10.3390/ani13233621 (2023).

54. Pandey, S. et al. Cutting-edge knowledge on the roles of phytobiotics and their proposed modes of action in swine. Front. Vet. Sci. 10, 1265689; 10.3389/fvets.2023.1265689 (2023).

55. Wang, J., Ghosh, S. S. & Ghosh, S. Curcumin improves intestinal barrier function: modulation of intracellular signaling and organization of tight junctions. Am. J. Physiol. Cell Physiol. 312, C438-C445; 10.1152/ajpcell.00235.2016 (2017).

56. Costa, M. O. & Harding, J. C. S. Swine dysentery disease mechanism: *Brachyspira hampsonii* impairs the colonic immune and epithelial repair responses to induce lesions. Microb. Pathog. 148, 104470; 10.1016/j.micpath.2020.104470 (2020).

57. Fodor, C. C. et al. Colonic innate immune defenses and microbiota alterations in acute swine dysentery. Microb. Pathog. 173, 105873; 10.1016/j.micpath.2022.105873 (2022).

58. Burrough, E. R., Arruda, B. L. & Plummer, P. J. Comparison of the luminal and mucosa-associated microbiota in the colon of pigs with and without swine dysentery. Front. Vet. Sci. 4, 139; 10.3389/fvets.2017.00139 (2017).

59. Daniel, A. G. S. et al. Synergic effect of *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* coinfection: anatomopathological and microbiome evaluation. Animals 13, 2611; 10.3390/ani13162611 (2023).

60. Helm, E. T., Lin, S. J., Gabler, N. K. & Burrough, E. R. *Brachyspira hyodysenteriae* infection reduces digestive function but not intestinal integrity in growing pigs while disease onset can be mitigated by reducing insoluble fiber. Front. Vet. Sci. 7, 587926; 10.3389/fvets.2020.587926 (2020).

61. Hansen, C. F. et al. A high dietary concentration of inulin is necessary to reduce the incidence of swine dysentery in pigs experimentally challenged with *Brachyspira hyodysenteriae*. Br. J. Nutr. 106, 1506-1513; 10.1017/S000711451100208X (2011).

62. Helm, E. T., Lin, S. J., Gabler, N. K. & Burrough, E. R. Brachyspira hyodysenteriae infection reduces digestive function but not intestinal integrity in growing pigs while disease onset can be mitigated by reducing insoluble fiber. Front. Vet. Sci. 7, 587926; 10.3389/fvets.2020.587926 (2020).

Shedder animals

SD positive animals

Shedding time (days)

30

25

20

15

10

5

0

mean=6.33

mean=9.25

mean=10

mean=11.25

n=12

n=12

n=5

n=8

Phytob

Positive Control

Phytob

Positive Control

Shedder animals

Wilcoxon p = 0.131

SD positive animals

Wilcoxon p = 0.796

Log_{10} *B. hyodysenteriae* (bacteria/g of faeces)

8

6

4

mean = 5.15

mean = 5.54

mean = 5.94

mean = 6.02

Phytob
(n = 12 pigs)

