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Effect of functional oil in piglet feeding and its effects on total blood count, health parameters, and gastrointestinal tract structure

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Abstract

This study was performed to evaluate the effects of a functional oil blend (castor oil plus cashew nutshell oil) on complete blood count, health parameters, and intestinal structure in nursery pigs. A total of 128 crossbreed piglets (6.79 ± 1.76 kg of body weight) were allocated in a randomized complete block design with two dietary treatments: a functional oil-free diet or a diet based on added functional oil (1500 mg functional oil blend/kg of diet). Both diets had 400–500 mg colistin sulphate/kg. Pigs fed functional oil had higher erythrocytes and monocytes on days 11 and 23, respectively. Lymphocyte concentration was reduced, and ileum pH was increased in pigs fed functional oil on day 23. On day 37, lower jejunal pH was observed. However, no treatment effect on diarrhoea occurrence was observed. The lactic acid bacteria count was greater in jejunum and ileum of pigs fed functional oil on day 23. Pigs fed functional oil had more jejunal lesions on day 37. Based on the criteria of this study, dietary supplementation to piglets with functional oil blend changed the concentration of erythrocytes and monocytes as an important role in the defence of the organism; however, it showed a reduced capacity to modify the pH in small intestinal contents, which was reflected in an absence in the incidence of diarrhoea. In addition, piglets fed the functional oil blend had improvements in lactic acid bacterial counts, without the ability to attenuate lesions in the jejunum.

Keywords: erythrocyte, feed additive, intestinal lesion, intestinal microbiology, leukogram, weaned piglet
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Introduction

The nursery phase is considered the most critical for piglets (Silva *et al.*, 2012; Li *et al*., 2019). During nursery, piglets are intensively challenged by stress factors such as sow separation, new social hierarchy formation, and environmental and dietary changes (Clouard *et al*., 2012). This can lead to growth reduction and poor performance. In this transition period, reduced feed intake, high morbidity, and susceptibility to enteric infections are commonly observed probably due to digestive immaturity of pigs (Dong & Pluske, 2007; Windisch *et al.*, 2008).

The gastrointestinal tract immaturity of piglets affects the intestinal architecture and compromises the absorption and secretion. This impairs intestinal health of piglets, favours pathogenic microorganisms proliferation (Oetting *et al*., 2006), and hence the occurrence of post-weaning diarrhoea (Vannucci & Guedes, 2009). Being responsible for a marked reduction in the health of pigs, post-weaning diarrhoea is a widespread

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problem that affects swine production in the whole world (Zhang *et al.*, 2012). As a preventive measure, antibiotic therapy is still used as the main therapeutic resource against diarrhoea (Rossi *et al.*, 2015). However, the emergence of regulations banning the use of growth-promoting antibiotics in animal feed (Mendel *et al.*, 2017) has led to the search for natural additives to replace antimicrobials, either partially or totally.

Thus, in the last decade, phytogenic additives, especially functional oils have been studied relative to their effectiveness to replace antimicrobials (Purevjav *et al.*, 2013; Jesus *et al.*, 2016; Ghizzi *et al.*, 2018; Torrent *et al.*, 2019). In fact, the effectiveness of functional oils in different species has been reported in previous studies (Purevjav *et al.*, 2013; Jesus *et al.*, 2016; Ghizzi *et al.*, 2018). Reports include a possible modulation effect on the intestinal mucosa (Murakami *et al.*, 2014). However, research on nursery pigs fed functional oils is scarce. The antimicrobial effect of functional oils could be beneficial when added to feed of weaned pigs since they have immature immune systems in the gastrointestinal tract (Li *et al.*, 2012).

Using phytogenic additives as antimicrobial alternatives in swine production is of scientific and industrial interest (Gois *et al.,* 2016). Thus, the search for natural products that contribute to the health and effective gastrointestinal tract functioning of piglets is paramount in intensive pig farming. Functional oils are a promising alternative. However, their effectiveness in intestinal modulation and the prevention of enteric pathogens when associated with conventional antimicrobials needs to be proven.

Thus, this study was performed to evaluate the effects of a functional oil blend (castor oil plus cashew nutshell oil) on complete blood count, health parameters, and intestinal structure in nursery pigs.

Material and Methods

This study was carried out in the swine facility of Universidade Estadual do Oeste do Paraná (UNIOESTE) – located in Marechal Cândido Rondon - Brazil. All animal procedures were approved by the local Ethics Committee on the Use of Animals - CEUA (approval no. 09/2019).

