

Impact of the physical structure of feed on performance, diet digestibility, and macroscopic anatomical changes in the oropharyngeal cavity of broilers

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Abstract: The study evaluated the impact of different pellet ratios on performance, diet digestibility, and changes in the oropharyngeal cavity in broiler chickens. One hundred sixty male broilers, aged 28 to 42 days, were distributed in a completely randomized design with four treatments and eight replicates of five birds each. These were M100 (control diet; 100% mash), P34 (34% pelleted), P66 (66% pelleted), and P100 (100% pelleted). At 28, 35, and 42 days of age, body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were assessed. At 42 days of age, ileal digesta was collected from two birds per repetition to determine the apparent ileal digestibility (AID) of dry matter (DM), crude protein (CP), and ileal digestible energy (IDE). In addition, the oropharyngeal cavity was analyzed to investigate possible alterations in these structures. At 42 days, samples of wood shavings were collected to determine the moisture content. The data was submitted to ANOVA and, when significant, compared using Dunnett's test at 5%. Birds in the P100 group had better FCR ($P < 0.05$) than birds in the M100 from 36 to 42 and 28 to 42 days. Birds fed the P100 and P34 diets had lower MS AID ($P < 0.05$), while birds in the M100 had better CP AID compared to those fed diets with some processing ($P < 0.05$). The treatments P66 and P34 had lower IDE than the control diet ($P < 0.05$). In conclusion, birds fed P100 performed better, while birds fed M100 had better AID.

Keywords: Fines, mash, pellet, poultry, processing.

1. Introduction

Feed processing can affect broiler performance, improving body weight gain and feed conversion ratio (Teixeira Netto et al., 2019). The process of pelleting broiler diets, in particular, has advantages compared to diets in mash form since the benefits include reducing the segregation of ingredients, making it easier to grasp the diet, reducing the presence of pathogens, increasing metabolizable energy due to the lower time taken for consumption (Jensen et al., 1962; Meinerz et al., 2001; Glover et al., 2018; Khalil et al., 2021; Lemons et al., 2021), and increased digestibility of some dietary components (Abdollahi et al., 2013). However, these benefits promoted by pelleting will only be achieved if the pellets maintain their physical quality until the animal ingests the feed (Abdollahi et al., 2013; Muramatsu et al., 2015). Pellets must resist fragmentation and friction during handling without breaking to avoid a high proportion of fines (Burin Junior et al., 2019). Several factors can affect the quality of pellets, such as the nutritional composition of the diet, the particle size of the ingredients, and variables in the production process, such as moisture content, temperature control, and conditioning time (Thomas & Van Der Poel, 1996; Cutlip et al., 2008; Vukmirović et al., 2017). In this context, McKinney & Teeter (2004) compared diets made up of 100% pellets and 100% fines, observing that broilers fed a total pelleted diet had more significant body weight gain and better feed conversion ratio when compared to diets with 100% fines. Likewise, Lilly et al. (2011) observed that feed consumption and body weight gain were higher when birds were fed a diet containing 60% pellets than those with 30% or 0%.

Although there are several studies evaluating the effects of processing and the physical form of diets on performance and diet digestibility in broiler chickens (McKinney & Teeter, 2004; Corzo et al., 2011; Glover et al., 2015; Massuquetto et al., 2019; McCafferty & Purswell, 2023), there are few studies evaluating the proportion of fine and pellets in the diet with performance and digestibility parameters, as well as assessing whether this disuniformity in the physical form of the feed can cause visible changes in the structures of the oropharyngeal cavity.

One of the hypotheses of this study is that diets in mash form or with higher proportions of fines can result in alterations or even obstruction in the salivary ducts of broiler chickens. However, further studies are needed to confirm this hypothesis and improve understanding of the effects of these diets on the oropharyngeal cavity of poultry.

Therefore, the objective of this study was to evaluate the different proportions of pelleted feed throughout 28 to 42 days and investigate the effect of these proportions on body weight gain, feed consumption, feed conversion ratio, apparent ileal digestibility, litter moisture, and possible macroscopic changes in the oropharyngeal cavity of broiler chickens.

2. Material and Methods

2.1. Animals, facilities, and experimental design

One hundred sixty-one-day-old male broiler chicks (Ross 308 AP95, Ross Brazil Ltda, São Paulo, Brazil) were obtained from a commercial hatchery. The experimental period lasted from 28 to 42 days of age.

