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Research Article

Effect of Palm Kernel Meal Inclusion on Growth Performance, Immune and Visceral Organ Weights and Cecal Lactic Acid Bacteria in Neonatal Broilers

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Abstract

Background and Objective: Feed represents up to 70% of the total cost of production in the poultry industry; the challenge is to find alternative sources available to increase economic profitability. The study aimed to evaluate growth performance, immune and visceral organ weights and cecal lactic acid bacteria in neonatal broilers fed 5, 10 and 15% of palm kernel meal in the diet.

Materials and Methods: Overall, 1,152 birds of the Ross 308® genetic line were used, randomly distributed in four treatments and six repetitions per treatment during the first 10 days old. Isoproteic and isoenergetic diets were made. **Results:** The dietary inclusion with 5 and 10% palm kernel meal increased ($p < 0.05$) the final body weight compared to the control treatment and the latter level of inclusion improved ($p < 0.05$) the feed conversion ratio. In addition, the greater contribution of fiber due to the higher inclusion of palm kernel increased the relative weight of the gizzard, however the relative weight of the small intestine decreased ($p < 0.05$) with the dietary use of palm kernel, whereas the other indicators measured did not change ($p > 0.05$) due to the effect of experimental diets. Likewise, palm kernel meal as an alternative feed in broiler diets increased the growth of cecal lactic acid bacteria, the green bacillus with white halo being the most representative. **Conclusion:** Palm kernel meal (5 and 15%) improved the performance, with a greater proliferation of cecal lactic acid bacteria and with few modifications in the relative weight of the digestive and immune organs.

Key words: Fattening bird, cecal beneficial bacteria, visceral and immune organ, growth performance, palm kernel

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Technological advances in poultry production developed in the United States of America and other countries in the last 40 years have been rapidly implemented and it has been necessary to handle birds in large sheds. The need for high quality nutrients, especially protein, increasing economic income and living standards has created a demand for poultry products¹. However, along with this, the need to maximize productivity for each kg of meat produced is intensified. Thus, there is a constant concern in reducing the expenses with the feeding of the birds, because this represents approximately 70% of the total cost of production².

Due to this, the need arises to find products or nutrients that reduce costs and at the same time meet the nutritional requirements necessary for the growth of broilers. These products must be cheaper compared to those commonly used in the poultry industry and they must be available in the production area³, thus the implementation of palm kernel meal in the diet could be a feasible alternative in the poultry industry⁴. Honduras is the third largest producer and exporter of palm oil in Latin America, surpassed only by Ecuador and Colombia but at the same time, it is the eighth largest in the world. According to data, the country currently has 190 thousand hectares planted with oil palm, with a production of 2.4 million tons of fruit and 480 thousand tons of crude oil⁵.

Research has agreed that it is necessary to use a product that provides benefits to intestinal health to maximize it and improve the performance of birds. In this sense, palm kernel is a byproduct of palm oil extraction; it provides approximately 16-18% crude protein and 13-20% crude fiber⁶. The use of palm kernel is very common in diets of ruminant animals to give volume to the diet and substitute the high-priced dietary ingredients⁷; however, in non-ruminant diets it should be limited especially in bird diets due to the high fiber content⁸. The presence of high levels of fiber in poultry diets can lead to a decrease in the surface, width and height of intestinal villi⁹, which affects the absorption and proper use of nutrients and causes a negative effect on weight gain and organ growth.

Studies by Sundu *et al.*¹⁰ found that the inclusion of up to 40% of palm kernel in the diet of broilers did not indicate a negative effect on the body weight of the birds. Also, the inclusion of a multi-enzymatic complex in these diets reduced fecal moisture. Likewise, feeding hens with palm kernel at different levels (up to 25%) improved the feed conversion ratio, production and quality of the egg¹¹. In ducks, the use of palm kernel did not affect the digestibility of dry matter and detergent neutral fiber, especially taking into account the physiological characteristics of this poultry species that does

not admit high levels of fiber¹². This study aimed to evaluate the effect of inclusion levels of palm kernel meal on growth performance, immune and visceral organ weights and lactic acid bacteria count in neonatal broilers.