A total of 128 crossbreed piglets (64 uncastrated males and 64 females, Landrace × Large White, Agroceres♂ × DanBred♀) weaned at 21 d, with an initial body weight (BW) of 6.79 ± 1.76 kg were used. Pigs were allotted randomly based on initial body weight and gender to one of the two dietary treatments in a randomized complete block design (two batches over time) with 16 replicates and four animals per experimental unit. In the beginning of the experiment, animals were weighed, tagged for identification purposes, and housed in slatted plastic floor pens (1.54 m^2) equipped with a semi-automatic feeder and nipple drinker. Pigs were allowed *ad libitum* access to feed and water throughout the experimental period.

Dietary treatments included a functional oil-free (FOF) diet or a diet based on added functional oil (FO). The functional oil blend was a commercially available product (Essential®, Oligo Basics Agroind. Cascavel, Brazil) produced from castor oil and cashew nutshell oil and contained 200 g cardanol/kg, 90 g ricinoleic acid/kg, and 40 g cardol/kg. The experimental dose of Essential® (1500 mg/kg feed) was based on the manufacturer's recommendations. Throughout the experiment, pigs received three diets: 1) pre-starter I (from days 1–11), 2) pre-starter II (from days 12–23), and 3) starter (from days 24–37). Diets were composed of corn, soybean meal, and synthetic amino acids (Table 1) and were formulated to meet requirements for nursery pigs (Rostagno *et al*., 2017) (Table 1). All diets were offered as mash.

Two animals from each experimental unit were selected for blood collection based on the closest BW relative to the average BW in each pen. Blood samples (~10 mL) were withdrawn from jugular vein (08h00) using 0.7 × 30 mm and 0.8 × 30 mm needles. Samples were collected at the end of each experimental phase after an 8-h period of feed fasting. Blood samples were transferred to test tubes containing anticoagulant (EDTA) and then placed on ice inside a thermal box (4 °C).

Blood samples were sent to the lab of Veterinary Hospital of the Universidade Federal do Paraná for complete blood count. Circulating blood cells, red cells (erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, and mean corpuscular haemoglobin concentration), white cells (leucocytes, segmented neutrophils, eosinophils, lymphocytes, and monocytes), and platelets were analysed using an automated haematology analyzer (BC-2800Vet, Mindray®, Shenzhen, China).

Table 1 Percent composition and calculated nutritional values of diets fed to animals in the experimental period (as fed basis)

Ingredients (%)	Functional oil-free				With functional oil			
	Pre I	Pre II	Male starter	Female starter	Pre I	Pre II	Male starter	Female starter
Ground corn, 7.88% CP	52.73	53.26	66.10	68.93	52.58	52.95	65.79	68.62
Soybean meal, 45.22% CP	8.00	18.18	23.88	21.64	8.00	18.24	23.94	21.70
Micronized soybean, 38% CP	8.00	6.00	3.00	3.00	8.00	6.00	3.00	3.00
Fish meal, 53% CP	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Whey concentrate	21.15	12.73	0.00	0.00	21.15	12.73	0.00	0.00
Cooking sugar	3.50	3.00	0.00	0.00	3.50	3.00	0.00	0.00
Common salt	0.40	0.34	0.39	0.39	0.40	0.34	0.39	0.39
Limestone	0.56	0.62	0.70	0.65	0.56	0.62	0.70	0.65
Dicalcium phosphate	1.21	1.10	1.21	1.09	1.21	1.10	1.21	1.09
Soybean oil	0.50	0.77	0.18	0.03	0.50	0.87	0.28	0.14
Lysine sulphate, 55%	0.08	0.09	0.61	0.46	0.08	0.09	0.61	0.46
L-threonine, 99%	0.10	0.13	0.18	0.12	0.10	0.13	0.18	0.12
DL-methionine, 99%	0.17	0.18	0.17	0.11	0.17	0.18	0.17	0.11
L-tryptophan, 98%	0.06	0.04	0.04	0.02	0.06	0.04	0.04	0.02
Mineral–vitamin premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Colistin sulphate, 8%	0.04	0.05	0.05	0.05	0.04	0.05	0.05	0.05
Essential functional oil®[2]	0.00	0.00	0.00	0.00	0.15	0.15	0.15	0.15
Calculated values								
ME (Mcal/kg)	3.41	3.37	3.23	3.23	3.41	3.37	3.23	3.23
Crude protein (%)	20.02	21.00	19.50	18.50	20.02	21.00	19.50	18.50
Total calcium (%)	0.85	0.82	0.82	0.77	0.85	0.82	0.82	0.77
Available phosphorus (%)	0.50	0.45	0.41	0.38	0.50	0.45	0.41	0.38
Lactose (%)	11.00	7.00	0.00	0.00	11.00	7.00	0.00	0.00
Total sodium (%)	0.28	0.23	0.20	0.20	0.28	0.23	0.20	0.20
Digestible lysine (%)	1.45	1.33	1.21	1.07	1.45	1.33	1.21	1.07
Digestible methionine + cysteine (%)	0.81	0.74	0.68	0.60	0.81	0.74	0.68	0.60
Digestible threonine (%)	0.91	0.84	0.76	0.68	0.91	0.84	0.76	0.68
Digestible tryptophan (%)	0.26	0.24	0.22	0.19	0.26	0.24	0.22	0.19