From 1 to 42 days of age, the birds were randomly allocated to 32 floor pens of 1.72 m² each (five birds/box) equipped with tubular feeders, nipple drinkers, and wood shavings as litter. Each box was considered an experimental unit. The birds were provided water and feed ad libitum, and the ambient temperature and animal mortality were checked daily. The temperature recommendations

were followed as described in the Management Guide. The air was renewed and circulated daily by opening and closing the curtains in the experimental shed.

The animals were distributed in a completely randomized design with four treatments and eight replicates. The treatments varied according to the proportion of pellets in the diets: 100% mash control diet (M100), diet consisting of 34% pellets and 66% fines (P34), diet composed of 66% pellets and 34% fines (P66), and 100% pelleted diet (P100).

To obtain the fines portion, the pelleted diets were sieved through a 1.5 mm wire mesh sieve. To avoid direct friction between the sieve and the pellet, 100 g of pellet was placed in the sieve so that it could only pass through once. In this way, the proportions of pelleted diets (P66 and P34) followed the percentage of fines according to each treatment during feed supply.

2.2. Experimental diets

Until they were 26 days old, the birds were fed a starter diet based on corn and soybean meal in mash form. At 27 days of age, the animals were subjected to 24 hours of adaptation to the experimental diets due to the different physical forms of the diet previously provided. From the 28th day, the other programs were implemented and followed until the birds were 42 days old. During the experimental period from 28 to 42 days, the diets were formulated with corn and soybean meal. Still, they differed only in the type of processing adopted: mash diet, pelleted, and fines obtained by sieving.

Table 1 describes the formulation, calculation, and analysis of the composition of the experimental diets. The pelleted diets were manufactured in a pellet mill (Koppers Junior C40-Koppers Company, Inc., Pittsburgh, PA) with a 50 hp Siemens motor and a pelleting die with 4.7 mm diameter holes and a thickness of 50 mm. The diets were conditioned for approximately 10 seconds at 75°C and a 1.5 kgf/cm² pressure. After pelleting, the feed was dried and cooled to an average temperature of 37°C.

Ingredient	Amounts (%)
Corn	66.900
Soybean meal	25.739
Soybean oil	3.403
Celite ¹	1.000
Dicalcium phosphate	0.902
Limestone	0.864
Sodium chloride (NaCl)	0.425
DL-Methionine	0.294
L-Lysine HCL	0.267
L-Threonine	0.106
Mineral premix ²	0.050
Vitamin premix ³	0.050
Phytase	0.001
Calculated chemical composition	
Metabolizable energy (kcal/kg)	3200
Crude protein, %	17.773
Ether extract, %	6.304
Crude fiber, %	2.573
Calcium, %	0.666
Available phosphorus, %	0.285
Sodium, %	0.197
Potassium, %	0.686
Digestible lysine, %	1.064
Digestible methionine, %	0.540
Digestible methionine + cysteine, %	0.787
Digestible threonine, %	0.702
Digestible tryptophan, %	0.192

Table 1 – Ingredient and composition of the experimental diets.

¹Celite 400 Insoluble marker (Celite, Celite Corp., Lompoc, FC).

²Supplied per kilogram of diet: Cu (copper sulfate), 10 mg; Fe (iron sulfate), 50 mg; Mn (manganese oxide), 80 mg; Co (cobalt sulfate), 1.0 mg; I (calcium iodate), 1.0 mg; Zn (zinc oxide), 50 mg.

³Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 9000 IU; vitamin D3 (cholecalciferol), 2,500 IU; vitamin E (DL- α -tocopherol), 200 IU; vitamin K3 (menadione nicotinamide bisulfite), 2.5 mg; vitamin B1 (thiamine- mononitrate), 1.5 mg; vitamin B2 (riboflavin) 6.0 mg; vitamin B6 (pyridoxine. HCl) 3.0 mg; vitamin B12 (cyanocobalamin) 12.0 mcg; pantothenic acid (D-Ca pantothenate), 12.0 mg; niacin (nicotinic acid), 25 mg; folic acid, 0.80 mg; biotin, 0.06 mg; selenium (sodium selenite), 0.25 mg.