MATERIALS AND METHODS

All the procedures adopted in carrying out this experiment were approved by the Pan-American Agricultural School, Zamorano, San Antonio Oriente, Honduras and conducted in accordance with the Guidelines for Experimental Animals.

Location: The study was conducted in July 2019 at the Poultry Research and Training Center of the Pan-American Agricultural School (Zamorano), located in Valle del Yegüare at 32 km of the Tegucigalpa road to Danlí. The place is 800 meters above sea level with an average temperature of 26°C.

Birds and diets: A total of 1152 chickens of the Ross 308® genetic line with a density of 11 chickens/m² were used in a complete randomized design with four dietary treatments, six repetitions and 48 birds per repetition. The experiment was lasted for 10 days. The treatments were control diet, 5, 10 and 15% palm kernel meal inclusion in the broiler diets. Diets were formulated according to the requirements acquainted by the manual for Ross 308® chicken broilers (Table 1). The metabolizable energy reported by FEDNA¹³ for palm kernel meal (1125 kcal kg⁻¹) was taken into account.

Each repetition consisted of a room with a deep wood chip bed and 11 birds m⁻². Feed and water *ad libitum* in hopper feeders and nipple drinkers were offered, respectively. The temperature and ventilation inside the shed by gas breeders, curtain handling and fans were controlled. The shed according to environmental quality standards was disinfected. No medications or therapeutic veterinary care during the entire experimental stage were used. The birds against Marek and Smallpox (first day) were vaccinated.

Performance parameters: The viability by live animals among those existing at the beginning of the experiment was determined. The feed conversion ratio as the amount of feed ingested, for a gain of 1 g of live weight (W) was calculated. The initial and final weight was carried out individually on the first day and at 10 days old, using a Mettler Toledo® IND226 industrial balance with an accuracy of ±1.00 g, respectively. Feed intake using the offer and rejection method was calculated.

Table 1: Ingredients and contributions of diets for broilers (0-10 days)

Ingredients	Inclusion levels with palm kernel meal			
	Control	5%	10%	15%
Cornmeal(CP, 7.79 %)	49.57	44.90	40.25	35.56
Soy meal (CP, 48.0 %)	39.54	38.69	37.84	36.99
Palm kernel meal	0.00	5.00	10.00	15.00
Mineral and vitamin premix	0.50	0.50	0.50	0.50
Sodium chloride	0.50	0.50	0.50	0.50
Palm oil	6.15	6.71	7.25	7.82
Choline	0.08	0.08	0.08	0.08
DL-Methionine	0.38	0.37	0.37	0.36
L-Threonine	0.10	0.11	0.12	0.13
L-Lysine	0.25	0.26	0.27	0.28
Calcium carbonate	1.13	1.12	1.07	1.03
Biphos	1.58	1.54	1.53	1.53
Mycofix plus 5.0	0.12	0.12	0.12	0.12
Lbzyme X50 Enzymes	0.05	0.05	0.05	0.05
Cocciostat	0.05	0.05	0.05	0.05
Proximal composition (%)				
Metabolizable energy (kcal kg ⁻¹ MS)	3000.00	3000.00	3000.00	3000.00
Crude protein	23.43	23.43	23.43	23.43
Crude fiber	2.39	3.64	4.41	5.82
Crude fat	9.57	10.23	10.88	11.55
Ashes	6.36	6.66	6.96	7.28
Ca	0.96	0.96	0.96	0.96
P available	0.48	0.48	0.48	0.48
Methionine+cystine	0.95	0.95	0.95	0.95
Threonine	0.86	0.86	0.86	0.86
Valine	0.91	0.90	0.89	0.88
Isoleucine	0.80	0.79	0.78	0.77
Leucine	1.60	1.59	1.59	1.58
Lysine	1.28	1.28	1.28	1.28
Histidine	0.51	0.50	0.50	0.49
Arginine	1.30	1.28	1.26	1.24
Tryptophan	0.24	0.24	0.24	0.24
Phenylalanine	0.80	0.79	0.79	0.78