[1]Mineral–vitamin premix (per kg of product): folic acid (103.12 mg); pantothenic acid (2249.99 mg); biotin (16.88 mg); chlorohydroxyquinoline (15.00 g); copper sulphate (22.07 g); ethoxyquin (206.00 mg); iron sulphate (6733.40 mg); iodine (37.51 mg); lysine (123.76 g); manganese sulphate (1866.71 mg); methionine (110.25 g); niacin (4687,50 mg); selenium (43.75 mg); threonine (46.64 g); vit. A (1437500,00 IU); vit. B1 (224.96 mg); vit. B12 (2537.50 mg); vit. B2 (537.50 mg); vit. B6 (437.50 mg); vit. D3 (262500,00 IU); vit. E (4250,00 IU); vit. K3 (375,00 mg); zinc (1000,00 mg); [2]Essential functional oil® (guarantee concentration per kg of product): cardanol (200 g), ricinoleic acid (90g), cardol (40 g), and vehicle

At the end of the pre-starter II (day 23) and starter (day 37) phases, piglets were weighed and fasted for 8 h before slaughter, followed by humane slaughter methods (electronarcosis with 240 volts for 3 s followed by exsanguination). Slaughtered animals had BW similar to the average of each treatment. Twenty-four pigs (12 per treatment) were slaughtered in each phase. Immediately after evisceration, the pH of the stomach and intestinal contents (jejunum, ileum, cecum, and colon) was measured using a portable pH meter (HI99163, Hanna Instruments, São Paulo, Brazil), as previously reported by Manzanilla *et al.* (2004).

Faeces were observed throughout the experiment to determine the effects of treatments on diarrhoea occurrence. Faeces were scored daily as preciously described by Oetting *et al.* (2006) on a 3-point scale (1 = solid faeces, 2 = soft faeces (pasty), and 3 = watery faeces). Faeces scored as 2 and 3 were declared diarrhoeal. Subsequently, the occurrence of diarrhoea was calculated as the proportion of diarrhoeal scores in each treatment throughout the experiment.

At the end of the pre-starter II (day 23) and starter (day 37) phases, pigs were weighed and then fasted for 8 h before slaughter. Slaughtered animals had BW similar to the average of each treatment. Twenty-four pigs (6 per treatment) were slaughtered in each phase. After evisceration, the intestinal mucosa (jejunum, ileum, cecum, and colon) was exposed with the aid of a sterile, disposable surgical blade. Intestinal content was sampled into sterile tubes and placed on ice inside a thermal box. Microbiological analyses were carried out in the microbiology lab of Unioeste.

Each sample of intestinal content (1 g) was added to a test tube containing 100 mL of sterile distilled water and stirred. Then, 1 mL of the solution was pipetted into a test tube containing 9 mL of sterile distilled water (10^{-1} dilution) and vortexed for 30 s. Further dilutions (10^{-5} to 10^{-7}) were vortexed for 10 s. A 100 µL aliquot of each diluted sample was spread evenly on the surface of a sterile polystyrene Petri dish conducive to lactic acid bacterial (LAB) growth with the aid of a Drigalski loop. To evaluate LAB, diluted samples were spread evenly on the surface of agar (Man, Rogosa, and Sharpe; MRS) and incubated upside-down (CO_2 incubator) at 35 °C for 48 h. After the incubation period, bacterial colonies were counted using a colony counter (J-3 automatic digital colony counter, Global Trade Technology, São Paulo, Brazil). Results were log-transformed, as previously described by Silva *et al.* (2017).