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2.3. Pellet durability index and geometric mean diameter

The pellet durability index (PDI) was assessed using the PDI determination device, which consisted of 5 rotating boxes (30 cm high; 12.5 x 12.5 cm base). Approximately 500 g of the pellets retained in a sieve (4.0 mm sieve, Talastem Peneiras para Análise LTDA, São Paulo, Brazil) were tested in the boxes that composed the PDI determination device at 50 rotations per minute for 10 minutes. The samples were then sieved (4.0 mm sieve) for approximately 30 seconds to remove the fines and broken pellets. The PDI was calculated as the number of intact pellets times 100 and divided by the total amount of the initial sample, resulting in a PDI of 81.6%.

To determine the geometric mean diameter (GMD) and geometric standard deviation (GSD) of the pelleted feed, 150 g of feed was used. It was dried in a forced ventilation oven at 105°C for 24 hours, equilibrated to room temperature, and weighed. The sample was then passed through a sieve kit (Bertel Ind. Metalúrgica Ltda., Caieiras, São Paulo, Brazil) with a set of six sieves (4.0; 2.0; 1.2; 0.6; 0.3; 0.15 and 0.0 mm) and shaken for 10 minutes. The sample retained on each sieve was weighed, and the GMD and GSD were calculated, 742 µm and 1.82, respectively.

2.4. Variables analyzed

2.4.1. Growth performance

Growth performance was evaluated from 28 to 35 days, 36 to 42 days, and the total period from 28 to 42 days of age. The birds, all the feed supplied, and the leftovers were weighed on days 28, 35, and 42 to calculate the average body weight gain (BWG), feed intake during the period (FI), and feed conversion ratio (FCR) by the ratio between FI and BWG, corrected for the weight of the dead birds.

2.4.2. Apparent Ileal digestibility

At 42 days of age, two birds per experimental unit were euthanized by cervical dislocation to collect ileal contents for nutrient digestibility analyses. The birds were eviscerated, and the ileum was separated to remove the ileal content, which was defined as 4 cm below Meckel's diverticulum and 4 cm above the ileum-cecum-colon junction. The ileal digesta was removed by light manual pressure from the intestinal fraction, homogenized, placed in their identified plastic containers, and frozen at -18°C. Before laboratory analysis, the samples were thawed at room temperature and freeze-dried (Modulyo D Freeze Dryer, Thermo Electron Corporation, Waltham, MA, USA) up to a 5×10^{-2} mbar vacuum pressure.

Feed and ileal content samples were ground to 1 mm and analyzed for dry matter (DM) content after drying in an oven at 105°C for 12 hours, and crude protein (CP, method 954.01) according to AOAC (1995). Gross energy (GE) was determined using a calorimetric bomb (Ika Werke C2000 Control Oxygen Bomb Clorimeter - Ika-Werke GmH&Co, Staufen, Germany). Acid-insoluble ash (AIA) was used as an insoluble marker for the digestibility calculations. The AIA content in the samples was determined according to the methodology of Scott & Boldaji (1997). The coefficient of apparent ileal digestibility (CAID) was determined according to the following calculation:

$$CAID = \frac{(\text{Nutrient in the diet}) - (\text{Nutrient in the ileal digesta} \times IF)}{\text{Nutrient in the diet}}$$

Where IF (indigestible factor) is the ratio between the diet AIA and the ileal content AIA. Ileal digestible energy (IDE) was calculated according to the following equation:

$$IDE \text{ (kcal/kg DM)} = GE \text{ in the diet} - (GE \text{ in ileal content} \times IF)$$

2.4.3. Litter moisture

At the end of the experiment, the moisture content of the poultry litter was determined. Five different points were standardized inside each pen as described in Figure 1, collecting all the material inside (around 500g at each point), avoiding the areas near and below the feeder and drinker. The samples were then homogenized, identified, and left at room temperature until the moment of analysis, about 4 hours after collection. To determine the moisture content, the litter samples were homogenized again, weighed, and placed in a 55°C oven for 12 hours. After this period, the samples were weighed to determine the moisture content (MO) according to the methodology described by AOAC (1995).

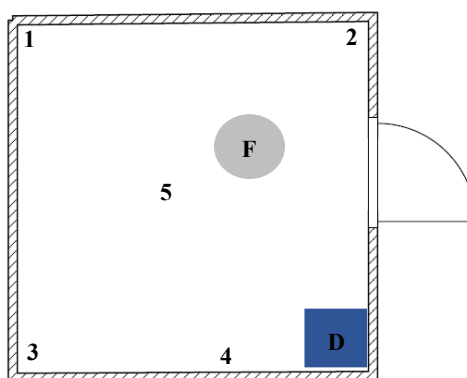


Figure 1 – Illustrative diagram of the pens, with litter collections identified by the numbers 1 to 5, representing the regions furthest from the feeding (F) and drinking (D) points.