¹Each kg contains; Vitamin A: 13,500 UI, Vitamin D3: 3,375 UI, Vitamin E: 34 mg, B2: 6 mg, Pantothenic acid: 16 mg, Nicotinic acid: 56 mg, Cu: 2,000 mg, Folic acid: 1.13 mg, Vitamin B12: 34 mg, Mn: 72 mg, Zn: 48 mg

Relative organ weight and cecal pH: At 10 days of age, 10 birds by fasting treatment for 6 hours were sacrificed by the bleeding method in the jugular vein, followed by the viscera (liver, heart and pancreas), immune organs (thymus, spleen and Bursa of Fabricius), intestines and cecum, were weighed on a digital scale Truweigh blaze digital SCALE BL-100-01-BK with accuracy ± 0.1 g. Following the sacrifice, the pH of the five-bird cecum by fasting treatment was determined using an Oakton® digital pH potentiometer model pH 700, calibrated with pH buffer solutions at 1.68, 4.01, 7.00, 10.01 and 12.45¹⁴.

Cecal lactic acid bacteria: In the sacrifice, the cecum of three birds/treatment was taken and the mucosa was scraped with a scalpel for microbiological culture. The cecal content of each sample (1 g), was deposited in a tube containing 9 mL of

sterile peptone water (*Cultimed Parnreac*-Chemical-SAU), homogenized in distilled water at a rate of 1/10 (w) and from it, serial dilutions (1/10) were made until dilution 10⁶. From each dilution, 0.1 mL was taken and seeded in Petri dishes with MRS agar (Difco Laboratories, Detroit, Mich.) and pH 5.6 at 37°C for 48 h in anaerobiosis (*Gas Pak system*, BBL, Cockeysville, USA).

For the determination of lactic acid bacteria, three repetitions were performed for each dilution; subsequently, the visual count of the colonies was performed. The tests were conducted in the microbiology laboratory of the Zamorano Pan American Agricultural School, Honduras.

Statistical analysis: The data were analyzed using one-way analysis of variance (ANOVA), before carrying out the analysis of variance we proceeded to verify the normality of the data by the Kolmogorov Smirnov test and for the uniformity of the variance, Bartlett's test was used, where necessary, the Duncan's test was used to determine the differences between means, with the help of statistical software SPSS version 17.1. Significance was evaluated at the level of $p < 0.05$.

RESULTS

Table 2 shows that inclusion levels of palm kernel meal up to 15% in broiler diets did not significantly change ($p < 0.05$) feed intake and viability. However, the inclusion of 5 and 10% of palm kernel meal significantly increased ($p < 0.05$) the body weight in relation to 0 and 15%. In addition, the group fed with 10% palm kernel showed the lowest feed conversion ratio ($p < 0.05$) compared to 0 and 15%, the latter being the highest value.

There was no significant differences ($p > 0.05$) in the relative weight of the digestive organs, digestive viscera, immune organs and cecal pH except for the relative weight of the gizzard that increased significantly ($p < 0.05$) with the highest inclusion of palm kernel meal (15%) in the diet of broilers up to 10 days, while the relative weight of the small intestine was reduced ($p < 0.05$) as the inclusion of palm kernel flour increased (Table 3).

Table 4 shows the count of cecal lactic acid bacteria of broilers fed with palm kernel meal up to 10 days of age. Four bacterial morphologies were found, such as green bacilli, white bacilli, green irregular planes bacilli and green bacilli with halo, the latter being statistically different ($p < 0.05$) between the control treatment and the inclusion of 5 and 15% palm kernel meal, with the highest count with 5% of palm kernel meal. In addition, the dietary use of palm kernel meal in bird diets increased total LABs with notable differences ($p < 0.05$) to control treatment.

