# Changes in growth, digestibility and gut anatomy by broilers fed diets containing ethanol-treated castor oil seed (*Ricinus communis* L.) meal

Cambios en el crecimiento, digestibilidad y anatomía intestinal de pollos de engorde alimentados con dietas que contienen harina de semillas de ricino (*Ricinus communis* L.) tratada con etanol

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### ABSTRACT

Eight weeks feeding trial was conducted to investigate the chemical composition, growth response, apparent nutrient digestibility, gut characteristics and morphology of broiler chickens fed four diets containing ethanol-treated castor oil seed meal (ECAM). Two hundred day-old Anak 2000 broiler chicks were then allotted to the four experimental diets containing ECAM at 0, 100, 150 and 200 g/kg, respectively. Each treatment was replicated five times with 10 birds per replicate. The final live weight, weight gain and feed intake reduced (P < 0.01) with increasing dietary inclusion of ECAM. Control fed broilers and those fed diets containing 100 g/kg and 150 g/kg ECAM had similar feed to gain ratio. Broilers fed diets containing 200 g/kg ECAM recorded the least (Linear and quadratic contrast, P < 0.05) crude protein and crude fibre digestibility, and the poorest (Linear contrast, P < 0.05) feed to gain ratio. Similar improved feed to gain ratio, apparent crude fibre, fat and ash digestibility were noticed with birds fed the control diet, diets containing 100 g/kg and 150 g/kg ECAM, respectively. Relative gizzard weight increased (P < 0.05) relative gizzard, pancreas, duodenum weight and the longest (P < 0.05) relative jejunum and ileum length. Villi length and crypt depth of the duodenum reduced (P < 0.05) with increasing dietary inclusion of ECAM. In conclusion, ethanol-treated castor seed oil meal can be included up to 150 g/kg diet in broiler ration without impairing growth and nutrient digestibility.

Key Words: Broiler performance, gut anatomy, ethanol, castor oil seed, apparent digestibility

### RESUMEN

Un ensavo de alimentación de ocho semanas se realizó para investigar la composición química, la respuesta del crecimiento, la digestibilidad aparente de nutrimentos, las características y la morfología intestinal de pollos de engorde alimentados con dietas que contienen harina de semillas de ricino tratada con etanol (HSRTE). Doscientos pollos de engorde Anak 2000 de un día de edad se asignaron a cuatro dietas experimentales que contienen HSRTE a 0, 100, 150 y 200 g/kg, respectivamente. Cada tratamiento se repitió cinco veces con 10 aves por repetición. El peso vivo final, la ganancia de peso y el consumo de alimento se redujo (P < 0.01) con la inclusión cada vez mayor de la dieta de HSRTE. Los pollos de engorde alimentados con el control y aquellos alimentados con dietas que contenían 100 y 150 g/kg de HSRTE tuvieron una relación alimento:ganancia similar. Los pollos de engorde alimentados con dietas que contenían 200g/kg de HSRTE registraron la menor proteína cruda y digestibilidad de fibra cruda (Contrastes lineal y cuadrático, P < 0.05) y la relación alimento: ganancia más pobre (Contraste lineal, P < 0.05). Se notó un mejoramiento similar en la relación alimento: ganacia y digestibilidad aparente de fibra cruda, grasa y cenizas con aves alimentadas con la dieta control, dietas que contenían 100 y 150 g/kg de HSRTE, respectivamente. El peso relativo de la molleja se incrementó (P < 0.05) con el aumento de los niveles de inclusión en la dieta de HSRTE. Los pollos alimentados con 200 g/kg de HSRTE registraron el mayor peso relativo de la molleja, el páncreas y el duodeno (P < 0.05) y la mayor longitud relativa del yeyuno y el íleon (P < 0.05). La longitud de las vellosidades y la profundidad de las criptas del duodeno se redujeron (P < 0.05) con un incremento de la inclusión en las dietas de HSRTE. En conclusión, la harina de semillas de ricino tratada con etanol puede ser incluida hasta 150 g/kg en dietas para raciones para pollos de engorde, sin perjudicar el crecimiento y la digestibilidad de los nutrimentos.

