

CORN WITH DIFFERENT PARTICLE SIZES IN LAYING HENS FEEDING: PERFORMANCE, EGG QUALITY AND INTESTINAL MORPHOLOGY

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ABSTRACT: This work was carried out to evaluate the effect of different particle size corn on the performance, egg quality and intestinal morphology of Hisex Brown laying hens. A total of 128 birds were used, in a completely randomized design with four treatments, four replicates and eight birds per experimental unit. Experimental rations had corn with different particle size: 565, 620, 781 and 1085 μm of mean geometric diameter (MGD). Feeding was *ad libitum* at 8:00 am and 4:00 p.m. The variables analyzed were: feed consumption, laying percentage, egg mass and weight, food conversion (was calculated by dividing the feed consumed by the total egg weight (kg.kg^{-1}) and the number of eggs produced (kg.dozen^{-1})), shell percentage and thickness, Haugh unit, yolk color index and morphometry analysis of the intestinal mucosa. The use of corn with MGD until to 1085 μm in rations does not affect the performance and eggs quality of Hisex Brown laying hens. However, there are indications that the intestinal morphometry of laying hens is negatively influenced by the increase in particle size of corn.

KEYWORDS: MGD. Egg quality. Intestinal morphology. Performance.

MILHO COM DIFERENTES TAMANHOS DE PARTÍCULAS NA ALIMENTAÇÃO DE POEDEIRAS: DESEMPENHO, QUALIDADE DOS OVOS E MORFOLOGIA INTESTINAL

RESUMO: Este trabalho foi realizado com o objetivo de avaliar o efeito de diferentes tamanhos de partículas de milho no desempenho, qualidade dos ovos e morfologia intestinal de poedeiras *Hisex Brown*. Foram utilizadas 128 aves, distribuídas em delineamento inteiramente casualizado, com quatro tratamentos, quatro repetições e oito aves por unidade experimental. As rações experimentais apresentaram milho de diferentes tamanhos de partículas: 565, 620, 781 e 1085 μm de diâmetro geométrico médio (DGM). A alimentação foi *ad libitum* às 8:00 e 16:00h. As variáveis analisadas foram: consumo de ração, porcentagem de postura, massa e peso dos ovos, conversão alimentar (calculada dividindo-se a ração consumida pelo peso total de ovos (kg/kg) e número de ovos produzidos (kg/dúzia)), porcentagem e espessura da casca, unidade de Haugh, índice e coloração de gema e análise morfométrica da mucosa intestinal. O uso de milho com DGM até 1085 μm nas rações não afeta o desempenho e a qualidade dos ovos de poedeiras *Hisex Brown*. No entanto, há indicações de que a morfometria intestinal de poedeiras é influenciada negativamente pelo aumento no tamanho das partículas do milho.

PALAVRAS-CHAVE: DGM. Qualidade dos ovos. Morfologia intestinal. Desempenho.

MAÍZ CON DIFERENTES TAMAÑOS DE PARTÍCULAS EN LA ALIMENTACIÓN DE PONEDORAS: RENDIMIENTO, CALIDAD DE LOS HUEVOS Y MORFOLOGÍA INTESTINAL

RESUMEN: Este estudio ha sido desarrollado con el objetivo de evaluar el efecto de diferentes tamaños de partículas de maíz en el rendimiento, calidad de los huevos y la morfología intestinal de gallinas ponedoras *Hisex Brown*. Se utilizaron 128 aves, distribuidas en un experimento completamente al azar, con cuatro tratamientos, cuatro repeticiones y ocho aves por unidad experimental. Las raciones experimentales fueron elaboradas con maíz de diferentes tamaños de partículas: 565, 620, 781, 1085 μm de diámetro geométrico medio (DGM). La alimentación fue *ad libitum* a las 8:00 y a las 16:00h. Las variables analizadas fueron: consumo de ración, porcentaje de postura, masa y peso de los huevos, conversión alimenticia (se calculó dividiendo el alimento consumido por el peso total de huevos (kg/kg) y el número de huevos producidos (kg/docena)), porcentaje y espesor de cáscara, unidad Haugh, índice y coloración de yema, y análisis morfométrica de la mucosa intestinal. La utilización de maíz con DGM de hasta 1085 μm en las raciones no perjudica el rendimiento y la calidad de los huevos de las ponedoras *Hisex Brown*. Sin embargo, hay indicaciones de que la morfometría intestinal de gallinas ponedoras es influenciada de forma negativa por el aumento del tamaño de las partículas del maíz.