Samples (3 cm) of small intestine (duodenum, jejunum, and ileum) were collected and then washed with saline solution (0.9%) and fixed in buffered formalin (10%) for histological slide preparation. Histological analyses were performed at the non-ruminant research lab of Universidade Federal de Sergipe, Brazil. Samples were inserted into individual histological cassettes, embedded in paraffin, and cut using a microtome (RM2125 RTS, Leica Biosystems, São Paulo, Brazil), as described by Gao *et al.* (2000). Slides were made for two replicates per sample (with no serial cuts) and then stained with haematoxylin and eosin.

Intestinal lesions were scored to compare possible histological alterations. Each lesion was scored by multiplying its severity by the extent, as previously reported by Kolf-Clauw *et al.* (2009). Lesion severity was scored as follows: 1 = mild lesion, 2 = moderate lesion, and 3 = severe lesion. Lesion extent was scored as follows: 0 = no lesion, 1 = low (<25% of intestine section with lesions); 2 = moderate (26% to 50% of intestine section with lesions), and 3 = high (>50% of intestine section with lesions) (Table 2). The final score was obtained by summing the lesion scores.

Table 2 Histological criteria used to establish the intestinal lesion score

Severity of injury	**Degree**
Lymphatic vessels dilation	1
Cellular vacuolization	1
Cube/shaped enterocytes	2
Villi atrophy	2
Villi fusion	2
Interstitial oedema	2
Villi apical necrosis	3
Extent of the injury	**Degree**
Absent	0
Low (up to 25% of bowel section affected)	1
Moderate (up to 26-50% of bowel section affected)	2
Large (more than 50% of the bowel section affected)	3

Prior to variance (ANOVA) or covariance (ANCOVA) analyses, residual error was evaluated for outliers using the Student residue (Rstudent) test. Rstudent values ≥ 3 SD (standard errors) were considered as significant. The normality of experimental errors and the homogeneity of variances between treatments for the different variables were previously evaluated using Shapiro–Wilk and Levene tests, respectively. Data on blood parameters were analysed using the following model:

$$Y_{ijk} = m + T_i + b_j + \beta\,(X_{ijk} - \bar{X}_{...}) + \varepsilon_{ijk},$$

where Y_{ijk} = the dependent variable in each plot, measured in the i-th class of diet, j-th block, and in the k-th replication; m = overall mean; T_i = effect of treatment classes (i = 1-2); b_j = effect of blocks (j = 1-2); β = regression coefficient of Y over X; X_{ijk} = average observation of the covariate (baseline of blood) in each plot, measured in the i-th treatment class, j-th block, and in the k-th replication; $\bar{X}_{...}$ = overall average for the covariate X and ε_{ijk} = random error of the plot associated with each Y_{ijk} observation.

Data on gastrointestinal tract and intestinal microbiology were analysed using the above-mentioned model without the covariate effect.

For data on diarrhoea occurrence (DO), the generalized linear model was adjusted using binomial distribution and a logit function:

$$\eta = \mu + T_i + b_j,$$

where: μ = overall mean; T_i = effect of treatment (i = 1-2); and b_j = effect of block (j = 1-2).

Treatment effects were tested via type III analysis. Data on DO were expressed as percentage. Treatment effects were analysed via ANCOVA or ANCOVA using the Fisher criterion. Statistical analyzes were performed using the general linear models procedure of SAS, University Edition (SAS Inst. Inc., Cary, NC, USA). All normally distributed data were presented as means with standard error of the mean.

Results and Discussion

Pigs fed FO had higher erythrocytes (P =0.023) and monocytes (P =0.023) on day 11 and day 23, respectively (Table 3 and 4). A lower lymphocyte concentration (P =0.035) on day 23 was observed in pigs fed FO.

