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Nutrient digestibility parameters as a tool for analysis of the intestinal health of broiler chickens

Ensaio de digestibilidade de nutrientes como ferramenta para avaliar a saúde intestinal de frangos de corte

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Abstract

The study was performed with the objective of verifying raw soy feed, oxidized oil feed, and a control group. Performance evaluation was done at 7, 14, and 21 days of age. Metabolism assay was carried out between the 17th and 20th days for nutrient digestibility analysis. On the 21st day, two birds per repetition were sent for necropsy and collection of intestine fragments (duodenum and jejunum) for histomorphometric analysis. Eight to 14 days after treatment with *Salmonella,* individuals showed lower feed intake and feed conversion than the control group. Treatment with coccidiosis decreased all performance parameters in the control. Raw soybeans and oxidized oil induce lower weight gain and higher feed conversion compared to the control feed. Unlike after 14 days, at 21 days treatment with salmonella a decrease in weight gain was noted. For the group challenged by coccidiosis feed intake, the feed conversion remained lower than the control group. Undesirable effects on performance in the groups fed raw soybean and oxidized oil remained at up to 21 days. In the evaluation of digestibility, it was observed that raw soy had lower values for digestibility of dry matter, ether extract, and nitrogen balance due to intake. In addition, a lower ratio of villus:crypt measurements was observed. Lower villus height was found in the duodenum of the group challenged by coccidiosis. This group presented a positive correlation between the digestibility of ether extract and the duodenum, indicating that increased villus height implies an increased digestibility of ether extract. The results obtained for the jejunum showed a positive correlation with villus height in groups challenged by coccidiosis, raw soybeans, and oxidized oil; and to crypt depth in the group challenged with oxidized oil. The information obtained in the present study demonstrates that nutrient digestibility parameters can be useful tools for the analysis of the intestinal health of broiler chickens.

Key words: *Eimeria acervulina*. *Eimeria máxima*. *Eimeria tenella.* Oxidized oil. Raw soy. *Salmonella enteritidis.*

Resumo

O experimento foi realizado com o objetivo de verificar os alimentos soja cru, óleo oxidado e um grupo controle. Foi avaliado o desempenho aos sete, 14 e 21 dias de idade. Foi realizado um ensaio de

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metabolismo do 17° ao 20° dia, para análise da digestibilidade dos nutrientes. No 21º dia, duas aves por repetição foram enviadas para necropsia e coleta de fragmentos do intestino (duodeno e jejuno) para análise da histomorfometria. De oito a 14 dias de idade o tratamento com *Salmonella* apresentou menor consumo de ração e conversão alimentar que o grupo controle. O tratamento com coccidiose apresentou redução em todos os parâmetros de desempenho em relação ao controle. A soja crua e o óleo oxidado apresentaram menores ganhos de peso e alta conversão alimentar em relação ao controle. Aos 21 dias o tratamento com *Salmonella* diferentemente dos 14 dias apresentou queda no ganho de peso. Para o grupo desafiado por coccidiose o consumo de ração e a conversão alimentar continuaram menores que o grupo controle. Os efeitos indesejáveis sobre o desempenho nos grupos alimentados com soja crua e óleo oxidado permaneceram até os 21 dias. Na avaliação dos coeficientes de digestibilidade, foi observado que a soja crua apresentou menores valores para digestibilidade da matéria seca, extrato etéreo e balanço de nitrogênio em função da ingestão. Além disso, foi observada uma menor relação vilo:cripta. Foi encontrada menor altura de vilosidade no duodeno, no grupo desafiado por coccidiose. Este grupo apresentou correlação positiva com a digestibilidade do extrato etéreo para duodeno, indicando que o aumento da altura de vilosidade implica em aumento da digestibilidade do extrato etéreo. Já os resultados obtidos para o jejuno, apresentaram correlação positiva para altura de vilosidade nos grupos desafiados por coccidiose, soja crua e óleo oxidado; e para profundidade de cripta no grupo desafiado com óleo oxidado. As informações obtidas no presente estudo permitem afirmar que os parâmetros de digestibilidade de nutrientes podem ser ferramentas úteis na avaliação das condições de saúde intestinal de frangos de corte.
Palavras-chave: *Eimeria acervulina*. *Eimeria máxima*. *Eimeria tenella*. Óleo oxidado. Soja crua. *Salmonella enteritidis.*

Introduction

Owing to the fast production cycle required to meet the increased demand for poultry products, the poultry industry has made efforts in recent years to maximize animal protein production over as short a period as possible. In addition, constant seasonal variations and market speculation have led to overvaluation of the raw materials, and consequently, to an inflation in feed production costs.