PALABRAS CLAVE: DGM. Calidad de los huevos. Morfología intestinal. Rendimiento.

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Introduction

In poultry production, food represents about 70% of the total costs and, among the ingredients, corn corresponds to 80% of the rations value being supplied in the meal form, which theoretically provides greater use by the birds due to the highest contact in the mucosa and area available for the action of digestive enzymes (AMERAH *et al.*, 2007). However, larger particles stimulate the digestive organs, especially the gizzard, providing ingredients maceration and facilitating the digesta permeability through the action of digestive enzymes (ROUGIÈRE *et al.*, 2009; SINGH *et al.*, 2014).

Although they prefer larger particles, the birds consume the smaller ones as the concentration of the larger is reduced, on the other hand, ingredients with different grain sizes make the mixing process difficult, leading to unbalance of the rations since they tend to separate the particles during transport or even inside the farm (GEWEHR *et al.*, 2011).

The determination of particle size corn that provides good acceptance and better digestibility by birds can potentially improve animal performance in addition to increasing the profitability of production (CARIOCA JUNIOR *et al.*, 2015).

A number of studies on corn grain size have been widely evaluated in broilers (DAHLKE *et al.*, 2003; PARSONS *et al.*, 2006; ZANOTTO *et al.*, 1996). Although little is known about the application of this knowledge to commercial laying hens and their effects on intestinal mucosa and egg quality (GEWEHR; FREITAS, 2008).

The objective of this work was to evaluate the effects of different particle size corn on the performance, eggs quality and intestinal morphology of *Hisex Brown* laying hens.

Material and Methods

The study was carried out in the Poultry Production Sector of the State University of Mato Grosso do Sul, Aquidauana Unit. A total of 128 laying hens, *Hisex Brown* strain, with 30-week-old were used during the 112-day period divided into four cycles of 28 days each. The birds were housed by pairs in galvanized wire cages with four divisions of 25 x 40 x 45 cm in a conventional laying shed with fiber cement tiles. The water drinker used was the trough type with running water that extend in the front of all cages, washed daily. Feeding was *ad libitum* at 8:00 am and 4:00 p.m., in feeders arranged under the drinkers, one for each experimental unit.

The lighting program adopted was 17 hours per day (natural + artificial lighting). The thermal conditions of the aviary were determined by means of a thermohygrometer at 8 h and 16 h, obtaining the minimum values of minimum temperature ($19.26^{\circ}\text{C} \pm 0.8^{\circ}\text{C}$), maximum ($23.4^{\circ}\text{C} \pm 2.7^{\circ}\text{C}$) and relative humidity of the air ($75.09\% \pm 6\%$).

The birds were distributed in a completely randomized experimental design consisting of four treatments, four replicates and eight birds per experimental unit.

The treatments consisted of rations formulated with corn from a single batch, which was fractionated in four parts and each fraction was submitted to different milling diameters (mm): 2.0 (MGD = 565 μm); 4.0 (MGD = 620

μm); 6.0 (MGD = 781 μm) and 8.0 (MGD = 1085 μm), obtained by means of a hammer type mill (28 hammers), driven by a 20CV engine and 3,550 rpm.

