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Effects of functional oil blend on performance, blood metabolites, organ biometry and intestinal morphometry in nursery piglets

Efeitos da mistura de óleo funcional no desempenho, metabólitos sanguíneos, biometria de órgãos e morfometria intestinal em leitões de creche

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Highlights

Piglets fed functional oil showed improved on performance in the pre-starter phase.
Piglets fed functional oil reduced stomach and liver weights.
Improvements were evidenced by intestinal histology in piglets fed functional oil.
Poorer feed conversion was observed in piglets fed functional oil in the total period.

Abstract

This study was conducted to assess the effects of functional oil (FO) blend on performance, blood metabolites, organ biometry and intestinal morphometry in piglets. A total of 128 crossbreed piglets (Landrace × Large White, 64 uncastrated males and 64 females, 21 d of age, and 6.79 ± 1.76 kg BW) were

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allocated in a randomized complete block design with two dietary treatments: a FO-free (FOF) diet or a diet based on added FO (1,500 mg/kg of diet with castor oil plus cashew nutshell oil). Piglets fed FO showed higher ($p \leq 0.05$) average daily feed intake, daily body weight gain and final body weight after 23 d of study. For the total period, piglets fed FO showed greater ($p = 0.007$) feed conversion ratio. On d 23, higher serum total protein ($p = 0.026$) and globulin ($p = 0.050$) concentration, lower liver ($p = 0.042$) and stomach ($p = 0.074$) weight, and greater ($p = 0.082$) villi height (VH) in duodenum were observed in piglets fed FO. Nonetheless, piglets fed FOF showed greater ($p = 0.054$) ileal VH, but greater ($p = 0.004$) crypt depth (CD) in jejunum. Piglets fed FO showed higher VH to CD ratio in jejunum ($p = 0.068$) and duodenum ($p = 0.074$) on d 23 and 37, respectively. Based on the results, FO blend improved the performance of weaned piglets; however, it negatively affected the feed conversion ratio in the total period. Moreover, FO blend promoted changes in total protein concentrations and improvements in digestive and absorptive capacity assessed through VH to CD ratio, with a significant reduction in organs.
Key words: Feed additive. Intestinal histology. Phytogenic. Plasma proteins. Weaned piglet.

Resumo

Este estudo foi conduzido para avaliar os efeitos de uma mistura de óleo funcional (OF) no desempenho zootécnico, nos metabólitos sanguíneos, na biometria de órgãos e na morfometria intestinal de leitões. Um total de 128 leitões mestiços (Landrace × Large White, 64 machos inteiros e 64 fêmeas; 21 dias de idade e peso corporal de 6,79 ± 1,76 kg) foram alocados em um delineamento de blocos casualizados completos, com dois tratamentos dietéticos: uma dieta sem OF (SOF) ou uma dieta baseada na adição de OF (1.500 mg/kg de dieta com OF de mamona e castanha de caju). Os leitões alimentados com OF apresentaram maior ($p \leq 0,05$) consumo de ração diário médio, ganho de peso corporal diário e peso corporal aos 23 dias de experimento. Entretanto, os leitões que consumiram dietas com OF tiveram conversão alimentar superior ($p = 0,007$) no período total. No dia 23, houve aumento na concentração de proteína total ($p = 0,026$) e globulina ($p = 0,050$), menor peso de fígado ($p = 0,042$) e estômago ($p = 0,074$), e maior ($p = 0,082$) altura de vilosidade (AV) no duodeno em leitões que consumiram OF; entretanto, os leitões SOF tiveram ($p = 0,054$) AV superior no íleo, mas apresentaram ($p = 0,004$) profundidade de cripta (PC) superior no jejuno. Uma maior relação AV:PC no jejuno ($p = 0,068$) e duodeno ($p = 0,074$) foi observada em leitões com OF nos dias 23 e 37, respectivamente. Com base nos resultados, a mistura de OF melhorou o desempenho dos leitões desmamados; no entanto, afetou negativamente a taxa de conversão alimentar no período total. Além disso, a mistura de OF promoveu alterações nas concentrações proteínas totais e melhorias na capacidade digestiva e absortiva avaliadas através da relação AV:PC, com uma redução significativa nos órgãos.
Palavras-chave: Aditivo alimentar. Histologia intestinal. Fitogênicos. Proteínas plasmáticas. Leitão desmamado.