Table 3 Means (± standard error) of the concentration of erythrogram indicators and platelets in piglets fed functional castor oil and cashew nutshell[1]

Variables	Diet[2]		SEM[3]	*P*-value[4]
	FOF	FO		
Pre-starter I phase (6.80 to 9.74 kg – 1 to 11 days)				
Red blood cells (millions/mm^3)	5.57 ± 0.07^b	5.90 ± 0.12^a	0.080	0.023
Haemoglobin (g/dL)	9.66 ± 0.22	9.77 ± 0.22	0.157	0.787
Haematocrit (%)	33.42 ± 1.07	33.08 ± 0.57	0.584	0.881
Mean corpuscular volume (fL)	57.98 ± 0.97	56.48 ± 0.99	0.700	0.273
Mean corpuscular haemoglobin concentration (%)	29.51 ± 0.21	29.49 ± 0.27	0.174	0.540
Platelets (mil/mm^3)	721512 ± 36020	681557 ± 52432	318.88	0.709
Pre-starter II phase (9.74 to 16.32 kg – 12 to 23 days)				
Red blood cells (millions/mm^3)	5.78 ± 0.08	5.95 ± 0.13	0.079	0.323
Haemoglobin (g/dL)	9.99 ± 0.14	10.31 ± 0.21	0.133	0.332
Haematocrit (%)	33.55 ± 0.49	34.50 ± 0.68	0.424	0.259
Mean corpuscular volume (fL)	58.31 ± 0.74	58.13 ± 0.75	0.522	0.990
Mean corpuscular haemoglobin concentration (%)	29.76 ± 0.27	29.91 ± 0.24	0.179	0.800
Platelets (mil/mm^3)	687166 ± 39112	695337 ± 28424	240.37	0.614
Starter phase (16.32 to 25.47 kg) – 24 to 37 days				
Red blood cells (millions/mm^3)	6.03 ± 0.07	6.28 ± 0.10	0.069	0.125
Haemoglobin (g/dL)	10.41 ± 0.22	10.75 ± 0.22	0.160	0.336
Haematocrit (%)	35.16 ± 0.63	35.40 ± 0.61	0.431	0.450
Mean corpuscular volume (fL)	57.96 ± 0.48	56.54 ± 1.02	0.609	0.684
Mean corpuscular haemoglobin concentration (%)	29.49 ± 0.38	30.39 ± 0.37	0.280	0.195
Platelets (mil/mm^3)	579916 ± 37857	561055 ± 36619	258.17	0.245

[1]Averages followed by different lowercase letters in the row differ according to the analysis of covariance at the 5% probability level. [2]Experimental diets – FOF: Functional oil-free, FO: With functional oil. [3]SEM: standard error of the mean [4]Significance level

The erythrogram consisted of counting erythrocytes (red blood cells). The higher erythrocyte concentration on piglets fed FO on day 11 was within the reference range for pigs, according to Weiss & Wardrop (2010). Similar results were observed by Yan *et al.* (2012) and Czech *et al.* (2009), who reported a significant increase in erythrocyte concentration in pigs fed phytogenic additives, also within the species reference range.

Platelets are blood cells responsible for clotting. In the present study, platelet concentration was kept within the reference range (100 to 900 thousand/mm^3) for pigs (Weiss & Wardrop, 2010) in all studied phases. Henn *et al.* (2010) also did not observe differences in blood platelet concentration in pigs fed dietary phytogenic additives (473 thousand/mm^3), which also remained within the reference range during the experimental period.

However, in other studies (Henn *et al.*, 2010; Yan *et al.*, 2011; Zhang *et al.*, 2012), dietary phytogenic additives did not affect erythrogram and platelet count in pigs. Throughout the experimental period, the erythrogram and platelet count remained within the reference range for pigs. This suggests the absence of anaemia and haemorrhages in the pigs.

The leukogram is the analysis of blood cells responsible for body defence. An increased monocyte concentration was observed in pigs fed FO on day 23 and values remained within the reference range (2 to 10%) for pigs (Weiss & Wardrop, 2010). However, in a study conducted by Henn *et al*. (2010), a dietary phytogenic additive did not affect monocyte concentration since it is altered in cases of viral or bacterial infections.