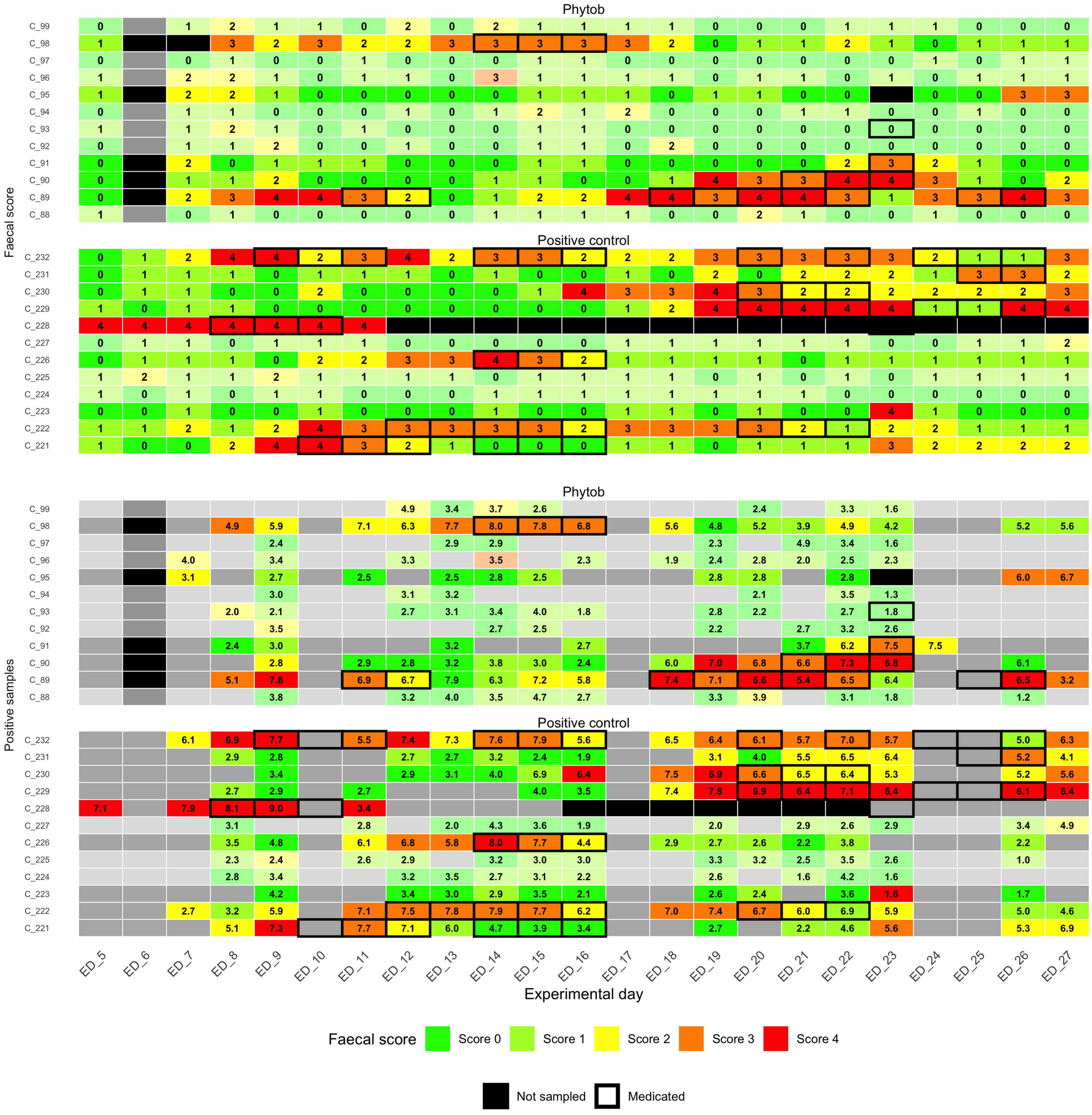
Positive Control
(n = 12 pigs)

Phytob
(n = 5 pigs)

Positive Control
(n = 8 pigs)