Introduction

Survival rate and health of piglets are important in intensive swine production systems. The most critical period for piglets is the one from weaning to the end of nursery phase. This is a period in which animals are more vulnerable to stressing factors such as changes in diet and physical and social environments (Clouard et al., 2012). In the nursery phase, diets are shifted from milk which is highly palatable and digestible to lower digestible solid feedstuffs.

Dietary changes coupled with an immature digestive system leads to low intake and absorption of nutrients (Rodrigues et al., 2020) making piglets more prone to digestive problems (Windisch et al., 2008). Gastrointestinal tract (GIT) immaturity deteriorates the intestinal mucosa by changing epithelium morphology. Changes such as villous atrophy, crypt hyperplasia (Montagne et al., 2007), and chronic impairment of the intestinal barrier function (Smith et al., 2010) have been reported. This condition renders piglets more prone to enteric infections and antibiotics have been extensively used over the years to prevent it.

Hence, research efforts have targeted finding natural dietary alternatives (Yang et al., 2015; Lan et al., 2016) that could replace the use of antibiotics in diets (Genova et al., 2021). In recent years, phytogenic feed additives, especially functional oils, have been widely studied (Zhang et al., 2012; Jiang et al., 2015). Functional oils are not essences- or spices-derived products (Bess et al., 2012). They have additional properties other than those relative to the nutritive values (Murakami et al., 2014). This is what makes them different from essential oils. The antimicrobial effects of functional oils have been previously reported in studies (Murakami et al., 2014; Cairo et al., 2018).

Previous studies have shown antimicrobial effects of phytogenic additives in pigs which benefit performance and intestinal health (Jiang et al., 2015; Mendel et al., 2017). The antimicrobial effect of functional oils could be boosted when added to piglets' diets because their immune and digestive systems are not fully developed (Li et al., 2012). Castor and cashew nutshell oils are examples of functional oils that have been reported to have an antimicrobial effect (Murakami et al., 2014). Studies with dietary castor and cashew nutshell oils in nonruminants are limited. However, a few promising data have been reported in poultry. In fact, dietary castor and cashew nutshell oils have shown to improve performance (Bess et al., 2012; Torrent et al., 2019) and intestinal health in broilers (Murakami et al., 2014).

Using phytogenic feed additives as antimicrobial alternatives to conventional antimicrobial in pig production is of scientific and industrial interest (Cairo et al., 2018). Thus, searching for dietary ingredients that promote health and effective GIT function in piglets is important for pig farming due to conventional antimicrobial feed additives prohibition (Genova et al., 2021). This study was conducted to assess the effects of functional oil blend (castor oil plus cashew nutshell oil) on performance, blood metabolites, organ biometry, and intestinal morphometry in nursery piglets.

Material and Methods

This study was conducted at swine center of the Antônio Carlos dos Santos Pessoa experimental farm belonging to Universidade Estadual do Oeste do Paraná - Unioeste, Marechal Candido Rondon, Paraná, Brazil. All animal procedures were approved by the local Ethics Committee on the Use of Animals - CEUA (approval no. 09/2019).

Animals, housing, and experimental design

A total of 128 crossbreed piglets (64 uncastrated males and 64 females, Landrace × Large White) weaned at 21 days and averaging 6.79 ± 1.76 kg BW were used. Piglets were allotted randomly based on initial BW and gender to one of the two dietary treatments in a randomized complete block design with 16 replicates and four animals per experimental unity.

At 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 semiautomatic feeder and nipple waterer. Pigs were allowed ad libitum access to diet and water throughout the experimental period.

Treatments and experimental diets composition

Dietary treatments included a FO-free (FOF) diet or a diet based on added FO. The FO blend was a commercially available product (Essential®, Oligo Basics Agroind., Cascavel, PR, Brazil) produced from castor and cashew nutshell oil, and contained 200 g cardanol, 90 g ricinoleic acid, and 40 g cardol per kg of product. The experimental dose of FO blend (1,500 mg/kg diet) was based on the manufacturer's recommendations.

Throughout the experiment, pigs received three diets: pre-starter I (from d 1 to 11), pre-starter II (from d 12 to 23), and starter (from d 24 to 37). Diets were composed of corn, soybean meal, and industrial amino acids (Table 1), formulated to be isonutritional and meet the requirements for nursery piglets (Rostagno et al., 2017). All diets were offered as mash.

Table 1
Composition of the experimental diets fed to piglets 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
Calcitic 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.