The experimental rations were based on corn and soybean meal and formulated to be isoenergetic and isonutritious, in order to meet the nutritional requirements of birds according to recommendations of Rostagno *et al.* (2011). All rations presented 17.20% of crude protein, 2780 kcal.kg⁻¹ of metabolizable energy, 3.80% of calcium and 0.45% of available phosphorus. The particle size of food and rations was determined according to the methodology described by Zanotto and Bellaver (1996).

Animal performance was evaluated by means of feed consumption (g.bird⁻¹.day⁻¹), laying percentage, egg mass (g.bird⁻¹) and feed conversion ratio (kg.kg⁻¹ and kg.dozen⁻¹). The feed intake was recorded weekly and calculated by the difference between the amount of feed supplied and the leftovers at the end of each week that composed the cycle. The eggs were collected and counted daily, obtaining at the end of each cycle the total egg production, egg laying percentage and egg mass of each experimental unit. The egg mass was obtained by the relation between the total production and the average weight of the eggs (total egg production x average egg weight) and expressed in grams.

Feed conversion was calculated by dividing the feed consumed (kg) by the total egg weight (kg) and the number of eggs produced (dozen), respectively, in each experimental unit.

In the last four days of the cycle were determined: mean weight the eggs (g), Haugh unit, yolk index (mm), crude yolk color, percentage and shell thickness (mm). Based on the data of total weight and number of eggs of the experimental unit, the average egg weight was obtained. After weighing, a sample of three eggs per experimental unit was randomly picked, which were individually weighed and then broken onto a on a flat, smooth glass surface and the height measurements of the albumin and yolk were determined and expressed in millimeters (mm) with the help of a digital caliper (Vonder™).

The Haugh unit values were determined by the equation described by Silversides and Budgell (2004): $\text{UH} = 100\log(\text{H} + 7.75 - 1.7\text{P}^{0.37})$; In which, H = albumen height (mm) and P = egg weight (g).

Then, with a digital caliper (± 0.05 mm), the diameter of the yolk was measured horizontally and, based on the average of the obtained values, the yolk index (mm) (height. diameter⁻¹) was calculated.

The crude yolk color was analyzed using a DSM (Yolk Color Fan™) colorimetric scale, ranging from 1 to 15, varying from light to dark yellow (orange).

The shells were then washed and subjected to ambient drying for 48 hours and weighed to determine the external quality by shell weight (g) and shell thickness (mm). The shell thickness, including the membranes, was measured by reading four distinct points of the equatorial region using a digital micrometer (Digimes™, ± 0.001 mm) and transformed into an average value per experimental unit.

For analysis of intestinal mucosal morphometry, after the last experimental cycle, two birds per replicates were fasting for eight hours, weighed and later desensitized and slaughtered by cervical dislocation.

After laparotomy, the small intestine was removed and its length (cm) was measured using a tape measure. Approximately 3 cm long segments of the small intestine (duodenum, jejunum and ileum) were collected, considering the duodenum from the pylorus to the distal portion of the duodenal loop, jejunum from the distal portion of the duodenal loop to the Meckel's diverticulum and ileum the portion anterior to the cecum.

The intestinal samples were washed in saline solution, fixed in 10% buffered formalin solution and subsequently dehydrated in series with increasing concentrations of alcohol, diaphanized in xylol, and included in paraffin according to the methodology described by Beçak and Paulete (1976). Longitudinal and semi-serial histological sections were obtained with seven μm thickness and subsequently stained by the Hematoxylin-Eosin method. During evisceration, the pro-ventricle and the gizzard isolated, emptied of contents and the absolute weights (g) were obtained by weighing the relative weights (%) as a function of the weight of the bird

(g).

For the morphometric study the images were captured by means of light microscopy (Olympus Bx 40), using the computerized image analyzer system (Image Proplus - version 5.2 - Cybernetic Media). The height of 30 villi and the depth of 30 crypts of each replicates were evaluated and from these values was obtained the mean.

Data were submitted to analysis of variance and polynomial regression analysis using the least squares method. The differences between the means were obtained by the *Tukey* test ($P < 0.05$).