Table 4 Means (± standard error) of the concentration of leukogram indicators in piglets fed functional castor oil and cashew nutshell[1]

Variables	Diet[2]		SEM[3]	*P*-value[4]
	FOF	FO		
Pre-starter I phase (6.80 to 9.74 kg – 1 to 11 days)				
Leukocytes (cells/mm^3)	16633 ± 721.47	17522 ± 1204	707.19	0.444
Segmented neutrophils (%)	45.67 ± 1.97	43.92 ± 2.43	1.557	0.511
Eosinophils (%)	2.08 ± 0.28	1.61 ± 0.26	0.197	0.272
Lymphocytes (%)	48.58 ± 2.24	48.92 ± 2.80	1.774	0.694
Monocytes (%)	3.83 ± 0.66	4.84 ± 0.59	0.447	0.063
Pre-starter II phase (9.74 to 16.32 kg – 12 to 23 days)				
Leukocytes (cells/mm^3)	14261 ± 624.54	14162 ± 560.59	412.89	0.718
Segmented neutrophils (%)	29.81 ± 2.72	34.43 ± 3.03	2.050	0.213
Eosinophils (%)	1.27 ± 0.25	1.37 ± 0.27	0.182	0.551
Lymphocytes (%)	64.62 ± 2.56^a	57.87 ± 2.54^b	1.878	0.035
Monocytes (%)	4.12 ± 0.50^b	5.13 ± 0.32^a	0.310	0.023
Starter phase (16.32 to 25.47 kg) – 24 to 37 days				
Leukocytes (cells/mm^3)	13911 ± 826.29	13694 ± 593.58	483.97	0.732
Segmented neutrophils (%)	43.90 ± 5.00	42.50 ± 3.29	2.832	0.903
Eosinophils (%)	1.80 ± 0.35	1.83 ± 0.36	0.252	0.873
Lymphocytes (%)	49.90 ± 4.84	49.83 ± 2.99	2.669	0.888
Monocytes (%)	4.50 ± 0.61	5.83 ± 0.60	0.446	0.238

[1]Averages followed by different lowercase letters in the row differ according to the analysis of covariance at the 5% probability level. [2]Experimental diets – FOF: Functional oil-free, FO: With functional oil. [3]SEM: standard error of the mean. [4]Significance level.

The lower lymphocyte concentration we observed in pigs fed FO on day 23 suggests a reduced ability to mobilize lymphocytes or a lower necessity of using immune defence mechanisms. However, values remained within the reference range (39–62%) for pigs (Weiss & Wardrop, 2010). Another point worthy of mention is that, despite not being affected, leukocyte concentration was above the reference range (11000 and 1200 cells/mm^3) for pigs (Weiss & Wardrop, 2010) in all studied phases in the present study. Similar results were reported in other studies (Henn *et al*., 2010; Yan *et al.*, 2012; Zhang *et al.*, 2012) when dietary phytogenic additives were offered to pigs. The observed leukocytosis may have been a response of the immune system due to intestinal pathologies or be related to the immune activity of the functional oil blend. However, we cannot fully state the effects of FO on the immune response in pigs.

Higher (*P* =0.019) ileum content pH on day 23 and lower (*P* =0.008) jejunum content pH on day 37 were observed in pigs fed FO (Table 5).

Table 5 Means (± standard error) of pH of the gastrointestinal tract contents of piglets fed functional castor oil and cashew nutshell[1]

Variables	Diet[2]		SEM[3]	*P*-value[4]
	FOF	FO		
Pre-starter I and II phase (6.80 to 16.32 kg) – 1 to 23 days				
Stomach	4.04 ± 0.24	4.06 ± 0.22	0.163	0.959
Jejunum	6.16 ± 0.16	6.35 ± 0.16	0.117	0.455
Ileum	6.01 ± 0.17^b	6.55 ± 0.19^a	0.139	0.019
Cecum	5.51 ± 0.10	5.67 ± 0.12	0.081	0.277
Colon	5.75 ± 0.11	5.86 ± 0.16	0.099	0.488
Starter phase (16.32 to 25.47 kg) – 24 to 37 days				
Stomach	4.85 ± 0.26	4.94 ± 0.25	0.179	0.783
Jejunum	6.45 ± 0.05^a	6.21 ± 0.08^b	0.056	0.002
Ileum	6.38 ± 0.13	6.28 ± 0.10	0.083	0.282
Cecum	5.50 ± 0.08	5.41 ± 0.08	0.059	0.423
Colon	5.61 ± 0.09	5.58 ± 0.10	0.070	0.836

[1]Averages followed by different lowercase letters in the row differ according to the analysis of variance at the 5% probability level. [2]Experimental diets – FOF: Functional oil-free, FO: With functional oil. [3]SEM: standard error of the mean [4]Significance level

Weaned piglets have a high gastric pH (ranging from 4 to 5) and it is affected by age and dietary changes (Brumano & Gattás, 2009). The values of stomach pH we observed, although not significant, were not optimal for pepsin activity (should range from 2–4; Rerat & Corring, 1991). Weaned piglets have low capacity to produce HCl (Brumano & Gattás, 2009), and hence to reduce stomach pH, which was observed in the present study.