Performance

Animals were weighed (digital stainless-steel scale, model UL50i) at the beginning and the end of each phase. Diet supply, leftovers, and waste were recorded daily. Based on those data, daily body weight gain (DBWG, g), average daily feed intake (ADFI, g), and feed conversion ratio (FCR, g:g) were calculated.

Blood metabolites

Two animals from each 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 the 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 8h-period of diet fasting. Blood samples were transferred to test tubes with no anticoagulant and then placed on ice inside a thermal box (4 °C).

Blood samples were then allowed to clot for 30 min. Serum was isolated from blood via centrifugation at 3,000 g for 15 min (80-2B, Centrilab) and stored at -5 ºC until analysis. Biochemical analyses were determined using commercial kits (Elitech®) and an automatic biochemical analyzer (Flexor EL 200, Elitech®, Puteaux, France). Serum total protein and albumin were determined via biuret and bromocresol green methods, respectively. Serum globulin was estimated based on the difference between total protein and albumin.

Organ weights and measurements, and intestinal morphometry

At the end of pre-starter II (d 23) and starter (d 37) phases, piglets were weighed and submitted to fasting for 8h before slaughter. Slaughtered animals had BW similar to the average of each treatment. Twenty-four pigs (12 per treatment) were slaughtered in each phase.

Intestine length and organ weight (empty stomach, spleen, liver with gallbladder, empty small intestine, empty cecum, and empty colon) were determined using a measuring tape and a digital scale, respectively. Organ weights relative to slaughter BW were calculated as previously described by Genova et al. (2021).

After evisceration, fragment (3 cm) of small intestine (duodenum, jejunum, and ileum) were collected and then washed with saline solution (0.9% NaCl) and fixed in buffered formalin (10%) to prepare histological slides.

Samples were inserted into individual histological cassettes, embedded in paraffin, and cut using a microtome (RM2125 RTS, Leica Biosystems, São Paulo, SP, Brazil) as described by Genova et al. (2021). Slides were made in two replicates per sample (with no serial cuts) and then stained with hematoxylin and eosin.

Morphometric analysis of villi was performed using a digital light microscope (DM2500 M, Leica Microsystems, São Paulo, SP, Brazil) at 10X magnification. The microscope was equipped with a digital camera (DFC295, Leica Microsystems, São

Paulo, Brazil) and images were analyzed using optical image software (Motic Images Plus 3.0). A total of 15 measurements (μm) of villi and their respective crypts were performed per slide. Villi height was measured from the basal region (upper portion of the crypts) to the apex. Crypts height was measured from the basal region to transition region between crypt and villi. These measures were used to calculate the villi to crypt ratio.

Statistical procedures

Statistical analyzes were performed using general linear models' procedure of SAS University Edition (SAS Inst. Inc., Cary, NC, USA). Residual error was evaluated for outliers via Student test. If studentized residuals exceeded 3, the sample was removed from statistical analysis. The normality of experimental errors and the homogeneity of variances between treatments for the different variables were previously evaluated using the Shapiro-Wilk and Levene tests, respectively.

For data on growth performance, blood metabolites, organ weights and measurements, and intestinal morphometry, the model included the fixed effect of dietary treatment and the random effect of block. Initial BW and gender were used as covariates in the performance data analysis. Blood baseline and gender were used as covariates for blood metabolites analysis. Data on organ weights, and measurements and intestinal morphometry were analyzed using only gender as a covariate. Treatment effects were analyzed via analysis of covariance using Fisher criterion. Differences were declared significant when $p < 0.10$ (Genova et al., 2021; Silva et al., 2021). All data were presented as means with standard error of mean.

Results and Discussion

Piglets fed FO showed higher ($p \leq 0.05$) ADFI, DBWG, and final body weight after 23 d of study. For the total period, piglets fed FO showed higher ($p = 0.007$) on FCR (Table 2).

