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Qualidade dos ovos e prevalência de endoparasitas na criação de galinhas caipiras em Apodi e Mossoró, Rio Grande do Norte, Brasil

Egg quality and endoparasite prevalence in free range chicken farming in Apodi and Mossoró, Rio Grande do Norte, Brazil

Calidad del huevo y prevalencia de endoparásitos en la cría de pollos de corral en Apodi y Mossoró, Rio Grande do Norte, Brasil

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Resumo

Esta pesquisa buscou diagnosticar a qualidade da produção de ovos caipiras nos municípios de Apodi e Mossoró no estado do Rio Grande do Norte, utilizando como parâmetros a qualidade microbiológica e física dos ovos, e a prevalência de endoparasitos nas aves. Na avaliação microbiológica dos ovos buscou-se detectar e quantificar coliformes totais e termotolerantes, bactérias mesófilas, *Staphylococus aureus* e *Salmonella* sp.. Os ovos foram classificados quanto a seu peso, coloração da gema, pH de gema e albúmen, unidade Haugh, índice de gema e espessura de casca. Para as análises parasitológicas, foram coletadas excretas frescas, e aplicada a técnica de sedimentação espontânea pelo método de Hoffmann e o método de flutuação de Faust, para identificação dos parasitos. As amostras de ovos foram negativas para coliformes totais, termotolerantes, mesófilas e *Salmonella* sp. em 100% das amostras. Quantos às características de qualidade física, os ovos apresentaram boa pigmentação da gema e boa qualidade interna e externa. Em relação a carga parasitaria das aves, a presença de *Eimeria* e *Heterakis* foram significativamente maiores nos meses com precipitação superior a 100 mm, e nos meses com umidade relativa superior a 80% houve prevalência de Ascarídeos sobre os outros parasitos.

Palavras-chave: Agricultura familiar; Avicultura; Parasitos intestinais; Ovos orgânicos.

Abstract

The objective of this work was to assess the production quality of free-range eggs in the municipalities of Apodi and Mossoró, in the state of Rio Grande do Norte, Brazil. The microbiological and physical quality of the eggs and the endoparasite prevalence in the chickens were used as parameters. The egg microbiological evaluation consisted of the detection and quantification of total and thermotolerant coliforms, mesophilic bacteria, *Staphylococcus aureus*, and *Salmonella* sp. The eggs were classified according to their weight, yolk color, yolk pH, albumen pH, Haugh unit, yolk index, and shell thickness. The parasitological analyses consisted of collection of fresh excreta and application of the spontaneous sedimentation technique by the Hoffmann method and the Faust flotation method to identify the parasites. All egg samples were negative for total and thermotolerant coliforms, mesophilic bacteria, and *Salmonella* sp. Regarding the physical quality, the eggs presented good yolk pigmentation and good internal and external quality. Regarding the parasitic load of the chickens, the presence of *Eimeria* sp. and *Heterakis* sp. was significantly higher in months with average precipitation greater than 100 mm; and in months with average relative air humidity above 80%, there was prevalence of *Ascaris* sp. parasites.

Keywords: Family farming; Aviculture; Intestinal parasites; Organic eggs.

Resumen

Esta investigación buscó diagnosticar la calidad de la producción de huevos de corral en los municipios de Apodi y Mossoró en el estado de Rio Grande do Norte, utilizando como parámetros la calidad microbiológica y física de los huevos y la prevalencia de endoparásitos en aves. En la evaluación microbiológica de huevos se buscó detectar y cuantificar coliformes totales y termotolerantes, bacterias mesófilas, *Staphylococus aureus* y *Salmonella* sp .. Los huevos se clasificaron según su peso, color de la yema, pH y albúmina de la yema, unidad Haugh, índice de espesor de la yema y de la cáscara. Para el análisis parasitológico, se recolectaron excretas frescas y se aplicó la técnica de sedimentación espontánea mediante el método de Hoffmann y el método de fluctuación de Faust, para identificar los parásitos. Las muestras de huevos fueron negativas para *Salmonella* sp. Total, termotolerantes, mesófilas y. en el 100% de las muestras. En cuanto a las características de calidad física, los huevos mostraron buena pigmentación de la yema y buena calidad interna y externa. En cuanto a la carga parasitaria de aves, la presencia de *Eimeria* y *Heterakis* fue significativamente mayor en los meses con precipitación mayor a 100 mm, y en los meses con humedad relativa mayor al 80%, hubo prevalencia de Ascáridos sobre los demás parásitos.

Palabras clave: Agricultura familiar; Avicultura; Parásitos intestinales; Huevos orgánicos.

1. Introduction

Chicken rearing is practiced in approximately 80% of family farms (Sobral et al., 2010); and has a promising market because the supply of this type of products do not meet the demand.