2.4.4. Macroscopic anatomical assessment of the oropharyngeal cavity

At 42 days of age, anatomical material was collected for macroscopic analysis of the birds' oropharyngeal cavity structure. For this purpose, one bird per repetition, previously used in the ileal digestibility analysis, and in a healthy physical condition, was selected and adequately identified. Each bird was then placed on a horizontal surface so the head could be cut off from the neck and put on a panel with standardized coloring for all the samples. A semi-professional camera (Sony Alpha ZV-E10, 16-50mm f/3.5-5.6 OSS lens, Kisarazu, Japan) was used to capture the images. Thus, the morphological structures recorded for the evaluations were: opening of the maxillary salivary gland, palatum durum, medial palatine salivary glands, choanal opening, infundibular cleft, sphenopterygoid salivary glands, plica transversa, radix linguae, tomium mandibulare, corpus linguae (tongue), apex linguae, and glottis (Figure 2).

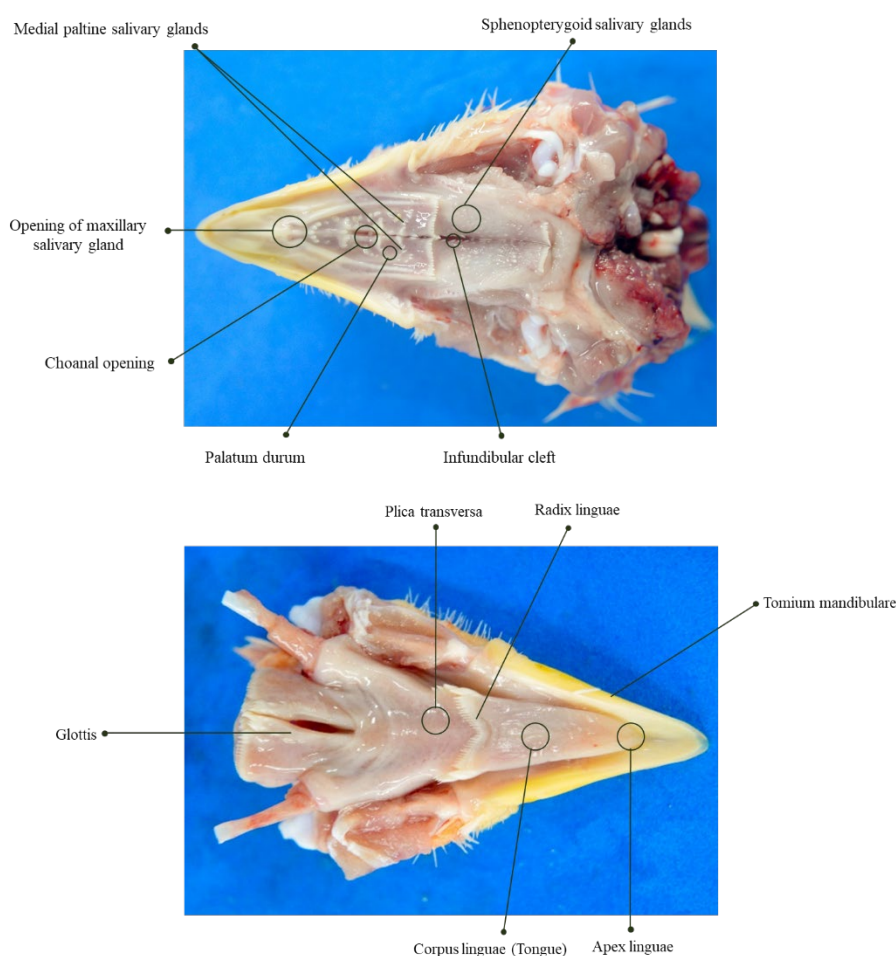


Figure 2 – Morphological structures recorded for the broiler evaluations at 42 days of age.