Table 2
Additional dietary effect of functional castor oil and cashew nut shell on the growth performance of piglets

Variables[1]	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)				
IBW (kg)	6.76	6.84	0.067	-
ADFI (g)	530[b]	578[a]	0.012	0.054
DBWG (g)	376[b]	417[a]	0.010	0.051
FCR (g:g)	1420	1390	0.022	0.377
FBW (kg)	15.79[b]	16.85[a]	0.269	0.050
Starter phase (16.32 to 25.47 kg – 24 to 37 days)				
ADFI (g)	1033	1007	0.018	0.328
DBWG (g)	663	649	0.013	0.530
FCR (g:g)	1560	1560	0.023	0.898
FBW (kg)	26.00	24.94	0.476	0.163
Total period (6.80 to 25.47 kg – 1 to 37 days)				
ADFI (g)	731	721	0.013	0.506
DBWG (g)	506	476	0.012	0.164
FCR (g:g)	1430[b]	1500[a]	0.017	0.007
FBW (kg)	26.00	24.94	0.476	0.163

[a,b]Averages followed by different lowercase letters in the row differ according to the analysis of covariance at the 10% probability level.
[1]IBW: initial body weight, FBW: final body weight, ADFI: average daily feed intake, DBWG: daily body weight gain, FCR: feed conversion ratio.
[2]Experimental diets – FOF: functional oil-free, FO: with functional oil.
[3]SEM: standard error of the mean.
[4]Significance level.

Feeding transition that occurs in nursery phase is very stressful for piglets. Weaning can delay growth rate due to digestive and immunological immaturity of animals (Li et al., 2012). The subsequent development can be directly determined by the performance parameters at the end of nursery phase.

Adding FO to piglets' diet during 23 d improved performance. Similar results were reported by Lan et al. (2016) who evaluated the effect of dietary essential oil (0.04%) on the performance of nursery piglets. The authors observed higher DBWG in piglets fed essential oil from d 8 to 21.

The use of functional oils in animal feeding has shown to improve digestibility and absorption of nutrients (Yan et al., 2012) due to their antimicrobial effect against intestinal pathogens. Moreover, functional oils have also been reported to improve diet palatability (De Lange et al., 2010), feed intake, digestive enzyme activity (Wenk, 2003), and intestinal health (Jiang et al., 2015). Altogether, these effects probably accounted for the positive response of post-weaning piglets fed FO.

The absence of effects in piglets fed FO on performance after 37 days may be associated with other factors that could have decreased FO effect as a growth promoter. Piglets showing worse FCR over the total period differs the study of Lan et al. (2016) who observed an improvement of 4.45% in FCR when nursery piglets were fed an essential oil blend (0.04%), and from those reported by Li et al. (2012) who observed improvement in FCR when nursery piglets received dietary levels of essential oil (0.05%, 0.1% 0.15%).

Despite the absence of significant performance improvement in starter piglets, dietary FO had no detrimental effect. However, the effectiveness of phytogenic additives as a growth promoter varies widely in pig farming. Its use as a performance enhancer in pigs (Jiang et al., 2015) may be limited by animal health status, housing conditions, dietary level (Feldpausch et al., 2018), diet nutritional quality, and even by FO stability in the diet or gastrointestinal tract (Li et al., 2012). Results suggest that further research is needed to determine the applicability of functional oils, as well as the adequate dosing and feeding strategies (Feldpausch et al., 2018).

On d 23, higher serum total protein (p = 0.026) and globulin (p = 0.050) concentrations were observed in piglets fed FO (Table 3).

Table 3
Additional dietary effect of functional castor oil and cashew nut shell on blood metabolites in piglets

Variables	Diet[1]		SEM[2]	p-value[3]
	FOF	FO		
Pre-starter I phase (6.80 to 9.74 kg – 1 to 11 days)				
Total protein (g/L)	49.13	51.13	0.829	0.132
Albumin (g/L)	28.72	28.81	0.475	0.881
Globulin (g/L)	20.52	21.73	0.570	0.186
Pre-starter II phase (9.74 to 16.32 kg – 12 to 23 days)				
Total protein (g/L)	53.44[b]	55.54[a]	0.526	0.026
Albumin (g/L)	31.78	32.01	0.356	0.786
Globulin (g/L)	21.66[b]	24.19[a]	0.666	0.050
Starter phase (16.32 to 25.47 kg – 24 to 37 days)				
Total protein (g/L)	62.22	60.99	1.160	0.646
Albumin (g/L)	37.19	36.2	0.678	0.360
Globulin (g/L)	25.03	24.79	0.686	0.938

[a,b]Averages followed by different lowercase letters in the row differ according to the analysis of covariance at the 10% probability level.
[1]Experimental diets – FOF: functional oil-free, FO: with functional oil.
[2]SEM: standard error of the mean.
[3]Significance level.