Palabras clave: Comportamiento de pollos de engorde, anatomía intestinal, etanol, semillas de ricino, digestibilidad aparente

### INTRODUCTION

Animal nutrition studies have shown that castor oil seed (Ricinus communis, L) meal can be used, to a limited extent, as oil seed cake in feeds for ruminants (Rao et al., 1988), rabbits (Adedeji et al., 2006), pigs (Geary, 1950) and poultry birds (Okorie et al., 1985). However, dietary inclusion at high levels in poultry rations suffered serious limitations due to the presence of residual ricin, ricinine and castor allergen (Puttaraj et al., 1993; Ani and Okorie, 2002). Deleterious factors contained in feed stuffs have been reported to modify mucosal structure and function, affect nutrient mobility, digestion and absorption of poultry birds (Reid, 1987; Klopfenstein, 1988). Other anti-nutritive factors like tannins have been implicated in changes relating to intestinal absorption of nutrient, reduction in villi width and length of duodenum and jejunum (Johnston et al., 2005; Kim and Miller, 2005) while, non-starch polysaccharides concentration has been implicated in changes relating to weights and length of the gastrointestinal tract (Johnson et al., 1984).

During the production of castor oil, undehulled seeds are normally used due to difficulties encountered in the dehulling process leaving behind a fibrous meal. The fibrous nature of the castor seed also constitutes a major constraint to its utilization in poultry nutrition. Fibrous ingredients have been reported to, negatively, affect nutrient utilization by diluting macronutrients (Longe and Ogedengbe, 1989), modifying gut characteristics and intestinal morphology (Wu et al., 2004) and modifying the upper and lower part of the digestive tract of poultry (Gabriel et al., 2003; Hetland et al., 2003). Although several processing methods have been attempted in the past, studies on dietary inclusion of ethanoltreated castor oil seed meal in feed for broilers are rare. The research was conducted to investigate the chemical composition, growth response, apparent digestibility, nutrient gut characteristics and morphology of broiler chickens fed diets containing varying levels of ethanol treated castor oil seed meal.

### MATERIALS AND METHODS

## Collection, processing and composition of test ingredients

The castor oil seed used in this study was obtained from the Forestry Research Institute of

Nigeria (FRIN) Ibadan, Oyo State, Nigeria. The seeds were sun-dried for 2-3 days (till it reached 10-11% moisture content), milled to pass through a 2.5 mm sieve using a laboratory hammer mill (Model W-6-H, Buffalo N. Y. 14206) and mixed with ethanol (75% V) in a mixing ratio of 100 mL of (75%) ethanol to 1 kg of ground seed. Mixing was done in a, locally, improvised rotational mixer (fabricated by the College of Engineering, University of Agriculture, Abeokuta, Ogun State Nigeria) for 15-20 minutes to produce the castor slurry. This was subjected to mild heat at 50 °C for 20 minutes. The mixture (slurry) was allowed to settle down and air-cooled. The upper clear liquid fraction was decanted and poured away while, the solid fraction was sieved using a muslin cloth and screw-pressed overnight to form the cake. The cake was thereafter sun-dried and subsequently ground using hammer mill (2.5 mm sieve) to yield the ethanol treated castor oil seed meal (ECAM). The milled sample was mixed along with other feed ingredients, which were used to formulate the experimental diets.

## Management of birds

The research was done at the Poultry Unit of the Teaching and Research Farm, University of Agriculture, Abeokuta, Nigeria, West Africa. Two hundred day old Anak 2000 male broiler chicks obtained from a commercial hatchery were used for the study. These were weighed individually distributed and balanced for weight amongst 20 brooding units of 1.5 m  $\times$  2.0 m dimension each housing 10 birds that were reared intensively on deep litter housing system. Brooding was done for 21 days. At the expiration of the brooding period, birds contained in each pen (10 in number) were transferred to other larger floor pens (each of 5  $m^2/pen$ ). Temperature was maintained at a stable ambient condition of 30.2 °C after brooding and throughout the study period.

### **Experimental diets**

Four experimental diets were formulated to include ECAM at 0, 100, 150 and 200 g/kg levels, respectively. The experimental diets are as presented in Table 1. Birds contained in each pen were fed with one of the experimental diets such that there were five pens (each containing 10 birds) assigned to each dietary treatment. A straight diet (with no partitioning of diet into starter and finisher) was used throughout the duration of the study (56 days). The crude protein and metabolisable energy contents of the feeds were balanced within the recommended range (NRC, 1994). Representative samples (n=4) of the experimental diets were later prepared and stored for proximate analysis later.