Results and Discussion

The regression analysis did not show effect ($P > 0.05$) of the different particle size corn on performance variables, egg quality (Table 1) and allometry of the organs of the hen digestive system (Table 2).

Table 1 - Performance and egg quality in laying hens fed on different particle size corn (MGD).

Variables	MGD* (μm)			
	565	620	781	1085
Feed consumption (g/bird day ⁻¹)	0.112±0.001	0.114±0.001	0.113±0.002	0.112±0.002
Food conversion (kg kg ⁻¹)	2.00±0.046	2.04±0.028	2.09±0.033	2.09±0.021
Food conversion (kg dozen ⁻¹)	1.46±0.027	1.44±0.016	1.49±0.017	1.49±0.021
Percentage of posture (%)	92.40±1.228	94.81±0.583	91.00±0.646	91.07±2.329
Egg mass (g ave ⁻¹)	55.22±1.028	57.03±0.633	53.98±1.214	53.92±1.138
Egg weight (g)	59.75±0.075	60.12±0.061	59.29±0.091	59.22±0.053
Shell percentage (%)	9.77±0.022	10.07±0.130	9.79±0.083	9.85±0.087
Shell thickness (mm)	0.43±0.002	0.43±0.005	0.44±0.003	0.44±0.004
Haugh Unit	95.31±1.335	92.71±0.859	94.41±0.910	95.67±1.097
Yolk index (mm)	0.44±0.004	0.45±0.003	0.45±0.002	0.45±0.003
Yolk color	5.25±0.112	5.17±0.094	5.19±0.041	5.25±0.055

*Mean geometric diameter.

Table 2 - Absolute and relative weights of gizzard and proventriculus, and length of small intestine in laying hens fed on different particle size corn (MGD).

Variables	DGM* (μm)			
	565	620	781	1085
Absolute weight of gizzard (g)	22.85±1.62	22.91±1.62	25.87±2.04	23.51±1.39
Relative weight of gizzard (%)	1.35±0.13	1.36±0.09	1.56±0.08	1.56±0.04
Absolute weight of proventriculus (g)	6.66±0.28	6.31±0.54	6.51±0.70	6.80±0.31
Relative weight of proventriculus (%)	0.39±0.02	0.38±0.03	0.39±0.03	0.40±0.02
Length of intestine (cm)	128.38±7.89	142.25±9.38	127.50±6.45	129.63±3.25

*Mean geometrical diameter.

Although research suggests that fine particles have greater contact with the mucosa and surface area available for enzyme action (AMERAH *et al.*, 2007), larger particles can stay longer in the gizzard until they are minimally reduced and routed to the duodenum (GEWEHR *et al.*, 2010; SVIHUS *et al.*, 2002), thus not altering the digestibility of nutrients and consequently the performance, a fact that could explain the lack of results in this experiment.

Similar results for performance were found by Gewehr

et al. (2010), which did not observe effect when feeding semi-heavy laying hens with whole corn and fine (660 μm), medium (1260 μm) and coarse (321 μm). Similarly, the use of corn with particle size of 1000, 1160 and 1380 μm in the diet of slow-growing broilers did not influence performance and carcass yield (CARIOCA JÚNIOR *et al.*, 2015).

However, Gewehr *et al.* (2011) reported an increase in the feed consumption of semi-heavy laying hens when using corn with a coarse (3198 μm) and mean (1254 μm)

grain size in relation to the thin (663 μm) and whole corn in diets without affect the performance and eggs quality of hens. These results resemble those obtained by Safaa *et al.* (2009), when feeding semi-heavy laying hens with coarse grain particles ($>2500 \mu\text{m}$), milled in 10 and 8 mm sieves, respectively.

The difference between the performance results in function of the different particle size corn observed in the studies above is possibly related by several factors such as the ingredients that make up the feed, corn cultivar, endosperm hardness, milling method and distribution of particle size, as mentioned by Singh *et al.* (2014).