This situation has induced an increase in research output relating to the fields of nutrition and physiology of the digestive system in poultry, with the objective of improving the utilization of nutrients derived from the feed. However, the susceptibility of the digestive tract to harm caused by various factors-chief among these being infectious agents such as *Eimeria* spp. and *Clostridium perfringens* (TIMBERMONT et al., 2011), and non-infectious factors such as change or interruption in diet, nutritional disequilibrium, non-starch polysaccharides, low quality fat, enzymatic dysfunction, and mycotoxins-makes the maintenance of a dynamic balance between the intestinal mucosa and luminal substance more difficult, interfering in digestive processes.

Since the 1950s, the use of antibiotics has represented a great advance in animal production concepts. Their usage in subtherapeutic levels has always been greatly disseminated in the poultry sector, since they help to achieve high uniformity in product batches and maximize feed conversion. The zootechnical benefits of antibiotics are explained through intestinal microbiota modulation, inhibition of the growth of undesirable microorganisms, and consequently, excessive challenges to the immune system, neutralizing toxins produced by biochemical activity of intestinal bacteria (ITO et al., 2005).

In spite of such improvements on the intestinal physiology level, since January 2006 the European Union has decided to completely ban the use of

growth-promoting antibiotics due to increasing concerns regarding the presence of residues in animal products for human consumption, that could produce allergic reactions, toxicity or induce the appearance of antibiotic resistance on pathogenic bacteria (SANTOS et al., 2000; HUYGHEBAERT et al., 2011).

In the face of such legislation, and in order to meet marketing demands, there is a necessity to create substitutes for growth-promoting antibiotics as well as to develop new procedures for the monitoring of intestinal health.

An understanding of mechanisms that regulate gastrointestinal tract health is of critical importance for manipulation of the different factors that lead to the maximization of broiler chicken performance. Nowadays, intestinal monitoring is based only on superficial observations that do not allow for a precise and reliable diagnosis capable of producing efficient solutions to resolve the problems. In modern poultry production, which values the maximum production of animal protein in as short a time as possible, there is a need for precise, practical and rapid tools to better assess intestinal health in situations that demand fast responses and application of emergency measures.

Thus, the new market climate combined with the lack of satisfactory results in the use of alternative additives confirms the need for constant monitoring of intestinal health and ratifies the importance of searching further for ways to control factors that affect the proper functioning of the digestive tract.

This study aimed to verify the efficiency of using nutrient digestibility values, determined through metabolism tests, for the evaluation of the intestinal health of broiler chickens challenged with microbial and chemical agents, compared to the parameters of intestinal histomorphometry.

Materials and Methods

Place of performance

The experiment was carried out in three isolation rooms at the Poultry Disease lab of the School of Veterinary, Federal University of Goiás, Brazil. In order to avoid possible cross contamination that could interfere with results, the birds were distributed so that all treatments remained separate.

The rooms had internal dimensions of 3 m x 4 m x 3 m (L x W x H), with masonry walls and covers, and two small extraction fans installed in the back. Five galvanized steel batteries of five floors and with divisions of 0.80 m x 0.75 m x 0.25 m (L x W x H), were distributed among the three rooms so there were three batteries in one room and one battery in each of the other rooms.

Experimental animals

The animals used were one-day-old broiler chickens of the Hubbard lineage, accommodated in groups of ten birds (five females and five males) per experimental unit. All of the birds used were vaccinated in the hatchery against Marek disease (Poulvac® Ovoline HVT) and Fowlpox disease (Chick-N-POX®).

Experimental design

The experimental design consisted of five randomized treatments divided into: control group, *Salmonella* Enteritidis, *Eimeria* sp. challenge, raw soy, and oxidized oil, with five repetitions per treatment, totaling 25 experimental units.

All diets consisted of corn-based bran feed and soy bran, and sought to meet the needs of each age, following the specifications of Rostagno et al. (2005). A pre-starter (from 1 to 7 days of age) feed was provided for all treatments and three starter (from 8 to 21 days of age) feeds provided according to the treatments (Table 1).

Table 1. Percentage composition of pre-starter (1 to 7 days of age) and starter (8 to 21 days of age) experimental feeds.