# Growth response and apparent nutrient digestibility

Average live weight per bird was measured at weekly intervals while, feed conversion ratio per pen was calculated as total feed consumed per weight gain. At the expiration of 56 days, two broilers from each pen (making a total of 10 birds per treatment) were selected and arranged in clean, separate and disinfected metabolic cages. Three davs of allowed acclimatization were before the commencement of the digestibility study. A known weight of feed, which matched their previous daily feed intake was fed during the metabolic trial. Excreta collection was done daily for a period of four days. The daily excreta voided for each bird was dried overnight (at 55°C) while, total collections per bird were pooled at the expiration of 4 days metabolic trial. Dried excreta samples were used to determine the proximate compositions according to the method of Association of Official Analytical Chemists (AOAC, 1995).

## **Gut characteristics**

At the end of the experiment, another set of two birds (apart from those used for metabolic trial)

making a total of 10 birds per treatment were selected from each pen and slaughtered. The digestive tract was opened up while, the digestive tract from the proventriculus to the end of the intestine was excised. The proventriculus, gizzard and pancreas were emptied, individually, and weighed. The small intestine was dissected into three segments: duodenum (from gizzard to pancreo-biliary ducts), jejunum (from pancreo-biliary ducts to Meckel's diverticulum). and ileum (from Meckel's diverticulum to ileo-caecal iunction). The gastrointestinal tracts of the birds were excised, cleaned and dried with dessicant paper then the lengths and weights of the various intestinal segments (proventriculus. gizzard, pancreas. duodenum. jejunum, ileum, ceaca and the small intestine) were measured. The lengths and weights were expressed as relative length (in cm/kg live weight) and weight (in g/kg live weight), respectively.

## Gut morphometry

About 0.5 cm portion taken at the medium part of each of the three intestinal segments (duodenum, jejunum and ileum) was used for histological measurements. The samples were opened longitudinally, rinsed with cold saline and fixed in a buffered formalin solution for about 4 h. Histological analysis was done according to the procedures of Goodlad *et al.* (1991). The preparations were mounted between slide and strip. Intestinal villi with their crypts were, individually, separated under a dissecting microscope while the length and width of the villi were measured according to the procedures described by Hampson (1986).

Table 1. Gross composition of early and the second	xperimental diets and chemical	composition of castor (Ricinus	<i>communis</i> L.) seed meal.
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Inclusion level of ethanol-treated castor oil	0	100	150	200
seed meal (ECAM) (g/kg)				
Ingredients				
Maize	52.00	52.00	52.00	52.00
Soya bean meal	32.00	22.00	17.00	12.00
ECAM	0	10.00	15.00	20.00
Fish meal (72% CP)	1.00	1.80	3.0	4.00
Wheat offal	14.00	13.20	12.00	11.00
Bone meal	3.00	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00	2.00
Lysine	0.20	0.20	0.20	0.20
Methionine	0.50	0.50	0.50	0.50
Vit/min premix	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00

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### **Chemical Analysis**

Representative samples (n = 4) of the dried unprocessed seeds were ground using a commercial kitchen blender, while, the ground samples of ECAM and dietary treatments were analyzed for their proximate constituents (AOAC, 1995). Samples were analysed for DM, ether extract (EE), crude fibre (CF) and ash (AOAC, 1995, ID 7.010, 7.016, 7.048). The nitrogen fraction was estimated in an automatic analyzer (Kieltech Auto 147 1013 Analyzer, Tecator) and the crude protein (CP) determined by multiplying the N value with 6.25. Gross energy of samples was determined using an adiabatic bomb calorimeter. Neutral detergent fibre (NDF) content was determined as described by Van Soest et al. (1991) with the use of sodium sulfite and heat stable  $\alpha$ amylase, and acid detergent fibre (ADF) following the method no. 973.18 of AOAC (1995). Acid detergent lignin (ADL) concentration was analyzed by the sequential method of Van Soest and Robertson (1980). Hemicellulose and cellulose were calculated as the differences: NDF-ADF and ADF-ADL, respectively. The mineral profile of the raw untreated seed and ground samples of ECAM were determined after wet-ashing by concentrated nitric acid and perchloric acid (1:1, v/v). Sodium, Potassium and Calcium were determined by flame photometry England), while Magnesium, (Corning 410. Manganese and Iron were determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). The ashed samples were analysed for copper using the atomic absorption spectrophotometry at wavelength of 324.7nm. Phosphorus was estimated photometrically via the Phosphorus molybdate complex described by Taussky and Shorr (1953). The chemical composition of ground samples of raw castor seed and ECAM were expressed using a simple average of four determinations.