Evaluating the particle size of cereals in the broiler diet, Rougière *et al.* (2009) attributed to gross particle size the development stimulation of the digestive organs, gizzard

and intestine, since they require greater activity of the organs in the digestion process with consequent hypertrophy, thus contributing to the improvement of protein and starch digestibility, corroborating with results obtained in several studies on broiler chickens (AMERAH *et al.*, 2007; AMERAH; RAVINDRAN, 2008; DAHLKE *et al.*, 2003; PARSONS *et al.*, 2006), a fact that was not observed in this study.

By means of the regression analysis there was no effect of the granulometries in the intestinal morphometry ($P>0.05$). However, it was observed that the mean values of villi height in duodenum, jejunum and ileum villi height as well as the crypt depth of ileum were altered ($P<0.05$) with the use of different particle size corn (Table 3).

Table 3 - Morphometry of duodenum, jejunum and ileum (villus height and crypt depth - μm) in laying hens fed on different particle size corn (MGD).

Variables	MGD* (μm)			
	565	620	781	1085
	Duodenum			
Villus height (μm)	752.86 \pm 12.99 ^a	793.92 \pm 17.91 ^a	681.62 \pm 8.27 ^b	745.92 \pm 10.49 ^a
Crypt depth (μm)	68.33 \pm 1.39	66.60 \pm 7.16	54.98 \pm 1.11	60.25 \pm 1.10
	Jejunum			
Villus height (μm)	489.87 \pm 6.10 ^a	462.65 \pm 6.61 ^b	487.88 \pm 8.47 ^a	471.50 \pm 5.23 ^{ab}
Crypt depth (μm)	48.81 \pm 1.06	38.99 \pm 0.60	48.90 \pm 1.60	48.63 \pm 5.76
	Ileum			
Villus height (μm)	330.17 \pm 5.11 ^a	271.41 \pm 7.57 ^c	285.85 \pm 7.27 ^{bc}	300.22 \pm 8.36 ^b
Crypt depth (μm)	56.25 \pm 1.27 ^a	47.91 \pm 1.64 ^{bc}	47.21 \pm 1.02 ^c	52.92 \pm 1.99 ^{ab}

*Mean geometrical diameter; Means in the same line followed by different letters differ from each other by *Tukey* test ($P<0.05$).

The results showed that in the duodenum, villus height reduced with the use of corn with 781 μm of MGD in the diet. In the jejunum in which the particle size of 620 was similar to MGD 1085 μm and lower than the other treatments. The highest levels of villi and crypt depth in the ileum were observed using the lowest corn grain size in the diets (565 μm), but the depth was similar to the results obtained for birds fed with the highest grain size (1085 μm).

The increase in villus height in the duodenum, jejunum and ileum may indicate an increase in the digestive and absorption capacity of the proximal small intestine in response to increased nutrient flux (LU *et al.*, 2011). Larger particles have a slower passage through the digestive tract, allowing a more effective enzymatic action of the digestive juices, increasing antiperistalsis and improving nutrient availability (NIR; SHEFET; ARONI, 1994). These authors observed that diets produced with fine particles flow more rapidly from the stomach into the duodenum and into the other parts of the small intestine.

Partially similar results were obtained by Lu *et al.* (2011), in which the villi height and intestinal wall thickness in the duodenum and intestinal villi height in the jejunum were higher in birds fed with diet containing whole corn as well as birds fed with diet containing ground corn (MGD = 565 μm) showed higher villi height and crypts depth of the ileum. On the other hand, when using corn with fine and coarse grinding in the diet of broilers, Santos *et al.* (2008) did

not observe effect of treatments on the height, width and area of the villi as well as in crypt depth of jejunum.

Conclusions

The use of corn with MGD until to 1085 μm in rations does not affect the performance and eggs quality of Hisex Brown laying hens. However, there are indications that the intestinal morphometry of laying hens is negatively influenced by the increase in particle size of corn.

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