Ingredients	Pre-starter	Normal starter	Raw soy starter	Oxidized oil starter
Corn (%)	57.850	61.669	58.752	60.709
Soybean meal 45% (%)	37.000	33.793	24.396	33.899
Whole soybean (%)	-	-	12.090	-
Broiler viscera and bone meal (%)	-	-	-	1.508
Vegetable oil	-	-	0.850	-
Dicalcium phosphate (%)	2.000	1.800	1.820	1.768
Limestone (%)	1.900	1.468	0.800	0.820
Salt (%)	0.400	0.424	0.442	0.425
Supplement Min - Vit (%)[1]	0.400	0.400	0.400	0.400
DL-Methionine 99% (%)	0.150	0.238	0.234	0.247
L-Lysine HCL 78.8% (%)	0.300	0.208	0.216	0.224
Total	**100.000**	**100.000**	**100.000**	**100.000**
Nutritional Levels				
ME (kcal kg^{-1})	2950	3000	3000	3000
Crude Protein (%)	22.04	21.00	21.00	21.00
Calcium (%)	0.939	0.880	0.880	0.880
Available phosphorus (%)	0.470	0.450	0.450	0.450
Sodium (%)	0.223	0.210	0.210	0.210
Digestible lysine (%)	1.466	1.260	1.260	1.260
Methionine + Cystine (%)	1.041	0.890	0.890	0.890
Digestible Methionine (%)	0.572	0.493	0.493	0.493
Digestible threonine (%)	0.997	0.859	0.859	0.859
Digestible tryptophan (%)	0.235	0.202	0.202	0.202

[1] Starter mineral vitamin supplement for birds, 4 kg/ton (guaranteed levels per kilogram of the product): Vitamin A: 2750000 UI, Vitamin D3: 500000 UI, Vitamin E: 4000 mg, Folic Acid: 100 mg, Calcium Pantothenate: 2500 mg, Biotin: 15 mg, Niacin: 8750 mg, Pyridoxine: 500 mg, Riboflavin: 1125 mg, Thiamin: 300 mg, Vitamin B12: 4000 mcg, Vitamin K3: 375 mg, Selenium: 62.5 mg, Choline: 65.25 g, Methionine: 420.75 g, Colistin: 2500 mg, Lincomycin: 1100 mg, Monesin: 17500 mg, Nicarbazin: 12500 mg.

Microbiological challenge - Salmonella enteritidis

The phagotype 4 used for the *Salmonella* Enteritidis challenge was obtained from broiler chicken samples from the state of Goiás. First, the strains were pricked out on MacConkey agar and incubated at 37 °C for 18 to 24 hours. After colony growth, the samples were removed and suspended in salt solution of 0.85% and kept at a temperature of 4 °C at a concentration of 6.4 x 10^4 UFC/ml. This concentration value was obtained from comparisons made to the MacFarland scale, following the instructions of Fernández et al. (2001). The concentration was confirmed by decimal dilutions plating serialized on MacConkey agar, followed by incubation at 37 °C and UFC counting of *Salmonella* sp. (BRADSHAW et al., 1990).

After preparation of the inoculum, it was administered to ten-day-old birds as an oral application. A 0.3 ml volume of the solution, containing 10^4 UFC/ml of *Salmonella* Enteritidis, was injected directly to the crop using a graduated pipette.

Caecal content samples, crop swabs, cloacal swabs, egg-yolk sack, spleen, and liver from control and infected birds were collected during necropsy of 21-day-old birds for microbiological and isolation analysis of *Salmonella* Enteritidis.

After organ harvest and swabbing, enrichment was carried out using selenite cystine and tetrathionate broth in 20-ml aliquots that were accommodated in conservatories at 37 °C for 24 hours. Each culture was then pricked out on Brilliant Green, Hektoen, and XLT4 agars, and again incubated at 37 °C for 24 hours. Later, a selection of suggestive colonies were chosen and cultivated on triple sugar iron agar (TSI). Samples that presented similar characteristics to the *Salmonella* type were submitted to the following biochemical tests for sub-species identification: indole production, H_2S production, urease, lysine decarboxylation, methyl red, malonate use, and motility.

Microbiological challenge - Eimeria sp

A commercial vaccine with a live attenuated oocysts suspension was used for the preparation of the inoculum for coccidiosis. The vaccine presented *Eimeria acervulina*, *Eimeria maxima*, *Eimeria mitts* and *Eimeria tenella* oocysts. A vaccine overdose was used to create a situation of challenge in order to lead to a discrete pathological situation and obtain a subclinical diagnosis.