2.5. Statistical analysis

The data were submitted to the Shapiro-Wilk normality test, and after the normal distribution was confirmed, the data were analyzed by one-way ANOVA using the ExpDes package linear model (Experimental Designs Package; Ferreira et al., 2014), using the R program version 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria; RStudio Team, 2020). When significant, the means were compared using Dunnett's 5% probability level test, using the mash diet as a control.

3. Results

From 28 to 35 days of age, there was no statistical difference in the proportion of pellets in the diet for the growth performance variables ($P>0.05$; Table 2). There was also no influence on FI and BWG in the periods from 36 to 42 and in the total period ($P>0.05$). Birds fed the 100% pelletized diet had better FI than birds fed the control diet from 36 to 42 and 28 to 42 days of age ($P<0.05$). The other treatments were similar to the control diet for both periods ($P>0.05$).

Item	Diets				SEM	p-Value
	M100 ¹	P34 ²	P66 ³	P100 ⁴		
28 to 35 days						
Feed intake, g	1263	1303	1314	1305	13.3	0.490
Body weight gain, g	812	824	878	848	12.2	0.327
Feed conversion ratio, g/g	1.555	1.554	1.513	1.501	0.014	0.367
36 to 42 days						
Feed intake, g	1074	1076	1073	1132	15.9	0.308
Body weight gain, g	746	720	778	800	14.7	0.426
Feed conversion ratio, g/g	1.484	1.472	1.405	1.359*	0.016	0.007
28 to 42 days						
Feed intake, g	2396	2377	2385	2433	24.7	0.360
Body weight gain, g	1540	1577	1639	1650	23.0	0.848
Feed conversion ratio, g/g	1.513	1.506	1.457	1.429*	0.011	0.007

Table 2 – Diets with different proportions of pellets on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broiler chickens from 28 to 35, 36 to 42 and 28 to 42 days of age.

SEM: Standard Error of Mean.

*Differ from control ($P<0.05$) by Dunnett's test.

¹M100 = 100% mash (control); ²P34 = 34% pellet e 66% fines; ³P66 = 66% pellet e 34% fines; ⁴P100 = 100% pellet.

The different proportions of pellets in the broiler diet affected apparent ileal digestibility ($P<0.05$; Table 3). Birds fed the 100% and 34% pelleted diets showed lower DM digestibility than the control group ($P<0.05$). On the other hand, the birds that received the 100% mash diet had higher ileal digestibility of CP than those that received the pelleted physical form in their treatments ($P<0.001$).

Birds fed different amounts of pellets, P66 and P34, showed lower IDE utilization compared to the control ($P<0.05$). On the other hand, the birds that received the 100% pelleted diets obtained similar IDE values compared to the birds in the group fed the 100% mash diet.

Item	Diets				SEM	p-Value
	M100 ¹	P34 ²	P66 ³	P100 ⁴		
DM, %	70.11	67.07*	68.65	67.57*	0.317	0.001
CP, %	83.84	79.22*	81.19*	82.42*	0.359	<0.001
IDE, kcal/kg	3550	3450*	3476*	3532	11.9	0.004

Table 3 – Diets with different proportions of pellets on the coefficient of apparent ileal digestibility of dry matter (DM) and crude protein (CP), and ileal digestible energy (IDE) in broilers at 42 days of age.

SEM: Standard Error of Mean.

*Differ from control ($P<0.05$) by Dunnett's test.

¹M100 = 100% mash (control); ²P34 = 34% pellet e 66% fines; ³P66 = 66% pellet e 34% fines; ⁴P100 = 100% pellet.

The litter moisture values for broilers at 42 days of age did not differ statistically ($P>0.05$; Table 4).

Item	Diets				SEM	p-Value
	M100 ¹	P34 ²	P66 ³	P100 ⁴		
Moisture	24.73	25.58	28.26	26.93	0.817	0.106

Table 4 – Diets with different proportions of pellets on litter moisture (%) in broiler chickens at 42 days of age.

SEM: Standard Error of Mean.

*Differ from control ($P < 0.05$) by Dunnett's test.

¹M100 = 100% mash (control); ²P34 = 34% pellet e 66% fines; ³P66 = 66% pellet e 34% fines; ⁴P100 = 100% pellet.