Phytob

Positive Control


Phytob
Faecal score
C_99 0 1 2 1 1 0 2 0 2 1 1 1 1 0 0 0 1 1 1 0 0 0
C_98 1 3 2 3 2 2 3 3 3 3 3 2 0 1 1 2 1 0 1 1 1
C_97 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0 0 0 0 1 0 1 1
C_96 1 2 2 1 0 1 1 0 3 1 1 1 1 0 1 1 0 1 0 1 1 1
C_95 1 2 2 1 0 0 0 0 0 1 1 1 0 1 1 0 0 0 0 3 3
C_94 0 1 1 0 0 0 1 0 1 2 1 2 0 0 0 1 1 0 0 1 0 0
C_93 1 1 2 1 0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0
C_92 0 1 1 2 0 0 1 0 0 1 1 0 2 0 0 0 0 0 0 0 0 0
C_91 0 2 0 1 1 1 0 0 0 1 1 0 0 0 0 0 2 3 2 1 0 0
C_90 0 1 1 2 0 0 0 0 1 1 0 0 1 4 3 3 4 4 3 1 0 2
C_89 0 2 3 4 4 3 2 0 1 2 2 4 4 3 4 4 3 1 3 3 4 3
C_88 1 0 1 0 0 0 0 0 1 1 1 1 0 0 2 1 0 0 1 0 0 0
Positive control
C_232 0 1 2 4 4 2 3 4 2 3 3 2 2 2 3 3 3 3 3 2 1 1 3
C_231 0 1 1 1 0 1 1 1 0 1 0 0 1 0 2 0 2 2 2 1 3 3 2
C_230 0 1 1 0 0 2 0 0 0 0 1 4 3 3 4 3 2 2 2 2 2 2 3
C_229 1 0 1 1 0 0 0 0 0 0 0 0 1 2 4 4 4 4 4 1 1 4 4
C_228 4 4 4 4 4 4 4
C_227 0 1 1 0 1 1 1 0 0 0 0 0 1 1 1 1 1 1 0 0 1 1 2
C_226 0 1 1 1 0 2 2 3 3 4 3 2 1 1 1 1 0 1 1 1 1 1 1
C_225 1 2 1 1 2 1 1 1 1 0 1 1 1 1 0 1 0 1 0 1 1 1 1
C_224 1 0 1 0 1 1 0 0 0 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0
C_223 0 0 1 0 0 1 0 0 0 1 0 0 1 1 0 1 0 0 4 1 0 0 0
C_222 1 1 2 1 2 4 3 3 3 3 3 2 3 3 3 3 2 1 2 2 1 1 1
C_221 1 0 0 2 4 4 3 2 1 0 0 0 1 1 0 1 1 1 3 2 2 2 2
Animal ID
Phytob
C_99 4.9 3.4 3.7 2.6 2.4 3.3 1.6
C_98 4.9 5.9 7.1 6.3 7.7 8.0 7.8 6.8 5.6 4.8 5.2 3.9 4.9 4.2 5.2 5.6
C_97 2.4 2.9 2.9 2.3 4.9 3.4 1.6
C_96 4.0 3.4 3.3 3.5 2.3 1.9 2.4 2.8 2.0 2.5 2.3
C_95 3.1 2.7 2.5 2.5 2.8 2.5 2.8 2.8 2.8 6.0 6.7
C_94 3.0 3.1 3.2 2.1 3.5 1.3
C_93 2.0 2.1 2.7 3.1 3.4 4.0 1.8 2.8 2.2 2.7 1.8
C_92 3.5 2.7 2.5 2.2 2.7 3.2 2.6
C_91 2.4 3.0 3.2 2.7 3.7 6.2 7.5 7.5
C_90 2.8 2.9 2.8 3.2 3.8 3.0 2.4 6.0 7.0 6.8 6.6 7.3 6.8 6.1
C_89 5.1 7.8 6.9 6.7 7.9 6.3 7.2 5.8 7.4 7.1 6.6 5.4 6.5 6.4 6.5 3.2
C_88 3.8 3.2 4.0 3.5 4.7 2.7 3.3 3.9 3.1 1.8 1.2
Positive samples
Positive control
C_232 6.1 6.9 7.7 5.5 7.4 7.3 7.6 7.9 5.6 6.5 6.4 6.1 5.7 7.0 5.7 5.0 6.3
C_231 2.9 2.8 2.7 2.7 3.2 2.4 1.9 3.1 4.0 5.5 6.5 6.4 5.2 4.1
C_230 3.4 2.9 3.1 4.0 6.9 6.4 7.5 6.9 6.6 6.5 6.4 5.3 5.2 5.6
C_229 2.7 2.9 2.7 4.0 3.5 7.4 7.8 6.9 6.4 7.1 6.4 6.1 6.4
C_228 7.1 7.9 8.1 9.0 3.4
C_227 3.1 2.8 2.0 4.3 3.6 1.9 2.0 2.9 2.6 2.9 3.4 4.9
C_226 3.5 4.8 6.1 6.8 5.8 8.0 7.7 4.4 2.9 2.7 2.6 2.2 3.8 2.2
C_225 2.3 2.4 2.6 2.9 3.2 3.0 3.0 3.3 3.2 2.5 3.5 2.6 1.0
C_224 2.8 3.4 3.2 3.5 2.7 3.1 2.2 2.6 1.6 4.2 1.6
C_223 4.2 3.4 3.0 2.9 3.5 2.1 2.6 2.4 3.6 1.6 1.7
C_222 2.7 3.2 5.9 7.1 7.5 7.8 7.9 7.7 6.2 7.0 7.4 6.7 6.0 6.9 5.9 5.0 4.6
C_221 5.1 7.3 7.7 7.1 6.0 4.7 3.9 3.4 2.7 2.2 4.6 5.6 5.3 6.9
ED_5 ED_6 ED_7 ED_8 ED_9 ED_10 ED_11 ED_12 ED_13 ED_14 ED_15 ED_16 ED_17 ED_18 ED_19 ED_20 ED_21 ED_22 ED_23 ED_24 ED_25 ED_26 ED_27
Experimental day
Faecal score
Score 0
Score 1
Score 2
Score 3
Score 4
Not sampled
Medicated