Each bottle had 4 ml of vaccine solution containing 1,000 doses. An individual dose (0.004 ml) had 2,300 oocysts. For the proposed challenge, a consensus was reached that the value of oocysts per bird would be close to 200,000. To reach these values, an oral inoculation of 0.34 ml vaccine solution per bird was necessary. The inoculation of the ten-day-old birds followed the same procedure as the *Salmonella* Enteritidis protocol, with the use of graduated pipettes and oral application until the crop was reached. The control birds were also manipulated, and received oral inoculation of sterile saline solution. Seven days after the inoculation, excreta was collected from all coccidiosis treatment repetitions to enable counting of oocysts. The McMaster method was used to reach this objective.

Chemical challenges - raw soy and oxidized oil

This challenge was made by incorporating raw soy without thermal processing in the feed composition, totaling 50% of the full amount of thermally processed soy bran. Selected whole soybeans were used for this. The administration occurred for ten days from the birds' 11th day of age.

The viscera oil without added antioxidants was obtained from a regional slaughterhouse. Four liters were removed and treated, divided into two beakers each having an individual capacity of two liters. To these vessels was added two grams of copper sulfate (oxidation catalyst), and they were stored in a conservatory at 60 °C for ten days. During this time, constant homogenizations were carried out three times a day using a glass core. Samples were collected daily for analysis of oxidation levels through the determination of Peroxide Value, according to the proposed methodology of Brasil (1998). After the oxidative challenge, the viscera oil was incorporated in the feed and administered to 11-day-old birds.

Experimental management

The rooms were cleaned and sanitized, with a sanitary break of seven to ten days. Incandescent lamps of 60 W were used in each floor of the battery and monitoring occurred daily in order to maintain appropriate room temperature for the birds. There was no need to use auxiliary heating equipment. Feeders and drinkers-a linear rail type installed on the side of the batteries-were cleaned and supplied with fresh clean water and ad libitum feed in the mornings and afternoons.

Analyzed variables

Performance parameters

Accumulated performances were analyzed at 7, 14 and 21 days of age. For that, the birds and the supplied and consumed feed were weighed weekly, and the number and weight of killed birds were

measured daily. The analyzed variables were weight gain (WG), final weight (FW), feed intake (FI), feed conversion (FC), and viability (VIAB).

To analyze weight gain, the birds were weighed at the beginning and end of each experimental period. Diet consumption was calculated as the difference between the full amount of supplied feed and the leftovers from each phase. Based on the diet consumption (corrected by the mortality date in each parcel, when it occurred) and on weight gain, the feed conversion was calculated. Viability was calculated as the percentage of living birds compared to the starter number of stored birds, then transformed into an arc sine, $(\%/100)^{0.5}$, prior to the statistical analysis.

Digestibility of supplied feed

In order to analyze the digestibility of supplied feed, excreta collection was done twice a day on the four consecutive days after the inoculation of biological and chemical challenges, in the beginning of the morning and at the end of the afternoon, from the birds' 17^{th} to 20^{th} days of age, as outlined in Sakomura and Rostagno (2007). At the end of the experimental period, the consumed feed and total excreta production were determined.

The bromatological analysis for ether extract (EE%), crude protein (PB%) and dry matter (DM%) of the feeds and excreta followed the method used by Silva and Queiroz (2002) at the Animal Nutrition Lab of the School of Veterinary from the Federal University of Goiás, Brazil. With the results from the ether extract, crude protein and dry matter analyses, the digestibility coefficients were assessed following the methodology described by Matterson et al. (1965).

Intestinal histomorphometry

At 21 days of age, two birds per experimental unit-a total of 50 birds-were taken to the Poultry Disease Lab and Histology Lab - of the School of Veterinary and Zootechnics, Federal University of Goiás, Brazil. The birds were euthanized by cervical dislocation and sent for necropsy, after which intestinal samples were collected for histomorphometry analyses. The duodenum portion was collected from the ascending part, two centimeters after the duodenum curvature. The jejunum portion was collected three centimeters from the Meckel diverticulum.

The three-centimeter-long standardized samples were cut longitudinally, opened through their mesenteric edges and stretched over a polystyrene panel held by five metal clamps. They were then immersed in a 10% formalin solution. After five days, they were immersed in 70% alcohol. The slide production for histomorphometry analysis (villus height and crypt depth) followed the proposed methodology of Luna (1968). The histological cuts were stained with hematoxylin-eosin (HE).