Biochemical profile analysis allows obtaining information on clinical, metabolic, and productive status of the animal (Gonzalez & Silva, 2017). Piglets fed FO showed higher total protein concentrations on d 23. However, values were lower than those in the basal range (79 to 89 g/L) in pigs (Radostits et al., 2002). Similar result was observed by Li et al. (2012) who reported higher total protein concentrations in nursery piglets fed with 0.05% and 0.15% of essential oils in the diet. However, Cho et al. (2006) reported dietary essential oils were not able to change total protein concentrations throughout nursery phase in piglets.

In all experimental phases, piglets showed total protein concentrations below normal. Gastrointestinal disorders reduce nutrient absorption efficiency in nursery piglets and this could have accounted for the reduced total protein concentration we observed. Total protein is an indicator of animal protein metabolism and low concentrations may be related to nutritional status and liver function (Gonzalez & Silva, 2017).

Albumin is the most abundant protein in the blood and basal concentrations for pigs range from 19 to 39 g/L (Radostits et al., 2002). In the present study, no changes in albumin concentration were observed in any of the experimental periods. Similar results were observed by Cho et al. (2006) who reported that nursery piglets fed an essential oil blend had no changes in albumin concentrations which met values recommended for pigs (38 g/L).

Changes in albumin concentrations were observed in the study of Li et al. (2012), where an increase in albumin concentration was reported when nursery piglets fed 0.15% of essential oil blend. However, it is well known that blood albumin concentration can be directly affected by factors such as dietary protein intake, liver function, dehydration, and gastrointestinal disorders.

Globulin is a poor indicator of protein metabolism with greater importance for the identification of inflammatory processes (González & Silva, 2017). In the present study, globulin concentration was lower than the recommended range (53 to 64 g/L) for pigs (Radostits et al., 2002) throughout the experiment. Globulin is negatively correlated with albumin where increased globulin in the blood restrains albumin synthesis in the liver. This is a biological strategy of the body to maintain protein level and blood osmotic pressure (González & Silva, 2017). Therefore, the lower globulin levels reported in the present study could be explained by the lower total protein concentration. Nonetheless, no inflammatory process was observed in animals in the present study.

Diet with functional oil reduced liver weight ($p = 0.042$) and empty stomach ($p = 0.074$) in piglets after 23 d of dietary treatment (Table 4).

When fed phytogenic additives, nutrient digestibility can be increased in piglets (Windisch et al., 2008) due to a higher activity of digestive enzymes (Wenk, 2003) which affect organ size. Piglets fed FO had reductions of 9.15% and 8.86% in liver and empty stomach weight, respectively. Different results were observed by Costa et al. (2011) and Oetting et al. (2006) who reported no effect of plant extracts in nursery pigs.

Table 4
Additional dietary effect of functional castor oil and cashew nut shell on biometry of digestive and non-digestive organs in piglets

Variables	Diet[1]		SEM[2]	p-value[3]
	FOF	FO		
Pre-starter I and II phase (6.80 to 16.32 kg – 1 to 23 days)				
Relative weight (%)				
Empty stomach	0.86a	0.79b	0.022	0.074
Spleen	0.22	0.23	0.007	0.348
Liver with gallbladder	3.22a	2.95b	0.073	0.042
Empty small intestine	6.92	6.27	0.217	0.131
Empty cecum	0.30	0.29	0.013	0.756
Empty colon	3.73	3.20	0.193	0.151
Small intestine, meters	11.39	10.91	0.433	0.579
Starter phase (16.32 to 25.47 kg – 24 to 37 days)				
Relative weight (%)				
Empty stomach	0.77	0.78	0.017	0.766
Spleen	0.22	0.23	0.008	0.586
Liver with gallbladder	2.79	2.83	0.068	0.697
Empty small intestine	5.19	5.24	0.163	0.867
Empty cecum	0.27	0.25	0.010	0.324
Empty colon	3.25	3.06	0.130	0.485
Small intestine, meters	11.62	12.09	0.318	0.414

[a,b]Averages followed by different lowercase letters in the row differ according to the analysis of covariance at the 10% probability level.
[1]Experimental diets – FOF: functional oil-free, FO: with functional oil.
[2]SEM: standard error of the mean.
[3]Significance level.

The liver is the main organ for nutrient metabolism. Liver weight or measurement changes could affect its capacity of supporting immune system through acute-phase protein synthesis or ensuring liver metabolism. Increased liver weight may suggest disease and/or toxicity, although this detrimental effect depends on other factors such as liver enzyme activity changes (Hou et al., 2020). Thus, adding FO did not cause toxicity or injuries to the liver of piglets in the present study.