Group Phytob Positive control

Survival probability

1.00
0.75
0.50
0.25
0.00

Log-rank: $\chi^2(1) = 2.06$, $p = 0.151$

0 5 10 15 20 25 27 28

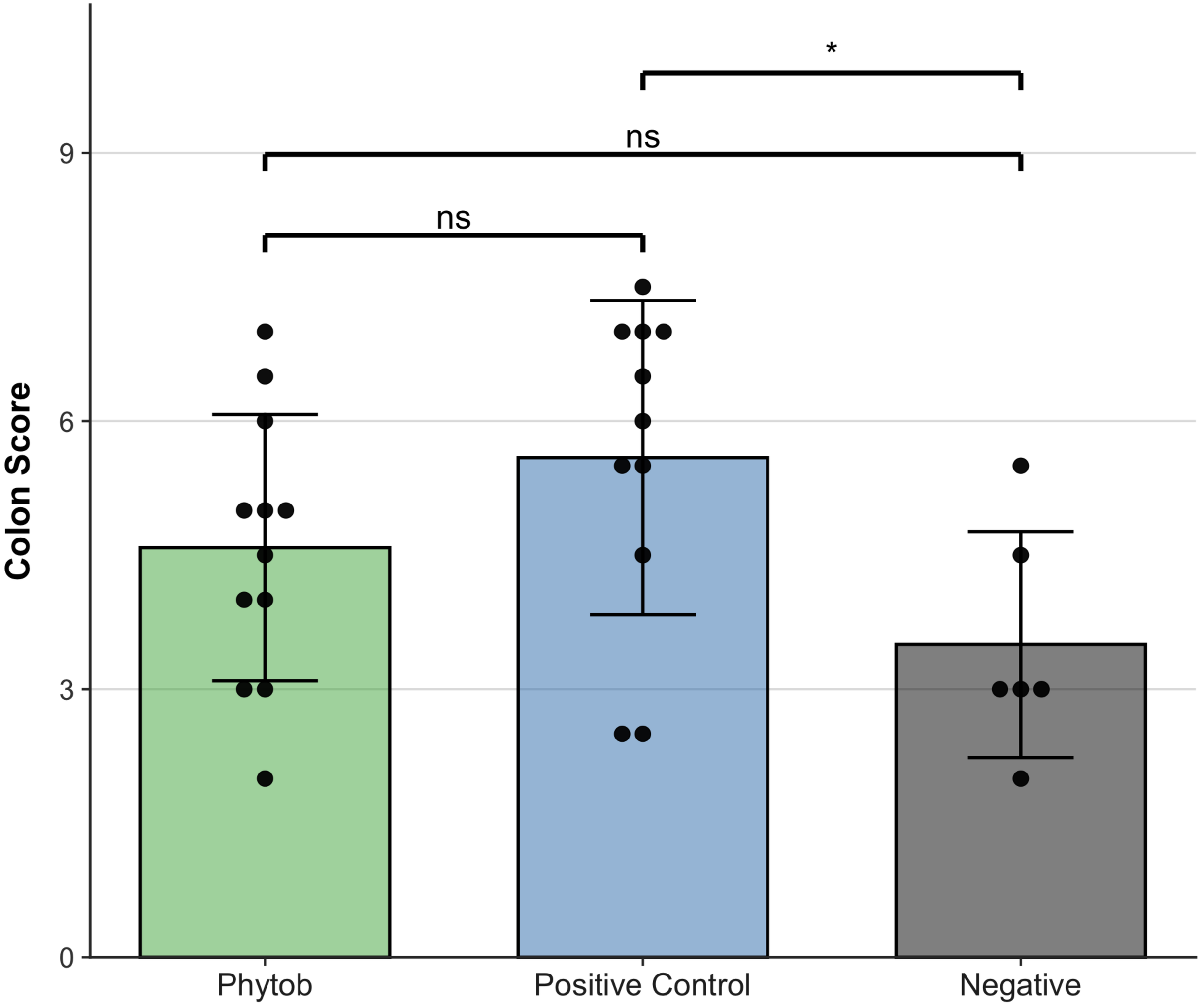