Table 2: Performance of broilers (1-10 days) fed with inclusion levels of palm kernel meal

Items	Palm kernel meal (%)				SEM±	p-value
	0	5	10	15		
Initial BW (g)	46.67	46.80	46.97	47.43	0.2780	0.242
Final BW (g)	165.15 ^b	174.66 ^a	180.89 ^a	158.74 ^b	2.7730	<0.001
FI (g)	142.45	149.03	146.03	142.45	2.2060	0.101
FCR (g g ⁻¹)	1.21 ^b	1.17 ^{bc}	1.11 ^c	1.28 ^a	0.0270	<0.001
Viability (%)	97.67	98.67	96.67	96.67	1.1589	0.388

^{a,b,c}Means with different letters in the same row differ to p<0.05. BW: Body weight, FBW: Final body weight, FI: Feed intake, FCR: Feed conversion ratio

Table 3: Relative weight of the digestive organs, digestive viscera, immune organs and cecal pH of broilers (10 days) fed with inclusion levels of palm kernel meal

Items (%)	Palm kernel meal (%)				SEM±	p-value
	0	5	10	15		
Proventriculus	1.05	0.96	0.98	0.96	0.050	0.547
Gizzard	6.58 ^b	6.58 ^b	7.01 ^{ab}	7.14 ^a	0.161	0.032
Small intestine	8.65 ^a	7.55 ^b	7.12 ^b	7.52 ^b	0.279	0.003
Cecum	1.09	0.93	0.92	0.93	0.065	0.205
pH cecal	6.67	6.51	6.76	6.45	0.116	0.260
Liver	3.34	3.48	3.32	3.22	0.112	0.458
Pancreas	0.46	0.51	0.46	0.48	0.024	0.543
Heart	0.77	0.73	0.76	0.76	0.030	0.696
Bursa of fabricius	0.18	0.20	0.17	0.20	0.016	0.339
Thymus	0.23	0.26	0.21	0.21	0.025	0.451
Spleen	0.10	0.12	0.10	0.10	0.008	0.344

^{a,b,c}Means with different letters in the same row differ to p<0.05

Table 4: Lactic acid bacteria count of broilers (10 days) fed with inclusion levels of palm kernel

Items (CFU g ⁻¹)	Palm kernel meal (%)				SEM±	p-value
	0	5	10	15		
Bacilli ¹	4.51	4.00	5.81	5.05	0.741	0.408
Bacilli ²	6.33	7.05	7.34	6.23	0.672	0.604
Bacilli ³	7.75	8.02	7.88	8.29	0.377	0.772
Bacilli ⁴	5.18 ^c	7.30 ^a	5.81 ^{bc}	6.61 ^{ab}	0.681	0.032
Total	6.59	6.72	6.77	6.63	0.620	0.997

^{a,b,c}Means with different letters in the same row differ to p<0.05. ¹Greens, ²Whites, ³Green irregular planes, ⁴Green

DISCUSSION

One of the main objectives of the study was to verify if the use of palm kernel meal up to 15% in diets does not depress the body weight of broilers up to 10 days. In this sense, Shakila *et al.*¹⁵ found that the inclusion of palm kernel meal up to 7.5% did not change the performance of broilers. In addition, Abdollahi¹⁶ obtained satisfactory results in the weight gain of broilers with the inclusion of 8 and 16% palm kernel with a multi-enzyme complex. Other results without the use of enzymes reported by Mardhati *et al.*¹⁷ found a reduction in weight gain with the inclusion of 20% palm kernel.

It is known that palm kernel meal has a high content of non-starch polysaccharides, thus the authors have recommended the use of this product with phytase enzymes, cellulases, carbohydrases, beta-glucanase and xylanase¹⁸. The diets formulated in this experiment contain an enzymatic complex capable of using the lignocellulosic structure as an

energy source that could cause an increase in the weight of the chickens with 5 and 10% inclusion level of palm kernel meal. However, the inclusion of 15% of palm kernel increased fiber content (3.43%) compared to the control group, which could reduce the weight of the birds, without affecting the viability of the lot, demonstrating the safety of the feed used. In addition, Olukomaiya *et al.*¹⁹ have recommended palm kernel meal as an alternative source of protein but anti-nutritional compounds bound to the cell wall and limit their use in high concentrations in broilers. On the other hand, Sundu *et al.*¹⁰ indicated that due to its high percentage of fiber the dietary use of palm kernel flour increases the rate of passage of the feed chyme through the gastrointestinal tract. Conversely, the results of Alshelmani *et al.*⁴ indicated that the inclusion of 15% of palm kernel meal decreased the digestibility of nutrients due to fibrous components, since these non-starchy polysaccharides have no enzymatic affinity and are transported to the cecum, this effect causes a lower

use of nutrients with a depression of the average weight of the birds²⁰. However, according to FAO²¹, levels of inclusion up to 30% in broiler diet do not affect biological performance.