Despite being significant, ileum and jejunum pH values observed on day 23 and day 37 were below the optimal range for trypsin and chymotrypsin activity (7.8 to 8.1); these are pancreatic enzymes responsible for protein digestion in the small intestine (Makkink *et al.*, 1994).

The dietary functional oil blend did not favour gastrointestinal content pH in the pigs, although a significant increase in jejunum and ileum LAB count was observed. Our findings may be explained by factors such as fasting time, amount of gastrointestinal content, and waiting period for pH measurement. It is possible that dietary buffering capacity prevented critical changes in pH (Costa *et al.*, 2011). There was no effect (P >0.1) of treatments on the occurrence of diarrhoea (Table 6).

Table 6 Observed proportions of diarrhoea in piglets fed functional castor oil and cashew nutshell[1]

Variable	Diet[2]		*P-value*[3]
	FOF	FO	
Pre-starter I phase (6.80 to 9.74 kg – 1 to 11 days)			
Diarrhoea occurrence (%)	62.50	75.00	0.123
Pre-starter II phase (9.74 to 16.32 kg – 12 to 23 days)			
Diarrhoea occurrence (%)	34.37	42.19	0.357
Starter phase (16.32 to 25.47 kg) – 24 to 37 days			
Diarrhoea occurrence (%)	31.25	34.37	0.699

[1]Observed proportions of diarrhoea occurrence, followed by different lower case letters in the row, differ from each other by the least significant means difference test at the 5% probability level. [2]Experimental diets – FOF: Functional oil-free, FO: With functional oil. [3]Significance level

This suggests that the dietary functional oil blend was not able to reduce the occurrence of diarrhoea in the pigs. This is supported by the results reported by Li *et al.* (2006) and Li *et al.* (2011). These authors observed no reduction in the occurrence of diarrhoea in pigs fed diets containing phytogenic additives. However, different results were observed by Oetting *et al.* (2006) and Silva *et al.* (2012), who reported a lower occurrence of diarrhoea in pigs fed dietary phytogenic additives. Post-weaning period is a stressful phase for piglets. During this phase, different factors, especially dietary change, can contribute to diarrhoea occurrence. Dietary transition causes morphophysiological changes in the gastrointestinal tract. This makes piglets more susceptible to diarrhoea caused by intestinal pathogens. The non-effectiveness of the functional oil blend to reduce diarrhoea in pigs may be related to its stability in the diet and gastrointestinal tract, the dietary dose, and the health challenge the pigs were subjected to. The LAB count was higher in jejunum (P =0.05) and ileum (P =0.041) of piglets fed FO on day 23 (Table 7).

Table 7 Means (± standard error) of the lactic acid bacterial count (log CFU/g) in piglets fed functional castor oil and cashew nutshell[1]

Variables	Diet[2]		SEM[3]	*P*-value[4]
	FOF	FO		
Pre-starter I and II phase (6.80 to 16.32 kg) – 1 to 23 days				
Jejunum	3.07 ± 1.37[b]	3.53 ± 1.76[a]	1.027	0.050
Ileum	6.03 ± 0.17[b]	6.59 ± 0.09[a]	0.140	0.041
Cecum	5.21 ± 1.05	6.09 ± 0.15	0.571	0.524
Colon	5.23 ± 1.05	5.39 ± 1.09	0.725	0.912
Starter phase (16.32 to 25.47 kg) – 24 to 37 days				
Jejunum	3.43 ± 1.21	3.68 ± 1.16	0.813	0.819
Ileum	4.79 ± 1.60	5.97 ± 0.17	0.695	0.389
Cecum	6.43 ± 0.13	6.39 ± 0.27	0.145	0.919
Colon	4.30 ± 1.09	6.00 ± 0.30	0.644	0.503

[1]Averages followed by different lowercase letters in the row differ according to the analysis of variance at the 5% probability level. [2]Experimental diets – FOF: Functional oil-free, FO: With functional oil. [3]SEM: standard error of the mean. [4]Significance level

Microorganisms are not randomly distributed in the intestine of animals. The different species and population density are established according to specific traits of gastrointestinal tract segments (Mackie *et al.*, 1999). The singularity of the gastrointestinal tract is due to changes in pH, the presence of digestive enzymes, and bile salts which create "microenvironments" that are suitable for a range of microorganisms (Pedroso *et al.*, 2005). In general, diets supplemented with phytogenic additives can positively affect intestinal microbial balance of pigs by favouring LAB colonization and reducing pathogenic bacteria (Zhu *et al.*, 2000). This was observed in the present study.