The different proportions of pellets in the diets evaluated did not macroscopically modify the structures of the broiler oropharyngeal cavity (opening of the maxillary salivary gland, palatum durum, medial palatine salivary glands, choanal opening, infundibular cleft, sphenopterygoid salivary glands, apex linguae, corpus linguae (tongue), glottis, plica transversa, radix linguae, and tomium mandibulare) at 42 days of age (Image 2 and 3). The visual evaluations showed that there were no noticeable changes in the structures that make up the oropharyngeal cavity of the chickens that received the diets with the lowest proportion of pellets (M100 and P34), as well as for the birds that received the diets with the highest proportion of pellets (P100 and P66).

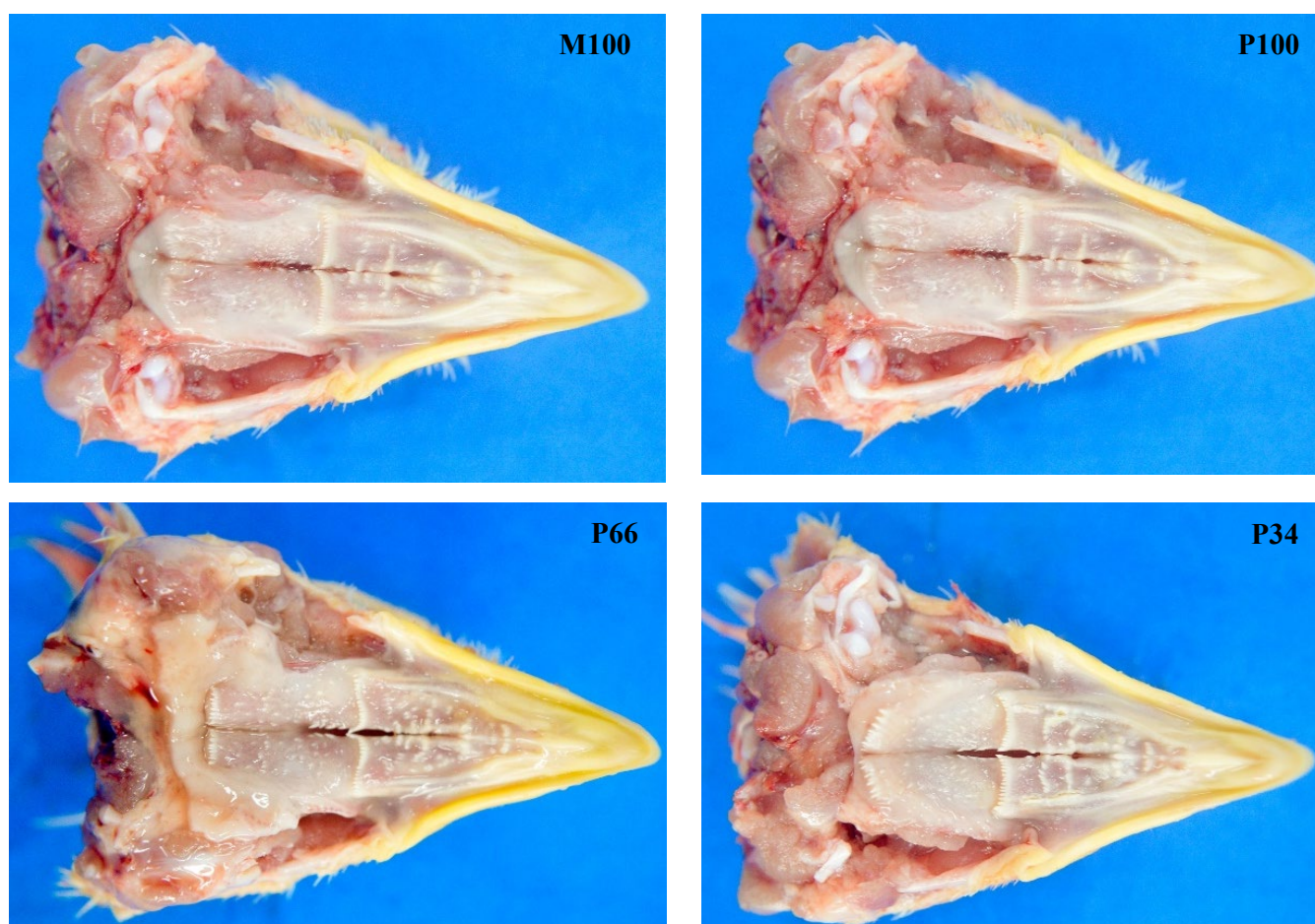


Figure 3 – Macroscopic anatomical assessment of the maxillary region of broilers at 42 days of age fed diets containing different proportions of pellets. F100 = 100% bran (control diet); P100 = 100% pellet; P66 = 66% pellet and 34% fines; P34 = 34% pellet and 66% fines.