Free-range chicken is usually reared in extensive system, with no facilities or proper management practices to meet the animals' nutritional, reproductive, and sanitary requirements. Therefore, the products from this rearing system can host pathogens, bringing losses to the poultry production and carrying foodborne diseases (Cardoso et al., 2001). Siqueira (2016), reported a worrisome fact, that 100% of the free-range poultry is exposed to parasitic infections.

Egg is an important product from free-range chicken farming; its characteristics make it a suitable medium for microorganism proliferation, thus, requiring special care to maintain its safety (Rêgo et al., 2012). The quality of this product may be affected by several factors, such as the chicken lineage, age, and diet, and the storage time (Barbosa et al., 2012). Thus, a set of characteristics is evaluated, which influence consumers' preferences, whether due to nutritional, sensory, or health attributes (Freitas et al., 2011).

Few studies characterize the production of free-range eggs in the semiarid region of the state of Rio Grande do Norte, Brazil. Thus, the objective of this work was to evaluate the quality of free-range eggs produced in the municipalities of Apodi and Mossoró, Rio Grande do Norte, Brazil, providing consumers, producers, and the scientific community with information on the microbiological and physical quality of eggs. In addition, this work provides information on the prevalence of endoparasites in chickens reared in feedlots in these municipalities, considering the local climatic conditions of the region.

2. Methodology

The research is qualitative and quantitative based on the methodology proposed by Pereira et al. (2018). A convenience non-probabilistic sampling was used to collect eggs and excreta samples, which were carried out in six properties in the municipalities of Mossoró and Apodi, in the state of Rio Grande do Norte (RN), Brazil.

2.1 Microbiological quality of eggs

The microbiological quality of eggs was evaluated using 108 eggs collected during visits to six properties. The eggs were collected on the same day of laying, placed in isothermal boxes, and taken to the Veterinary Microbiology Laboratory of the Federal Rural University of the Semi-Arid Region (UFERSA) for quantification of total and thermotolerant coliforms, mesophilic bacteria, *Staphylococcus aureus*, and *Salmonella* sp., following the methodology described in BRASIL (2003).

2.2 Physical quality of eggs

The internal and external quality of the eggs was evaluated using 135 fresh eggs collected in six properties (average of 22 eggs per property). The eggs were evaluated for specific gravity (at 9 different concentrations), absolute egg weight (g), yolk color (using a Roche color fan), yolk pH and albumen pH (using a pH meter), Haugh unit, yolk index (height to width ratio), and shell thickness (using a digital caliper at three different points). The Haugh unit was calculated using the equation HU = 100 LOG (h + 7.57 - 1.7 * p0.37, where h is the height of the dense albumen (mm), and p is the egg weight (g) (Nesheim et al., 1979).

2.3 Parasitological analysis

The presence of intestinal parasites in the chickens was evaluated monthly in each property for five months (February to June). Fresh excreta were collected directly from the poultry shed floor, which was previously covered with a tarp. The collected samples were packed in plastic bags, placed in isothermal boxes, and taken to the Laboratory of Biotechnology Applied to Infectious Diseases of the UFERSA for analysis. The samples were analyzed using the spontaneous sedimentation technique by the Hoffmann method (Hoffman 1987), and light eggs were evaluated using the Faust flotation method to assess the capacity of floating on hypersaturated saline solutions that some helminth eggs have (Faust et al., 1938). The correlation of parasite prevalence with rainfall and air temperature and humidity of the region was also evaluated. The meteorological data was obtained from the UFERSA weather station, in Mossoró, RN (5°12'48"S, 37°18'44' W, and altitude of 37 m).

2.4 Statistical analysis

Statistical analysis of egg physical quality data consisted of descriptive analysis, calculating the absolute and relative frequencies for each variable, using simple means. Parasitological data were subjected to Fisher's exact test, using the statistical program SPSS 20. Statistical differences were considered for $p<0.05$ (95% confidence).

3. Results and Discussion

The results for *Salmonella* sp., *Staphylococcus aureus*, total and thermotolerant coliforms, and mesophilic bacteria were negative for all 108 eggs collected for microbiological evaluation.

Similar results were reported by Melo et al. (2015), who evaluated the microbiological quality of free-range eggs and found all eggs within the established safety standard.

Among the microorganisms evaluated, *Salmonella* spp. is the main pathogen associated with egg contamination (Stringhini et al., 2009); it must be absent in 25-gram samples, according to Brazilian law. Therefore, the eggs collected were within the standards established by the Resolution 12 of 2001 of the Brazilian Health Regulatory Agency.