In pre-starter phases I and II, the dietary functional oil blend contributed to intestinal microbial balance and favoured the development of LAB in jejunum and ileum. Distal segments of small intestine have the greatest microbial diversity (Mackie *et al.*, 1999). Similar results were observed by Li *et al.* (2012), who reported lower enterobacteria counts and higher LAB in the faeces of piglets fed dietary essential oils.

However, Gois *et al.* (2016) and Li *et al.* (2012) did not observe changes in the total bacterial count, enterobacteria, and LAB in the intestinal content of pigs fed dietary essential oils. The gastrointestinal tract is fully developed and has better digestive efficiency in starter piglets. This reduces the amount of feed residues that could favour pathogenic microorganism development. Functional oils aid gastric secretion and pepsin activation and increase pancreatic juice production and digestive enzymes (Costa *et al.*, 2011).

Although the observed intestinal lesion score results suggest mild lesions, pigs fed FO had an increase (*P* =0.001) in jejunum lesions after 37 d on dietary treatment (Table 8).

Table 8 Means (± standard error) of the intestinal lesion score (%) in piglets fed functional castor oil and cashew nutshell[1]

Variables	Diet[2]		SEM[3]	*P*-value[4]
	FOF	FO		
Pre-starter I and II phase (6.80 to 16.32 kg) – 1 to 23 days				
Duodenum	12.58 ± 2.05	10.67 ± 1.63	1.300	0.468
Jejunum	11.42 ± 1.98	13.67 ± 2.83	1.709	0.508
Ileum	13.00 ± 2.29	13.83 ± 3.18	1.920	0.843
Starter phase (16.32 to 25.47 kg) – 24 to 37 days				
Duodenum	15.33 ± 2.31	13.92 ± 2.01	1.509	0.653
Jejunum	11.25 ± 1.31[b]	17.42 ± 1.11[a]	1.061	0.001
Ileum	11.00 ± 1.86	11.75 ± 2.41	1.492	0.801

[1]Averages followed by different lowercase letters in the row differ according to the analysis of variance at the 5% probability level. [2]Experimental diets – FOF: Functional oil-free, FO: With functional oil. [3]SEM: standard error of the mean. [4]Significance level

The integrity of the intestinal epithelium is indicative of intestinal health (Xiong *et al.*, 2015). The gastrointestinal mucosa serves as a dynamic barrier that regulates nutrient and water absorption and excludes potential pathogens. In the nursery pre-starter phase, the gastrointestinal tract of piglets is not fully developed and it is easily modified by environmental stimuli. Changes compromise the barrier function and hence cause intestinal inflammation (Wijtten *et al.*, 2011). The jejunum is the small intestine segment with the highest concentration of microorganisms (Mackie *et al.*, 1999). Therefore, the jejunum is the intestinal segment most prone to lesions. Undigested feed residues favour the adherence of pathogenic bacteria to the intestinal mucosa of piglets, which are more resistant to the antimicrobial action of the functional oil; however, this fact was not demonstrated in the present study. However, the intestinal lesion score can be evidenced in situations of local acidification when functional oils do not modulate intestinal pH. Other factors such as infection, desquamation, association with antibiotics, and mucus production can also be relevant.

Conclusions

Dietary supplementation of piglets with a functional oil blend (castor oil plus cashew nutshell oil) changed the concentration of red blood cells and monocytes, with an important role in the defence of the organism. However, they showed a reduced capacity to modify the pH in small intestinal contents, which was reflected in an absence of influence on the incidence of diarrhoea. In addition, piglets fed the functional oil blend had improvements in LAB counts, but with higher jejunum lesion scores due to the reduced pH in this intestinal segment.

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Authors' Contributions

All the authors contributed equally and commented on the early and final version of the manuscript.

Conflict of Interest Declaration

There are no conflicts of interest.

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