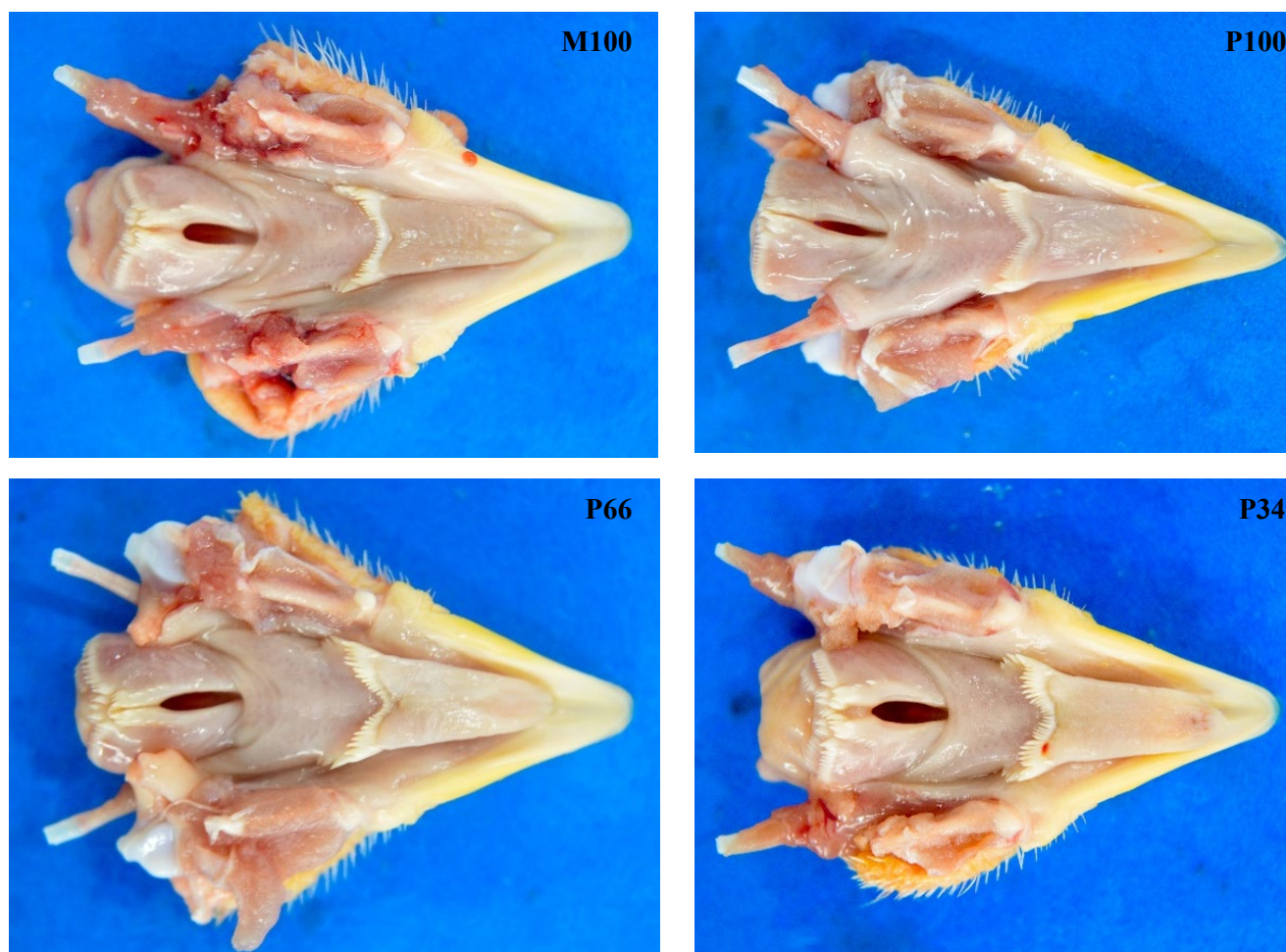


Figure 4 – Macroscopic anatomical assessment of the mandibular region of broilers at 42 days of age fed diets containing different proportions of pellets. F100 = 100% bran (control diet); P100 = 100% pellet; P66 = 66% pellet and 34% fines; P34 = 34% pellet and 66% fines.