### Experimental design and statistical analysis

The design of the experiment was a completely randomized design. For the growth performance, replicate units (n = 50 per treatment) served as the experimental units for statistical analysis. For apparent nutrient digestibility study (n = 10 per treatment), gut characteristics (n = 10 per treatment), individual birds selected were considered as the experimental unit. Data obtained were analysed using the general linear models procedures of SPSS (1997).

Polynomial contrast (Linear and quadratic) was applied to determine the effects of varying inclusion levels (0, 50, 100 and 150 g/kg) of ECAM. A probability of P < 0.05 was considered to be statistically significant.

### RESULTS

## Chemical composition

The proximate composition, fibre fraction analysis and mineral profile of the unprocessed seeds and ECAM (expressed in average of four determinations) are as presented on Table 2. The ECAM contained a higher dry matter and crude protein content than the raw seed. The proximate assay showed that ECAM has a lower crude fibre and ether extract than the raw seed. The estimated metabolisable energy of ECAM was higher than values obtained for the unprocessed seed. The mineral profile of ECAM however, showed a higher Ca, Mg, P and a relatively lower Na and K as compared with raw castor seed.

# Growth response and apparent nutrient digestibility

The weight gain, feed conversion ratio and apparent nutrient digestibility of broiler chickens fed diets containing ECAM are as displayed on Table 3. The weight gain and feed intake reduced [Linear (LC) and quadratic contrast (OC), P < 0.01 with increasing dietary inclusion of ECAM. Broilers fed with control diet, diets containing 100 g/kg and 150 g/kg ECAM showed improved feed to gain ratio while, birds fed diets containing 200 g/kg ECAM recorded poor feed to gain ratio. Broilers fed with diets containing 200 g/kg ECAM recorded the highest apparent ash digestibility (LC and QC, P < 0.05), ether extract digestibility (QC, P < 0.05) and least (LC and QC, P < 0.05) crude protein and fibre digestibility. Apparent dry matter digestibility showed no effect (P > 0.05) following dietary inclusion of ethanol-treated castor oil seed meal in feed for broilers.

## Gut characteristics and morphometric studies

Gut characteristics of broilers fed diets containing varying inclusion levels of ECAM is shown in Table 4. The relative weight of gizzard and pancreas increased (P < 0.05) with increasing levels of dietary inclusion of ECAM. Broilers fed the diet containing 200 g/kg ECAM recorded the highest (P < 0.05) duodenal weight and the longest (P < 0.05)

#### DISCUSSION

relative length of jejunum and ileum. Gut morphology of broiler chickens fed diets containing varying inclusion levels of ECAM is as shown on Table 5. The villi length (LC and QC, P < 0.01) and crypt depth (LC, P < 0.01; LC, P < 0.05) of the duodenum reduced with increasing dietary inclusion of ECAM. Jejunal crypt depth however, increased (LC and QC, P < 0.01) with increasing dietary inclusion of ECAM. Birds fed diet containing 200 g/kg ECAM had the highest (LC and QC, P < 0.01) jejunal crypt depth. Villi length, crypt depth and villi/crypt ratio of the ileum were not affected (P > 0.05) by dietary inclusion of ECAM.

The ethanol treatment of castor oil seed improved dry matter, crude protein and ash contents of castor oil seed meal. The high crude protein recorded for castor oil seed meal (when compared with the raw seed) confirmed it as a potential oil seed cake for poultry nutrition while, a relatively high Ca, Mg, P content assayed for ECAM shows that it contains essential minerals needed in poultry nutrition for bone development in broilers and also egg shell development in layers.