The slide photogrammetry was done using a Cybershot P-71 digital camera attached to a bright-field Carl Zeiss Germany microscope. Measurement of the villus height (VH) and crypt depth (CD) was determined at 250X magnification, where three villi (one central and two peripheral) per image were observed, and posterior analysis of the measurements in the Axion-Vision Carl Zeiss Germany program was carried out.

Statistical analysis

The data were submitted to variance analysis and the averages were compared through the orthogonal contrast test ($p < 0.05$) according to the GLM procedures of SAS (2008). In order to assess the correlation between digestibility and the intestinal histomorphometry data, correlation testing was used.

Results and Discussion

The seven-day-old birds' performance results did not present differences ($p > 0.05$) between treatments for starter weight (IW), weight gain (WG), final weight (FW), feed intake (FI), feed

conversion (FC), and viability (VIAB) (Table 2). These values are compatible with the study's objective, as the first experiment period was meant for standardization of birds' before the beginning of the proposed challenges. This procedure has permitted consistency in conditions across all treatments without any interference from factors inherent in the inoculation process.

Table 2. Starter weight (IW), weight gain (WG), final weight (FW), feed intake (FI), feed conversion (FC) and viability (VIAB) of seven-day-old broiler chickens.

Treatments	IW (g)	WG (g)	FW (g)	FI (g)	FC (g/g)	VIAB (%)
Control	45.14	149.94	195.08	115.24	0.77	100
Salmonella	45.08	144.58	189.66	116.22	0.80	100
Coccidiosis	45.06	147.98	193.04	107.56	0.72	100
Raw soy	44.24	144.28	188.52	107.16	0.74	100
Oxidized oil	44.46	143.98	188.44	108.84	0.75	100
CV (%)	2.34	3.44	2.98	9.28	8.82	0
Contrasts						
Control x Salmonella	NS	NS	NS	NS	NS	NS
Control x Coccidiosis	NS	NS	NS	NS	NS	NS
Control x Raw soy	NS	NS	NS	NS	NS	NS
Control x Oxidized oil	NS	NS	NS	NS	NS	NS

NS: non-significant, according to contrast test ($p > 0.05$).

The period from eight to fourteen days of age encompasses the inoculation process, and the results obtained allow for starter effect analysis of the proposed challenges. The reference values for this period are found in Table 3, where differences can be observed in DC ($p < 0.05$) among all treatments. For FC, differences were observed ($p < 0.05$) between the treatments Control x Salmonella and Control x Coccidiosis. There was a difference ($p < 0.05$) for WG and FW between Control x Coccidiosis, Control x Raw Soy, and Control x Oxidized oil.

Table 3. Weight gain (WG), final weight (FW), diet consumption (DC), feed conversion (FC) and viability (VIAB) of 14-day-old Hubbard broiler chickens submitted to different intestinal challenges.

Treatments	WG (g)	FW (g)	DC (g)	FC (g/g)	VIAB (%)
Control	428.1	473.3	514.8	1.20	100
Salmonella	413.8	458.9	389.0	0.94	100
Coccidiosis	411.1	456.1	380.7	0.92	100
Raw soy	400.8	445.1	470.5	1.17	100
Oxidized oil	405.6	450.1	480.1	1.18	100
CV (%)	2.91	2.73	4.58	3.69	0
Contrasts					
Control x Salmonella	NS	NS	< 0.0001	< 0.0001	NS
Control x Coccidiosis	0.0360	0.0423	< 0.0001	< 0.0001	NS
Control x Raw soy	0.0018	0.0019	0.0027	NS	NS
Control x Oxidized oil	0.0076	0.0082	0.0144	NS	NS

NS: non-significant, according to the contrast test ($p > 0.05$).

The *Salmonella* Enteritidis challenge did not affect the performance, diet consumption, or feed conversion when compared to the control group. The data are comparable with those of Berchieri Júnior (2000), who reported a lack of negative impact on productivity when birds are challenged with paratyphi salmonella.

The inoculum concentration might have influenced performance values, as inoculation amount was limited to a level that could lead to a subclinical framework on the birds, simulating field situations. Another factor that can be taken into account-so that the challenge would not negatively affect the performance-is that *Salmonella* did not cause cellular destruction of the enterocytes, and did not affect the birds' performance in this way over a short amount of time.