4. Discussion

The performance results obtained in this study are consistent with McCafferty and Purswell (2023), who evaluated different proportions of pellets (100% pellets, 75% pellets and 25% fines, 56% pellets and 44% fines, 42% pellets and 58% fines, 31% pellets and 69% fines, 100% fines, and 100% mill fines that passed through a 2,000 μm sieve) in broiler chickens from 1 to 42 d of age, reporting no significant differences. On the other hand, the birds showed better BWG and FI when fed a higher proportion of pellets than diets with a lower proportion of pellets (Dozier et al., 2010).

Burin Junior et al. (2019) evaluated broilers fed diets with mash, pelleted 50:50, and pelleted 70:30, and they found greater FI and BWG of chickens fed with pelleted diets, regardless of the percentage of fines. In the same study, the best proportion of FCR was with the highest inclusion of pellet, when compared with the mash feed. McKinney & Teeter (2004), evaluating different concentrations of pelleted diets (20 to 80% pellets) compared to a mash diet, found a 6% increase in body weight gain and a 5% improvement in feed conversion ratio. The authors observed that from 40% onwards, pelleting provided more significant BWG and a tendency to improve FCR compared to a mash diet.

Corzo et al. (2011) evaluated the performance of broiler chickens fed a pelleted diet with different proportions of pellets (32% and 64%) and found that diets containing higher proportions of pellets performed better than the mash diet. These findings reinforce that including pellets in the diet optimizes performance in broiler chickens.

Our study observed that the diets with 66% and 34% pellets did not affect the BWG and FI of the broilers in all the periods evaluated. This suggests that, even though the proportion of pellets was higher than McKinney & Teeter's (2004) results, our diets were similar to those of mash diets regarding animal performance. This indicates that the quality of the thermal processing may influence the final result. Low-quality pellets cause breakdowns, leading to a higher proportion of fines at the time of ingestion, which results in worse FCR in birds.

Pelleting, resulting from the combination of pressure, moisture, and temperature, requires optimized processing conditions to maximize nutritional attributes, such as nutrient digestibility. During this process, protein denaturation and starch gelatinization affect the particle's binding properties and nutritional values (Scott et al., 1997; Abdollahi et al., 2010; dos Santos et al., 2020). Massuquetto et al. (2019) found no difference between pelleted and mash diets in digestibility parameters, demonstrating that the

pelleting process was not enough to change the use of the diet's nutritional fractions. Other studies have also shown that pelleting has a low influence on nutrient digestibility (Svihus et al., 2005; Zimonja et al., 2008).

The digestibility of the diet can be influenced by the animals' consumption. Birds fed 100% pelleted diets showed lower diet utilization than those fed 100% mash diets. Chickens prefer larger particles over smaller ones (Nir et al., 1994; Nir & Ptichi, 2001), probably because they find it easier to apprehend the diet (Massuquetto et al., 2019). This preference can lead to higher consumption, which is associated with reduced feed loss and lower energy costs during feed consumption (Jensen, 2000). McKinney & Teeter (2004) observed that feeding diets with more fines resulted in a lower resting frequency, demonstrating that diets with low physical quality can influence broiler feed consumption. The bird uses less energy to consume the pellet than a diet with a large proportion of fines, ensuring adequate consumption of the nutrients needed for maintenance.

A macroscopic evaluation of the animals' oropharyngeal cavity was carried out to identify possible alterations in the differences in processing between the treatments, but no visible alterations were observed. Microscopic analysis is recommended to detect potential changes in the oropharyngeal cavity of broilers that are not identifiable through macroscopic evaluation. These analyses can contribute to a more detailed understanding of the effects of different diets on broiler health, such as mash, pelleted, and diets with varying amounts of fines. However, further studies are needed to confirm this hypothesis.

5. Conclusion

The 100% pelleted diets resulted in a better FCR between 36 and 42 days and from 28 to 42 days of age for broiler chickens compared to the animals fed the mash diet. The other performance results, BWG and FI, showed no difference between the treatments. The 100% mash (M100) promoted greater apparent ileal digestibility. The anatomical evaluation of the oropharyngeal cavity was not influenced by the different pellet proportions, indicating that this variable should be evaluated in more detail.

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