S. aureus is a pathogenic microorganism that, in some cases, can be used as indicators of food sanitary conditions; it causes food deterioration and health problems to consumers that ingest these pathogens or the toxins produced by them (Stringhini et al., 2009). Thus, regarding *S. aureus*, the eggs collected in the present study were safe for consumption.

The Brazilian legislation has no established parameters regarding the presence of total and thermotolerant coliforms and mesophilic bacteria in raw eggs. According to Forsythe (2013), this food is considered unfit for consumption when presenting values of mesophilic bacteria above 106 CFU g-1. Thus, the collected eggs were fit for human consumption, since that 100% of the samples were negative for these microorganisms.

Considering that the eggs are not contaminated up to oviposition, the hygiene of the laying place and at handling are factors that directly affect the contamination level of this food (Mottin et al., 2016). Melo et al. (2015), emphasize that egg contamination by the environment can occur by contact of eggs with feces or contaminated areas, with microscopic organisms entering the shell through cracks. The shell, cuticle coating the shell, and shell membranes are natural protective barriers that effectively prevent internal egg contamination

(Téo & Oliveira, 2005); thus, the use of eggs without cracking in the shell influenced the negative results for presence of microorganisms in the eggs analyzed.

In addition, the use of eggs that were laid within one day for the analysis may have also influenced the results obtained. This is because the vitelline membrane of older eggs tends to deteriorate, allowing bacteria to enter the albumen, and later to penetrate the yolk, whose membrane permeability increases over time at air temperatures above 10 °C (Gantois et al., 2009; Martelli & Davies, 2012).

The eggs can also be contaminated during their passage through the cloaca when in contact with the natural flora of this region; through the transovarian route during the egg formation due to non-symptomatic infections of the chickens; and through microorganisms present in the chickens' food and drinking water, which reach their bloodstream and, later, the reproductive tract of chickens, contaminating the egg yolk during its formation (Mottin et al., 2016). This type of contamination did not occur in the samples evaluated, since microorganisms were not found in the egg yolks.

In addition to microbiological quality, the eggs were evaluated for internal and external quality, considering their weight, yolk color, albumen pH, yolk pH, Haugh unit, and yolk index.

The egg weights were classified according to the recommendation of the current Brazilian legislation (Brasil, 1991), resulting in 23 small eggs (17.04%) with 45 to 49 grams, 39 medium eggs (28.89%) with 50 to 54 grams, 38 large eggs (28.15%) with 55 to 60 grams, and 35 extra large eggs (25.93%) with 60 to 65 grams.

Differences in weight among eggs are usually due to the nutritional issues of the chickens, since nutrition during their laying phase directly affects egg weight. Free-range production systems without a controlled, balanced diet to meet the nutritional needs of the chickens tend to be negatively affected by this, compromising not only the yield of eggs but their quality (Nys, 2011).

However, in the present study, most eggs were classified as medium or large, which is satisfactory, indicating that the nutrient requirements of the chickens are being met, and the egg weight differences were due to other factors. According to Barbosa et al. (2012), the weights of free-range chicken eggs are different due to the genetic diversity of chickens used by the producers, which was confirmed in the properties visited for the present study.

Egg weight is also dependent on the age of the chickens; the weight of the eggs tends to increase as the age of the chicken increases (Padhi, 2016; Silversides & Scott, 2001). This also explains the different sizes of the eggs, since the production is usually from chickens of

different ages.

Regarding the yolk color, 88 eggs (65.67%) presented yolk pigmentation between 7 and 10; 8 eggs (5.97%) above 10, and 38 eggs (28.35%) below 7, according to the Roche color fan.

Yolk color is a characteristic considered by the producer due to its importance in the acceptability of the product by consumers. Eggs from chickens reared in alternative systems tend to have yolks with more pigmentation due to their diverse diet, since they have access to forage and carotenoid-rich components, which results in yolks with orange shades (Rizzi & Marangon, 2012), explaining the results found in the present study.

Albumen pH presented values above 7.9 for 71 eggs (52.92%) and below 7.9 for 63 eggs (47.08%). Yolk pH presented values above 6.2 for 43 eggs (32.57%) and below above 6.2 for 89 eggs (67.42%). These results are within the fresh egg standards described by Alleoni and Antunes, (2001), who reported that these pH values should be approximately 7.9 for the albumen and 6.2 for the yolk.

Significant increase in albumen pH may be related to longer storage periods under high air temperatures (Jin et al., 2010). Some studies report that the yolk pH is little changed (Mayes & Takeballi, 1983; Silversides & Scott, 2001). The eggs evaluated in the present work were fresh and were stored properly, thus, the albumen pH and yolk pH were not affected.