The use of palm kernels decreased the relative weight of the small intestine (Table 3). As abovementioned, palm kernels are rich in fibrous compounds without enzymatic affinity in birds¹⁰; thus, the use of exogenous enzymes with palm kernel in the diets could increase the assimilation of the nutrients and decrease intestinal inflammation linked to the postprandial activity²². In this sense, Sarica *et al.*²³ found a decrease in the relative weight of the small intestine when using a multienzyme complex in the diets of broilers. Furthermore, Soltan²⁴ showed that the use of palm kernel meal up to 20% with enzymes did not reduce the utilization of nutrients and carcass traits in broilers, as well as Alshelmani *et al.*⁴ reported that contribution of crude fiber up to 4.41 % with enzymes in the diets of broilers improved the growth performance.

The proventriculus and gizzard are two compartments of the stomach of the birds, the first secretes hydrochloric acid and pepsinogen and the second has the function of crushing, pulverizing, compressing and grinding of the bolus for better use in the small intestine²⁵. The volume and weight of the gizzard increase substantially when the diets contain whole grains and insoluble fiber²⁶. In this sense, the inclusion of a 15% palm kernel meal rich in insoluble fiber increased the relative weight of the gizzard. Similarly, Olukomaiya *et al.*¹⁹ reported higher activity and relative weight of gizzard when high levels of palm kernel meal and higher contributions of crude fiber were used in broiler diets. Hetland *et al.*²⁷ reported that high fiber diets increased the gizzard's weight by 60% due to the greater functionality of this organ. Additionally, it is expressed that fiber intake is directly related to gizzard development²⁸.

Although contribution of fiber in the diet was higher (Table 1) but this was not enough to increase the relative weight of the cecum, perhaps a broader contribution and permanence of this fibrous compound is necessary to cause the increase in the weight of this organ. Likewise, authors such as Martínez *et al.*²⁹ and Dihigo *et al.*³⁰ have reported that feeding broilers with fibrous feeds such as *Lablab purpureus* and *Morus alba*, did not change the relative weight of cecum. Also, our study did not find changes in cecal pH in chickens fed with palm kernel meal that is rich in fiber. Similar results have been reported by Mateo *et al.*²⁸ and Martínez *et al.*¹⁴ who used different fibrous sources in the diets of broilers. Other studies using phytochemical compounds rich in polyphenols and organic acids in chicken diets reported a decrease in cecal pH related to an increase in volatile fatty acids³¹⁻³³. The liver plays an important role in bile secretion, fat

and protein metabolism³⁴. In our experiment, no statistical variation was observed in the relative weight of the liver (Table 3), whereas it undergoes a few changes in the first ten days as reported by Fontana *et al.*³⁵. However, the growth of this organ has been associated with hypocholesterolemic chemical products or compounds³⁶; apparently, palm kernel does not have these chemical structures, capable of changing the lipid metabolism-related to the surrounding cholesterol, especially since the metabolism of this biomolecule is entirely hepatic³⁷, however, to corroborate this hypothesis it is necessary to make further studies.

The pancreas produces pancreatic juice, which is a mixture of digestive enzymes and also produces hormones such as insulin and glucagon, which are involved in carbohydrate metabolism³⁸. Mateos *et al.*²⁸ reported no significant difference ($p > 0.05$) in the weight of the pancreas of broilers fed with whole wheat and/or oatmeal (rich in insoluble fiber). In addition, the pancreatic hypertrophy is related to an excess of secondary metabolites such as trypsin and chymotrypsin inhibitors³⁹, yet these anti-nutritional factors have not been reported in palm kernel meal.

The heart of the birds has four compartments, just like the human heart, it has the ventricles and atria, where the blood is pressurized to the rest of the body's organs⁴⁰. According to Chinajariyawong and Muangkeow⁴¹ the relative weight of the heart in broilers was affected by the inclusion of 10 and 40% palm kernel meal, however, similar to our experiment (Table 3), Bello *et al.*⁴² found no notable difference for the relative weight of the heart in broilers when they included 20% palm kernel. In this sense, Cañete *et al.*⁴³ have described that an increase in the weight of the organ is associated with the activity of the circulatory system caused by hemoconcentration and hypovolemia, perhaps due to the loss of fluid or the growth of an organ, it is necessary to increase the blood supply.