Sampling day

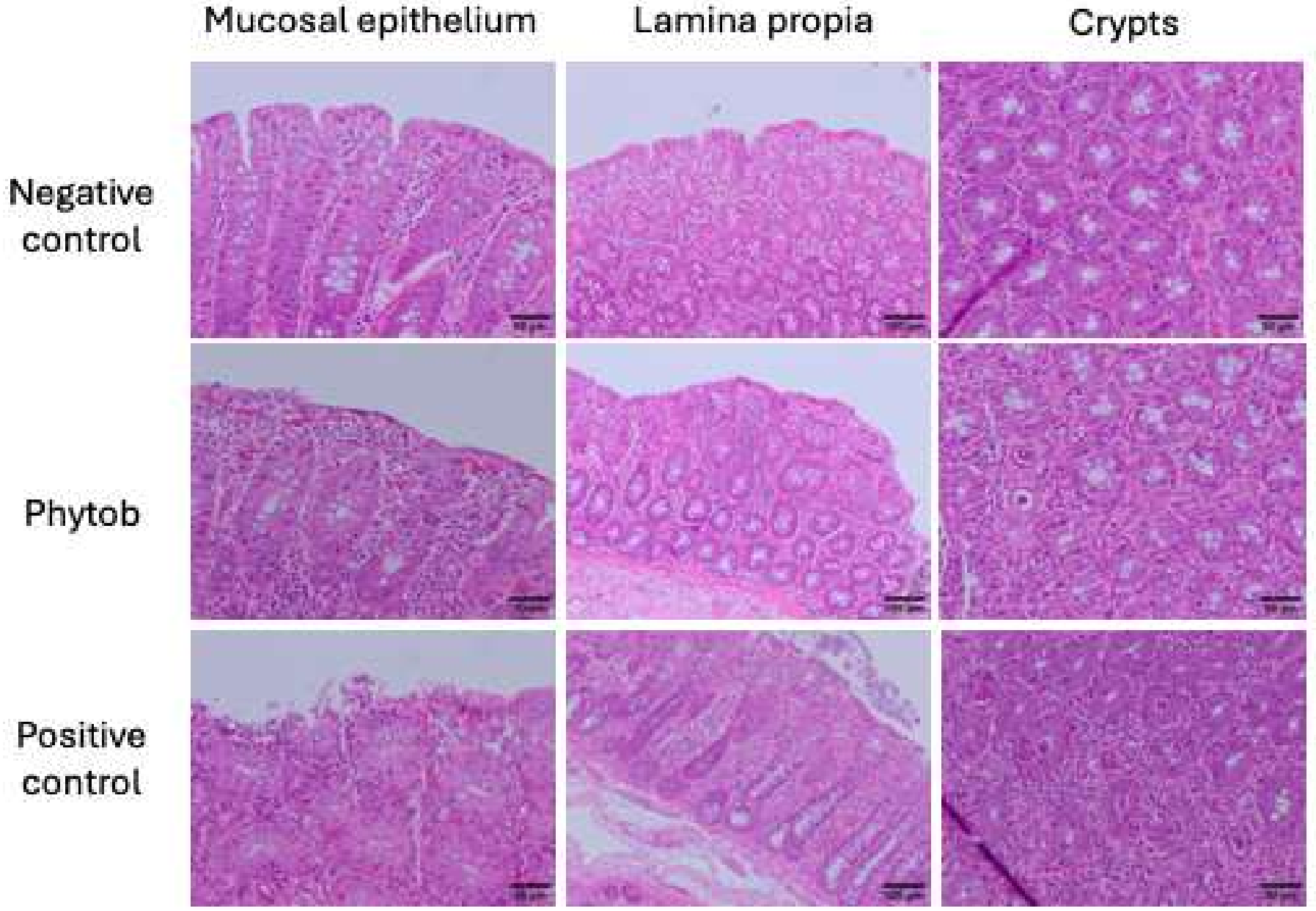
Number at risk

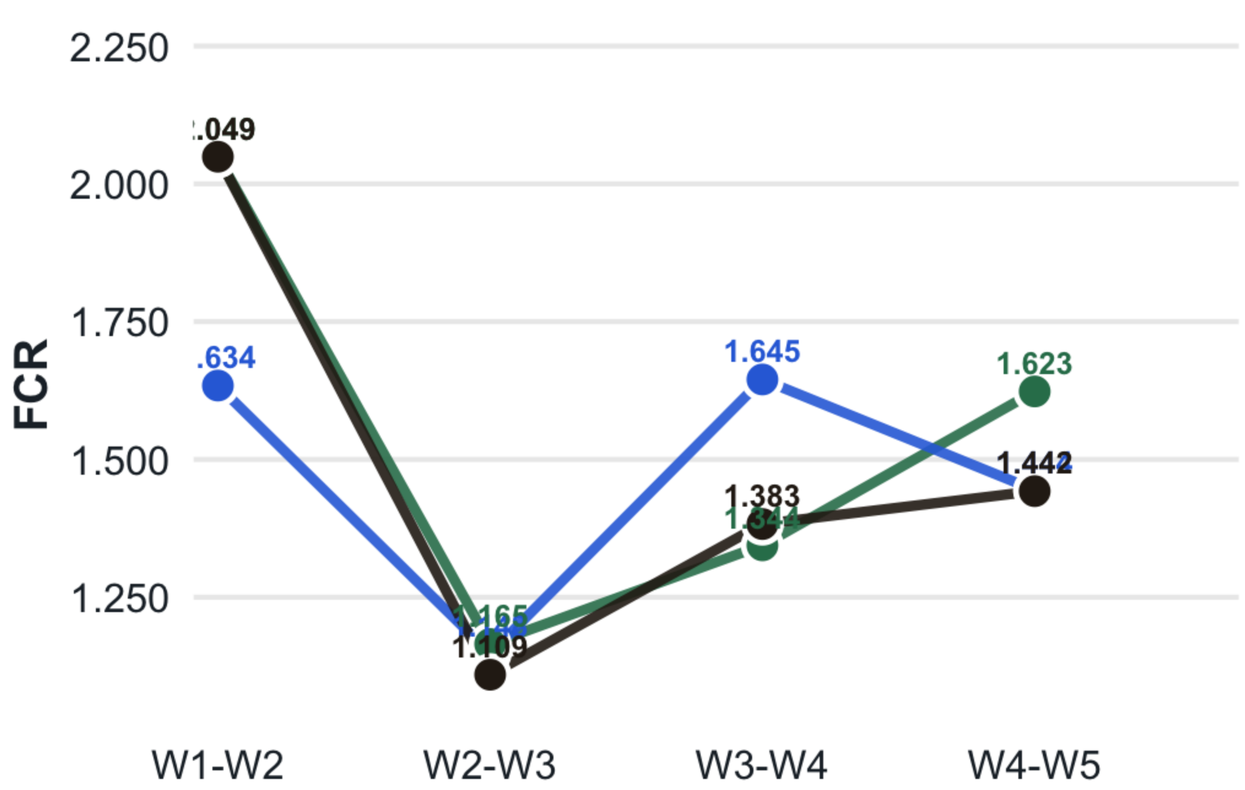
Group																											
Phytob	12	12	12	12	12	12	12	12	12	10	10	10	10	10	10	10	10	10	10	9	9	9	8	8	8	8	8	7	0
Positive control	12	12	12	12	12	12	11	11	10	9	8	8	7	7	7	7	6	6	6	5	5	5	5	4	4	4	4	4	0