Parameters †	0	100	150	200	Raw castor seed	*ECAM
Dry matter (DM)	95.06	95.67	95.52	95.75	88.26	89.54
Crude protein	22.00	21.28	21.04	20.75	24.41	28.24
Ether extract (EE)	3.63	4.31	4.64	4.98	12.38	9.75
Crude fiber (CF)	3.84	4.52	4.86	5.20	27.14	11.78
Ash	3.49	3.54	3.65	3.66	6.05	5.58
<sup>a</sup> ME (MJ/Kg)	11.90	11.91	11.91	11.92	7.98	10.60
Mineral (mg/100g)						
Calcium	0.24	0.25	0.26	0.26	30.00	32.10
Phosphorus	0.20	0.27	0.30	0.33	96.11	97.34
Magnesium	ND	ND	ND	ND	8.51	8.95
Manganese	ND	ND	ND	ND	0.02	0.02
Potassium	ND	ND	ND	ND	112.10	110.40
Sodium	ND	ND	ND	ND	100.20	99.60
Iron	ND	ND	ND	ND	0.97	0.99
Copper	ND	ND	ND	ND	0.05	0.04

Table 2. Chemical composition of dietary treatments and test ingredients.

† Analysis were expressed in average of four determinations

<sup>a</sup>Metabolisable energy (ME) estimated using the formula of NRC (1994): ME = 26.7(DM) + 77(EE) - 51.22(CF). \*ECAM: Ethanol treated castor oil seed meal. ND= Not determined

Table 3. Growth response and nutrient digestibility of broilers fed diet containing ethanol-treated castor oil seed meal (ECAM) in Abeokuta, Nigeria.

ECAM inclusion (g/kg)	0	100	150	200	Standard error of	Significance of treatment effects $(P > F)$	
					the mean	Linear	Quadratic
Parameters							
Final live weight (g/bird)	2060.67	1784.33	1601.00	1422.00	98.75	0.002	0.007
Weight gain (g/bird)	2015.60	1739.33	1556.00	1376.90	62.44	0.003	0.006
Total feed intake (g/bird)	6329.10	5415.27	5352.64	5149.89	155.97	0.002	0.004
Feed to gain ratio	3.14	3.11	3.44	3.74	0.10	0.041	0.057
Mortality (%)	0.00	0.00	0.00	3.33	0.97	0.044	0.07
Apparent nutrient digestibi	ility:						
Dry matter (%)	84.49	84.58	84.55	84.91	5.33	0.950	0.750
Crude protein (%)	73.99	73.04	70.74	67.59	4.06	0.042	0.030
Crude fibre (%)	63.24	63.65	62.95	60.02	4.11	0.019	0.020
Ash (%)	61.66	61.85	61.91	66.32	3.78	0.020	0.022
Ether extract (%)	75.20	75.08	74.53	76.98	2.99	0.052	0.039

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ECAM inclusion (g/kg)	0	100	150	200	Standard error of	Significance of treatment effects (P>F)	
					the mean	Linear	Quadratic
Relative weights (g/kg):							
Proventriculus	4.37	4.80	5.20	5.41	0.42	0.880	0.645
Gizzard	15.53	18.81	20.12	22.61	1.24	0.033	0.060
Pancreas	2.52	3.10	3.26	5.11	0.72	0.024	0.049
Duodenum	9.76	10.20	10.00	12.40	1.01	0.044	0.055
Jejunum	16.12	16.40	17.10	17.60	0.97	0.066	0.063
Ileum	13.11	13.20	14.00	14.10	1.22	0.910	0.876
Relative length (cm/kg):							
Duodenum	2.96	3.57	3.69	4.30	0.27	0.071	0.088
Jejunum	6.80	8.17	8.43	9.43	0.38	0.044	0.079
Ileum	7.57	8.34	9.49	10.47	0.41	0.033	0.065

 Table 4. Gut characteristics of broilers fed diets containing different proportions of ethanol-treated castor oil seed meal (ECAM) in Abeokuta, Nigeria.

 Table 5. Gut morphology of broilers fed diets containing different proportions of ethanol-treated castor oil seed meal (ECAM) in Abeokuta, Nigeria.