The performance results from the group challenged with coccidiosis presented a decrease in all the assessed parameters compared to the control group ($p < 0.05$). Assessing that the inoculation period (11th day of age) and the birds weighing (14th day of age) took place within four days, this period, according to Kawazoe (2009), corresponds only to the prepatent period of the *Eimeria acervulina,* not encompassing other Eimerias used in the inoculum. The same author also reports that the action of *Eimeria acervulina* causes injury to the intestinal cells, leading to poor food digestion and causing direct impacts on overall performance. On the other hand, Persia et al. (2006) reported that coccidiosis infection could lead to a performance decrease, owing to a metabolizable energy reduction in diet and lower amino acid digestibility.

According to research by Costa et al. (2006), that assessed the effect of different soy extrusion temperatures over 21-day-old chicks' performance, lower extrusion temperatures caused lower diet consumption and weight gain values. These results are the basis of the data obtained at 14 days of age after the administration of raw soy feed. The presence of anti-nutritional components reduces the availability of nutrients.

The lowest weight gain and worst feed conversion observed in the treatments that consisted of 40% or more of raw whole soy, are probably the result of anti-nutritional factors found in this legume, mainly lectins and protease inhibitors (LIMA et al., 2011). On the other hand, results suggest that 20% of the protein from raw whole soy does not seem to represent a level of inclusion capable of exerting the harmful effects of the bioactives present in soy seeds.

The overall effect of oxidized oil throughout the birds' performance agrees with the results of Anjum et al. (2004), who supplied feed consisting of 2% of fresh oxidized soy oil and observed that the weight gains were superior in chickens that received a fresh soy oil feed. However, the results obtained from Racanicci et al. (2008) are different from those of the present study, in that by assessing the oxidized viscera oil effect on poultry performance, did not come by any difference in the performance when compared to poultry which received diets without oxidized viscera oil. Despite the existing difference between peroxide levels (Table 4) found and the level of inclusion in the feed, these same authors report that undesirable effects on performance are not always noted.

Table 4. Peroxide values (mEq/Kg) found in poultry viscera oil samples before the feed incorporation.

Peroxide Values	0	0	1.39	1.40	1.79	1.59	2.00	2.19	5.59	6.76
Days	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th

Table 5 shows the reference data for the 21-day-old birds. Statistical differences ($p < 0.05$) can be observed in all parameters of birds challenged with *Salmonella* Enteritidis. Weight gain and final weight values presented a drop when compared to the control group, differing from the results obtained on the 14th day of age. The explanation for this finding lies in the colonization process of the *Salmonella:* The low concentration of the inoculum could have slowed down the colonization process on the bowel surface and consequently, affect the performance results at 14 days of age. However, at 21 days of age the effects on performance were more intense, indicating a higher microorganism action that suggests the colonization process presented a greater effectiveness.

According to Lamont et al. (2002) and Beaumont et al. (2003), both the intensity and duration of colonization of *Salmonella* Enteritidis are influenced by the host's immunity, bird's age, inoculation route and serotype. Berchieri Júnior (2000) states that paratyphi *Salmonella* can be detected up to nine weeks after infection. All reports corroborate the performance parameters found, suggesting microorganism persistence in birds.

There was no difference ($p > 0.05$) observed in weight gain and final weight when evaluating the obtained data from the group challenged with coccidiosis; however, feed intake and feed conversion continued to be statistically different from levels in the control group. Despite being superior to the control group, feed conversion followed the relationship between diet consumption and weight gain. The relative improvement of performance can be related to the life cycle of the eimeria. As reported by Kawazoe (2009), the parasite can be in the extracellular phase of the reproductive cycle, being more susceptible to the defense mechanisms of the immune system. Another factor to be addressed refers to the attenuating vaccine characteristics: After vaccination, immunity is firstly stimulated by the development of the life cycle of vaccine strains; this effect is maintained and increased by reinfection.

Table 5. Weight gain (WG), final weight (FW), feed intake (FI), feed conversion (FC) and viability (VIAB) of 21-day-old Hubbard broiler chickens submitted to different intestinal challenges.

Treatments	WG (g)	FW (g)	FI (g)	FC (g/g)	VIAB (%)
Control	726.86	772.00	1100.92	1.51	100
Salmonella	688.38	733.46	941.26	1.37	100
Coccidiosis	708.64	753.70	944.82	1.33	100
Raw soy	676.56	720.80	1038.48	1.53	100
Oxidized Oil	690.74	735.20	1026.50	1.48	100
CV (%)	3.09	2.95	3.59	2.59	0
		Contrasts			
Control x Salmonella	0.0107	0.0117	< 0.0001	< 0.0001	NS
Control x Coccidiosis	NS	NS	< 0.0001	< 0.0001	NS
Control x Raw soy	0.0015	0.0015	0.0133	NS	NS
Control x Oxidized oil	0.0157	0.0154	0.0041	NS	NS

NS: non-significant, according to the contrast test ($p > 0.05$).