On the other hand, the development of the thymus is parallel to the development of the Bursa of Fabricius⁴⁴. Both are the primary lymphoid organs with significant participation in the adaptive immunity of birds⁴⁵. In this sense, a relationship between feed intake with palm kernel meal and the development of the thymus and bursa of Fabricius was not found. Other studies have reported that broiler chickens challenged with ochratoxins and fed with palm kernel meal fermented with *Aspergillus niger* increased the relative weight of the bursa of Fabricius⁴¹. Likewise, the relative weight of the spleen was not different between treatments (Table 3), similar results were reported by Sadeghi *et al.*⁴⁶ and Zúñiga *et al.*⁴⁷, when they used various fiber types and dehulled lupine (*Lupinus angustifolius*) in broiler diet, respectively. This secondary lymphoid organ is more active

with the involution of the primary lymphoid organs (thymus and bursa of Fabricius)⁴⁸. It is known that the activity of these immune organs depends on the conditions of tenure, management, nutrition and health of birds⁴⁹.

The lactic acid bacteria (LAB) that make up the digestive tract microflora have a positive role in the health and productivity of broiler chicken^{4,50}. According to Latorre *et al.*⁵¹, the colonization of lactic acid bacteria is directly related to the composition of the diet. In this sense, Zulkifli *et al.*⁵² found a higher proliferation of cecal lactic acid bacteria in broilers at the level of 25% palm kernel inclusion to replace partially soybean meal and corn. The previous results coincided with those presented in Table 4, since the use of palm kernel meal favored the growth of bacteria that grow in MRS (Man, Rogosa y Sharpe), due to a broader contribution of fiber in the diet (Table 1), mainly insoluble. The fibrous compounds cannot be digested and absorbed in the small intestine and are transported to the large intestine where a part is usable by the lactic acid bacteria⁵³.

Furthermore, Sugiharto and Ranjitkar⁵⁴ have stated that the use of dietary prebiotic compounds in the diets of broilers could increase the population of cecal lactic acid bacteria. Van Immerseel *et al.*⁵⁵ mentioned that palm kernel meal has a high content of beta glucans, which could favor the growth of cecal lactic acid bacteria (LAB). Carlson *et al.*⁵⁶ have recommended the use of prebiotics such as beta glucans and insulin to improve the host's intestinal health. Prebiotics tend to increase the number of beneficial bacteria in the intestine such as *Lactobacillus* spp. and *Bifidobacterium* spp., which reduces the growth of pathogenic bacteria by competitive exclusion^{52,57,58}.

The use of palm kernel meal in broiler diets increased the green bacillus count by 2.12 (T1) CFU g⁻¹ compared to the control treatment, however the experimental treatments did not change total LAB count. This result confirms that the use of fibrous sources in adequate amounts such as palm kernel meal favors the proliferation of LABs and perhaps better intestinal health, which was reflected in a better productive response (Table 4). In this sense, Fernandez *et al.*⁵⁹ found that diets formulated with palm kernel meal and with high contributions of mannan-oligosaccharides increased the population of *Bifidobacterium* spp. and *Lactobacillus* spp. while decreasing the Enterobacteriaceae groups. Intestinal health in the early days of the bird is determined by anaerobic bacteria present in the digestive tract, which tend to become dominant bacteria in the cecal due to the handling and nutrition conditions of the chicken⁶⁰, as well prebiotic sources can ensure a better genetic expression of the chicken during its fattening stage⁶¹.

CONCLUSION

The inclusion levels of palm kernel meal up to 10% in broiler diets to replace partially soybean meal and corn improved the body weight and feed conversion up to 10 days of age, without affecting the other measured indicators. The relative weight of the gizzard increased with the higher inclusion level of palm kernel meal, whereas the relative weight of the small intestine reduced as the inclusion level of palm kernel meal increased. Dietary inclusion of palm kernel meal favored the growth of the green bacilli with white halo.

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