ECAM inclusion (g/kg)	0	100	150	200	Standard error of	Significance of treatment effects (P>F)	
					the mean	Linear	Quadratic
Duodenum:							
Villi length (µm)	1450	1447	1294	1140	64.89	0.002	0.004
Crypth depth (µm)	202	201	196	190	20.98	0.001	0.020
Villi/Crypth ratio	7.18	7.20	6.60	6.00	0.46	0.030	0.060
Jejunum:							
Villi length (µm)	1090	1091	1089	1092	52.73	1.205	1.900
Crypth depth (µm)	172	176	178	201	19.03	0.004	0.002
Villi/Crypth ratio	6.34	6.20	6.12	5.43	0.34	0.940	0.730
Ileum:							
Villi length (µm)	602	596	600	604	29.65	1.110	0.990
Crypth depth (µm)	166	170	167	167	19.72	1.110	0.915
Villi/Crypth ratio	3.63	3.51	3.59	3.62	0.76	1.660	1.760

Increased dietary inclusion of processed castor oil seed meal caused a progressive increase in fibre content of the diet. This is due to the fact that undehulled castor oil seed (high in fibre content) was used in the production of the feed. The reduced feed intake recorded with increased dietary inclusion of ECAM was due to poor palatability of the diet and increased fibre content, which probably led to early gut fill (Longe and Ogedengbe, 1989). This increased bulkiness of the diet affects the gut transient time of the digesta and reduced nutrient digestibility. This resulted in poor feed to gain ratio, apparent crude protein and crude fibre digestibility observed with broilers fed with diets containing 200 g/kg ECAM. A similar trend was reported by Okorie *et al.* (1987). The reduced weight gain noticed with increasing dietary inclusion of ECAM could be linked with the poor amino acid profile of castor oil seed meal and dietary ricin content. Castor oil seed meal was reported to contain low lysine, methionine, tryptophan, and cystine contents (Vilhjalmsdottir and Fischer, 1971). Okorie and Anugwa (1987) also confirmed that dietary ricin content (in castor oil seed meal) adversely affected the feed consumption and nutrient utilization in chicks. The fibrous nature of dietary ingredients fed to poultry birds have been reported to modify the upper and lower parts of the digestive tract

(Gabriel *et al.*, 2003). The high relative weight of gizzard, pancreas and duodenum noticed with broilers fed with the diet containing 200 g/kg ECAM could be due to the fibrous nature of ECAM. Heavy gizzard weight was also noticed with broilers with 4% wood shavings (Hetland *et al.*, 2003). Higher gizzard weight obtained could be due to the increasing frequency of contraction of this organ to reduce the fibre particles. The longest relative length of jejunum and ileum were also recorded for birds fed with diets containing 200 g/kg ECAM.

The density and size of villi and microvilli of small intestine are directly related to the absorption capacity of the birds. The villi length and crypt depth of the duodenum reduced with increasing dietary inclusion of ECAM. Previous works confirmed that crypt depth and villi length are related to the rate of sensory activity and increase/decrease in cell turn over (Langhout et al., 1999; Hedemann et al., 2003). High duodenal villi length and crypt depth obtained with broilers fed with control diet and the diet containing 100 g/kg ECAM showed indications of improved absorption and hydrolysis potential of nutrient. Reduction in crypt depth as seen for broilers fed with diets having more than 100 g/kg ECAM is indicative of lower secretory activity such as lower mucus production (Langhout et al., 1999). Engberg et al. (2004) reported that reduced intestinal crypt depth could be linked with reduced intestinal microbial population. Lowered intestinal bacteria population may be due to accumulated dietary ricin content which increased feed toxicity. In conclusion, dietary inclusion of ethanol treated castor oil seed meal in broiler diets could be up to 150 g/kg diet in broiler ration without negative effect on the performance and nutrient digestibility and also led to modifications of the digestive tract.

## CONCLUSIONS

The current study confirmed ethanol treated castor oil seed meal (ECAM) as a potential, alternative oil seed meal which poses no deleterious effect on growth, nutrient digestibility and gut morphology when included up to 150 g/kg in rations for broilers. Dietary inclusion in commercial broiler production at levels higher than 150 g/kg should therefore be discouraged due to its negative effect on growth, gut morphology and nutrient utilization. Exploration of other processing methods with a view to successfully incorporate castor seed meal at higher inclusion levels in poultry nutrition is therefore recommended.

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