There was a gradual aggression regarding the *Salmonella* Enteritidis, corresponding to the degree of inoculum concentration and meeting the study's objectives, creating a subclinical context. Oppositely, the group challenged with coccidiosis presented a reverse behavior, with less aggressive

results at the 21st day of age. The life cycle characteristics of this microorganism directly influence the performance parameters since the aggressions are more profuse in the intracellular phases, when the destruction of the villus is more intense.

Undesirable effects on the performance of the groups fed with raw soy and oxidized oil remained until 21 days of age. The continuous administration of these agents could have contributed to the duration of deleterious effects over the weight gain and diet consumption.

During the assessment of the digestibility coefficients at 21 days of age, it was observed that the group with a diet consisting of raw soy presented lower values ($p < 0.05$) for dry matter metabolization coefficient (DMMC), nitrogen balance in ingestion (NB/Ing) and metabolization coefficient ether extract (EEMC) in relation to the control group (Table 6). These findings are comparable to the effects caused by anti-nutritional agents. According to Fasina et al. (2004), lectin activities provoke disorder and destruction of the intestinal villi which interferes in the digestion and absorption of nutrients, reduces enzyme secretion by enterocytes, provokes hypersecretion of endogenous protein, and causes loss of plasma protein for the intestinal lumen and an increased mucus secretion from intestinal cells.

The group challenged with oxidized oil showed a difference ($p > 0.05$) on nitrogen balance (NB). These isolated data render it difficult to make conclusive statements, but can suggest a depletion of intestinal villi, reinforcing the findings of Dibner et al. (1996), who observed the same experimental results when assessing the intestinal histomorphometry of broiler chickens fed with oxidized animal fat.

No difference was found in the other treatments ($p > 0.05$) regarding the parameters of dry matter, nitrogen balance, nitrogen balance in ingestion, and ether extract.

During the intestinal histomorphometry assessment (Table 7), differences ($p < 0.05$) regarding villus height (VH) in the duodenum and in the jejunum were noticed in the groups challenged with coccidiosis and salmonella, respectively. There was a statistical difference concerning crypt depth (CD) only in the jejunum for the group challenged with raw soy.

Table 6. Dry matter metabolization coefficient (DMMC), nitrogen balance (NB), nitrogen balance in ingestion (NB/Ing) and ether extract metabolization coefficient (EEMC) of 21-day-old Hubbard broiler chickens, subjected to different intestinal challenges.

Treatments	DMMC (%)	NB (g)	NB/Ing (%)	EEMC (%)
Control	75.29	7.02	67.68	85.10
Salmonella	76.42	6.68	69.32	85.43
Coccidiosis	75.98	6.96	69.41	85.35
Raw soy	73.07	6.68	65.06	78.98
Oxidized oil	74.52	6.16	65.80	84.87
CV (%)	1.77	4.52	2.85	1.55
Contrasts				
Control x Salmonella	NS	NS	NS	NS
Control x Coccidiosis	NS	NS	NS	NS
Control x Raw Soy	0.0158	NS	0.0441	<0.0001
Control x Oxidized Oil	NS	0.0002	NS	NS

NS: non-significant, according to the contrast test ($p < 0.05$).

Table 7. Villus height (VH) and crypt depth (CD) of the duodenum and jejunum from 21-day-old Hubbard broiler chickens, subjected to different intestinal challenges.

Treatments	Duodenum		Jejunum	
	VH (µm)	CD (µm)	VH (µm)	CD (µm)
Control	2493.16	580.47	1747.19	502.44
Salmonella	2409.69	553.80	2024.16	459.28
Coccidiosis	2215.08	542.07	1838.00	489.07
Raw soy	2309.97	633.70	1687.88	642.25
Oxidized oil	2310.02	594.92	1672.22	483.75
CV (%)	7.76	14.28	9.55	19.82
Contrasts				
Control x Salmonella	NS	NS	0.0189	NS
Control x Coccidiosis	0.0255	NS	NS	NS
Control x Raw soy	NS	NS	NS	0.0427
Control x Oxidized oil	NS	NS	NS	NS

NS: non-significant, according to contrast test ($p < 0.05$).