We cannot state the higher stomach weight improved feed intake or digestion in piglets fed FOF because we did not measure the histochemical and morphological traits of the stomach. However, Jamroz et al. (2006) reported increased mucus secretion and formation of a thick mucus layer in the stomach of poultry fed 100 ppm of carvacrol, cinnamaldehyde, and capsaicin blend. On the other hand, Cairo et al. (2018) observed no effect on organ weight in piglets fed phytogenic feed additive (0.5; 1.0; or 1.5 g red pepper essential oil/kg of diet).

Piglets fed FO showed higher (p = 0.082) duodenal villi height (VH), while those fed FOF showed (p = 0.054) higher ileal VH and higher (p = 0.004) jejunal crypt depth (CD). A higher VH to CD ratio in the jejunum (p = 0.068) and duodenum (p = 0.074) was observed in piglets fed FO on d 23 and d 37, respectively (Table 5).

Table 5
Additional dietary effect of functional castor oil and cashew nut shell on intestinal histology of piglets

Variables[1]	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				
VH (µm)	168.01[b]	176.36[a]	3.153	0.082
CD (µm)	66.63	68.47	1.163	0.358
VH:CD	2.54	2.58	0.021	0.348
Jejunum				
VH (µm)	155.54	148.1	4.96	0.241
CD (µm)	66.68[a]	59.68[b]	2.395	0.004
VH:CD	2.40[b]	2.55a	0.047	0.068
Ileum				
VH (µm)	185.13[a]	167.22[b]	4.639	0.054
CD (µm)	68.58	66.26	1.202	0.269
VH:CD	2.70	2.53	0.051	0.120
Starter phase (16.32 to 25.47 kg – 24 to 37 days)				
Duodenum				
VH (µm)	172.34	170.46	2.342	0.806
CD (µm)	66.15	65.97	0.825	0.976
VH:CD	2.56[b]	2.62[a]	0.019	0.074
Jejunum				
VH (µm)	167.15	161.85	4.534	0.621
CD (µm)	67.5	62.61	1.943	0.128
VH:CD	2.56	2.64	0.057	0.268
Ileum				
VH (µm)	182.32	172.71	4.559	0.261
CD (µm)	68.22	67.23	1.319	0.651
VH:CD	2.69	2.58	0.033	0.109

[a,b]Averages followed by different lowercase letters in the row differ according to the analysis of covariance at the 10% probability level.
[1]VH: villus height, CD: crypt depth, VH:CD: villus height to crypt depth ratio.
[2]Experimental diets – FOF: functional oil-free, FO: with functional oil.
[3]SEM: standard error of the mean.
[4]Significance level.

According to Tucci et al. (2011), an adequate cell replacement provides a higher VH to CD ratio due to greater number of mature and functional enterocytes. The higher VH to CD ratio we observed in jejunum and duodenum on d 23 and 37, respectively, may indicate a possible cell proliferation-stimulating effect of dietary FO (Costa et al., 2011).

Jejunum is the main site of intestinal absorption. Thus, a higher VH to CD ratio could improve nutrient digestibility and absorption (Shen et al., 2009). This would explain the performance results in pre-starter piglets we observed. Similar results were reported by Murakami et al. (2014) in broilers challenged with coccidiosis that showed higher jejunal VH and VH to CD ratio when fed functional oil (1.5 kg of castor oil /ton and 2 kg of castor oil/ton).

However, different results were observed by Oetting et al. (2006) and Li et al. (2012) who reported no effect of dietary plant extracts and essential oils on morphological small intestine traits in nursery pigs. Villi atrophy and increased crypt depth can occur in the intestinal epithelium of piglets due to higher epithelial shedding (Genova et al., 2021). This is supported by the results we observed in piglets fed FOF. Maintaining an adequate density of mature and intact villi allows better contact of the epithelial surface with the digesta, which makes digestion and absorption more efficient.

It seems that FO can contribute to intestinal mucosa structure in piglets, especially when added to pre-starter nursery diets. In this period, the gastrointestinal tract undergoes physiological transition and possesses a greater capacity for morphometric changes.

Conclusions

Based on the results in the present study, dietary functional oil blend (castor oil plus cashew nutshell oil) improved the performance of weaned piglets however, it negatively affected feed conversion in the total period. Moreover, dietary FO promoted changes in total protein concentrations and improvements in digestive and absorptive capacities assessed via VH to CD ratio, with a significant reduction in organs.

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