0 5 10 15 20 25 27 28

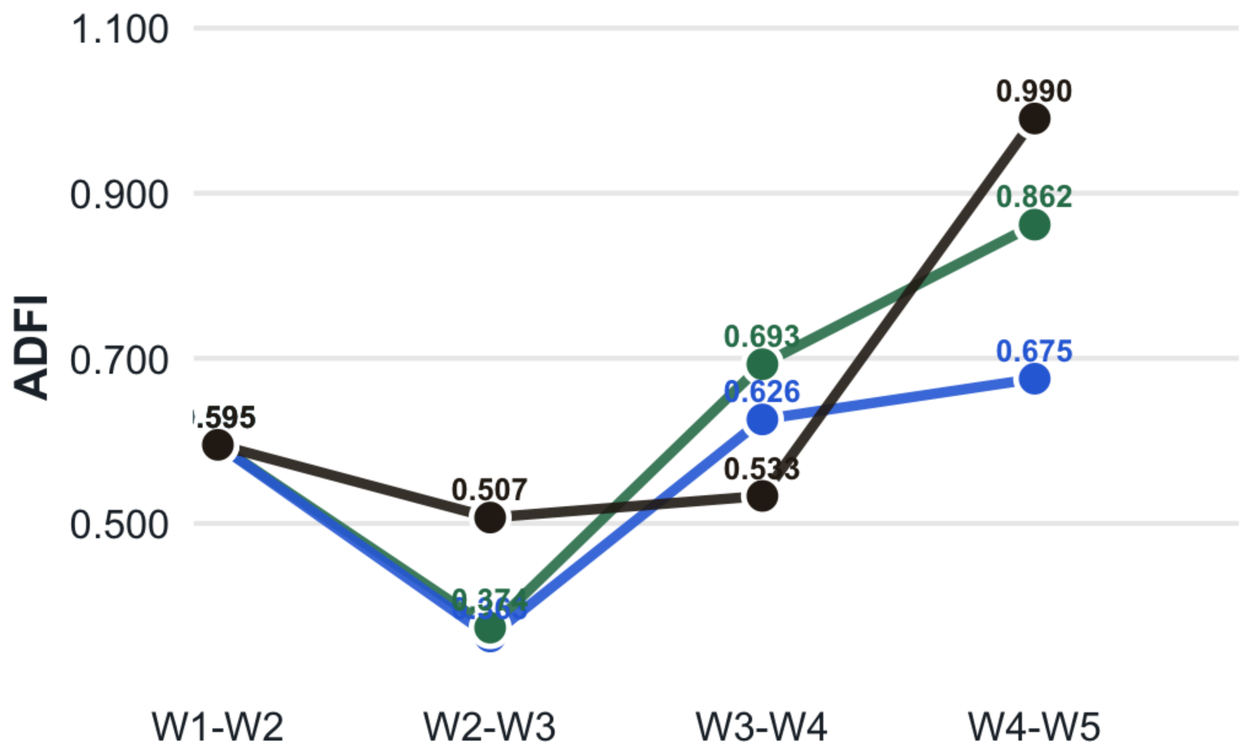
Sampling day


*
ns
ns
9
6
3
0
Colon Score
Phytob
Positive Control
Negative

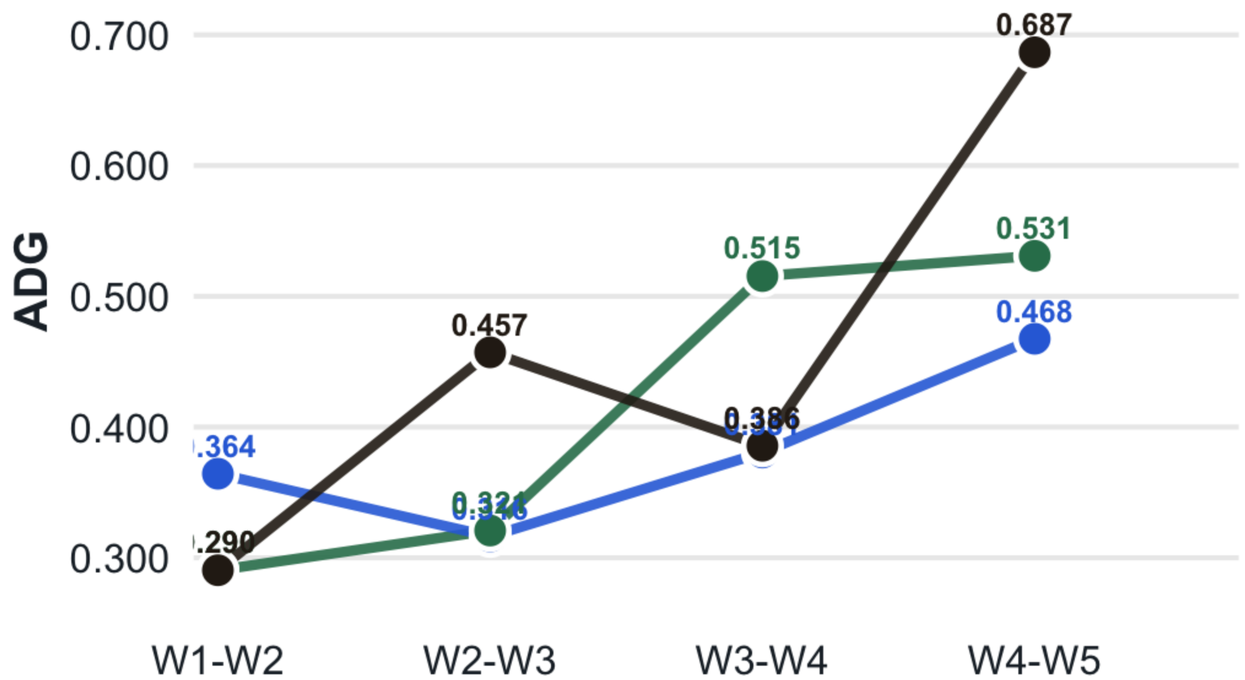

Mucosal epithelium
Lamina propia
Crypts
Negative control
Phytob
Positive control



	W1-W2	W2-W3	W3-W4	W4-W5	Total
Positive control	-20.3%	3.5%	19.0%	0.1%	8.0%
Phytob	0.0%	5.0%	-2.8%	12.5%	6.4%



	W1-W2	W2-W3	W3-W4	W4-W5	Total
Positive control	0.0%	-28.4%	17.4%	-31.8%	-12.5%
Phytob	0.0%	-26.3%	29.9%	-13.0%	-7.5%



	W1-W2	W2-W3	W3-W4	W4-W5	Total
Positive control	25.4%	-30.8%	-1.3%	-31.9%	-19.0%
Phytob	0.0%	-29.8%	33.6%	-22.7%	-13.1%

Phytob Positive control Negative control

-30.0% 0.0% 30.0%

Group Positive control Phytob

All animals

PC - Phyt: Δβ = -0.003
p = 0.762

SD positive animals

PC - Phyt: Δβ = -0.021
p = 0.515

Non-SD-positive animals

PC - Phyt: Δβ = -0.002
p = 0.929

PC - Phyt: Δβ = -0.016
p = 0.120

PC - Phyt: Δβ = -0.081
p = <0.001

PC - Phyt: Δβ = -0.081
p = 0.009

PC - Phyt: Δβ = -0.027
p = 0.030

PC - Phyt: Δβ = -0.032
p = 0.422

PC - Phyt: Δβ = -0.086
p = <0.001

ED 0-14

ED 14-21

ED 21-27

ADG (kg/day, per pig)

Faecal score sum per interval

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- suple2.docx
- Suple1.docx