The lowest values found concerning villus height in the duodenum of the group challenged with coccidiosis, could be related to a longer acting time of the *Eimeria acervulina*. This microorganism has the shortest pre-patent period (96 hours) and presents tropism on the duodenum cells (KAWAZOE, 2009). These characteristics suggest a longer period of parasite action and consequently, greater destruction of the duodenum villi.

The high VH values for the jejunum found in the group challenged with salmonella ratify the claims made by Hofer et al. (1997), which highlighted the high colonizing ability of *Salmonella* Enteritidis, without entailing alterations in the gastrointestinal tract cells' integrity.

While assessing all the digestibility parameters from the group challenged with raw soy, the only noticeable result was the crypt depth in the jejunum. The intestinal mucus presents a constant cell renewal, this process being associated with the two cytological events of proliferation and differentiation. According to Maiorka et al. (2002), these processes determine a turnover characterized by a balance between cell synthesis and extrusion in the bowel surface microvillus.

The fact that only the crypt depth variable showed a statistical difference suggests a high cellular production (synthesis) and the conclusion is that there is a renovation process of the intestinal villi, since no difference ($p > 0.05$) was seen in the villus height of the jejunum. This hypothesis gains new shapes through the data in Table 8, which are demonstrated by the relation between villus height and crypt depth.

Table 8. Relation between villus height (VH) and crypt depth (CD) of duodenum and jejunum from 21-day-old Hubbard broiler chickens subjected to different intestinal challenges.

Treatments	Duodenum	Jejunum
	VH:CD	VH:CD
Control	4.34	3.88
Salmonella	4.43	4.47
Coccidiosis	4.09	3.80
Raw soy	3.67	2.68
Oxidized oil	4.01	3.47
CV (%)	15.37	22.58
Contrasts		
Control x Salmonella	NS	NS
Control x Coccidiosis	NS	NS
Control x Raw soy	NS	0.0329
Control x Oxidized oil	NS	NS

NS: non-significant, according to contrast test ($p < 0.05$).

By comparative assessment of digestibility values and morphometric parameters, it is possible to delimit the action of raw soy over intestinal health at 21 days of age. Dry matter results, nitrogen balance in ingestion, and ether extract suggest aggressions-and a consequent drop in the digestive and absorption performances-of the gastrointestinal tract. These data, added to the low values found for jejunum crypt depth, and for relationship between villus height and crypt depth, suggest a situation of villus renewal after the harmful action of the anti-nutritional agents found in raw soy.

Coefficient of determination "r" values obtained from the correlations between the digestibility values from the ether extract and the intestinal parameters of histomorphometry are shown in Table 9, however, other digestibility values did not show any correlation with parameters of intestinal histomorphometry. The correlation results obtained for the duodenum in the groups challenged with *Salmonella*, raw soy and oxidized oil were inconclusive. As the duodenum is a place of low lipid absorption, the association of the two variables did not present a correlation. Only the group challenged with coccidiosis presented a positive correlation, indicating that the increase of villus height implies an increase of the digestibility of ether extract.

As for the results obtained for the jejunum, positive correlations were found for villus height in groups challenged with coccidiosis, raw soy, and oxidized oil; and for crypt depth in groups challenged with oxidized oil. These data suggest that the digestibility of the ether extract is directly related to villus height and crypt depth.

Table 9. Correlation between ether extract digestibility and villus height (VH), and crypt depth (CD) of duodenum and jejunum from 21-day-old broiler chickens.

Treatments	r value							
	Duodenum				Jejunum			
	VH		CD		VH		CD	
Control	- 0.26	-	0.86	-	0.58	-	0.98	+
Salmonella	0.71	-	0.15	-	0.06	-	0.04	-
Coccidiosis	0.96	+	0.77	+	0.81	+	0.01	+
Raw Soy	0.14	-	0.61	-	0.59	+	0.07	+
Oxidized oil	0.88	-	0.87	-	0.93	+	0.70	+
Total	0.86	-	0.03	-	0.27	+	0.02	-

The information obtained in this study demonstrates that parameters of nutrient digestibility can be useful tools for assessing intestinal health conditions and, when associated with intestinal histomorphometry values, amplify the possibilities of understanding the function of the gastrointestinal tract when facing chemical and biological challenges, as suggested by the correlation found between values of ether extract metabolism and intestinal histomorphometry. It can also be concluded that there is a positive correlation between the digestibility of ether extract and the intestinal histomorphometry of the jejunum of broiler chickens challenged with coccidiosis, oxidized oil, and raw soy.

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