The Haugh unit values found were above 72 for 122 eggs (96.06%), which classify them as excellent in quality, according to the US Department of Agriculture (Hauver & Hamann John, 1990). Barbosa et al. (2008), reported that long-period storage of eggs in uncontrolled environments can reduce Haugh unit values. However, due to the higher turnover of free-range eggs, they present better Haugh unit, i.e., good quality.

The yolk index is another measure that can be used to assess the internal quality of eggs (Souza et al., 2014). The yolk index of the eggs evaluated in the present work was higher than 0.30 for 96.92% of the eggs; thus, they were within the standard range for fresh eggs, which varies from 0.30 to 0.50, indicating good internal quality (Kraemer et al., 2015).

The egg shell is the first barrier that protects the egg against microorganisms, which is very important for consumers and producers, since cracks in eggshells can bring financial losses or indicate handling problems in the production process (Barancelli et al., 2012).

According to Oliveira et al. (2019), the egg shell quality depends on the egg size and weight. Among the 135 eggs evaluated, 112 (82.96%) presented shell thickness greater than 0.33 mm. According to Icken et al. (2006), the quality of the egg is classified as good when it

has shell thickness of 0.35 to 0.40 mm. Based on this range, the eggs evaluated were within the standard and, therefore, had good quality.

Another important criterion adopted in this research to profile the production of free-range eggs in the municipalities of Apodi and Mossoró was the evaluation of endoparasites in the chickens, considering their presence and climate variables for the study site.

The highest rainfall depths were found in February and March, exceeding 100 mm; and the lowest in April to June, below 100 mm. Considering this factor, a statistical difference was found between these two periods only for presence of *Heterakis* sp. and *Eimeria* sp., which showed greater presence in the months with the highest rainfall depths (Table 1).

Table 1. Correlation between prevalence of endoparasites in free-range chickens and rainfall depths in the municipalities of Apodi and Mossoró, RN, Brazil.

Parasites	**Rainfall depth > 100 mm**		**Rainfall depth < 100 mm**	
	Presence (%)	**Absence (%)**	**Presence (%)**	**Absence (%)**
Ascaridia	14.28	85.72	34	66
Heterakis	57.14 [a]	42.85	19.04 [b]	80.95
Eimeria	35.71 [a]	64.28	4.76 [b]	95.23
Capilaria	21.42	78.58	14.28	85.72

Means followed by different letters in the rows are different by the Fisher's exact test (p<0.05). Source: Authors.

March and April had higher average air humidity (above 80%) than February, May, and June, which presented average air humidity below 80%. Thus, the prevalence of ascarids was significantly higher in months with higher air humidity, with no difference between months for the other parasites (Table 2).

Table 2. Correlation between prevalence of endoparasites in free-range chickens and relative air humidity in the municipalities of Apodi and Mossoró, RN, Brazil.

Parasites	Air relative humidity > 80%		Air relative humidity < 80%	
	Presence (%)	Absence (%)	Presence (%)	Absence (%)
Ascaridia	64.28 [a]	35.71	0 [b]	100
Heterakis	42.85	57.15	28.57	71.43
Eimeria	14.28	85.72	19.04	80.96
Capilaria	21.42	78.58	14.28	85.72

Means followed by different letters in the rows are different by the Fisher's exact test (p<0.05). Source: Authors.

Regarding the air temperature, February, May, and June had the highest averages, above 27.5 °C, and March and April presented air temperatures below this value. These months with lower air temperatures presented prevalence of ascarids (Table 3).

Table 3. Correlation between prevalence of endoparasites in free-range chickens and air temperature in the municipalities of Apodi and Mossoró, RN, Brazil.

Parasites	Air temperature > 27.5 °C		Air temperature < 27.5 °C	
	Presence (%)	Absence (%)	Presence (%)	Absence (%)
Ascaridia	0 [b]	100	64.28 [a]	35.71
Heterakis	28.57	71.43	42.85	57.15
Eimeria	19.04	80.96	14.28	85.72
Capilaria	14.28	85.72	14.24	85.72

Means followed by different letters in the rows are different by the Fisher's exact test (p<0.05). Source: Authors

The air temperature and relative humidity of the studied regions throughout the year showed that periods with low air temperature and high air humidity have prevalence of *Ascaridia galli* over others parasite found in the analyses. These findings confirm the results found by Tarbiat et al. (2015), who reported than ideal air humidity and temperature are essential for this parasite to reach its infectious stage, which occurs in a wider air temperature range, between 15 and 35 °C, and humidity above 70%.

Ascaridia sp. is reported in the literature as the predominant parasitosis in free-range poultry farming; the eggs of these parasites can survive for years in their host, compromising the production by causing weight loss in chickens, low egg production, and poor nutrient

absorption (Bowman, 2009; Thapa et al., 2017).

Heterakis gallinarum and *Eimeria* sp. were the most frequently found parasites in the months with the highest rainfall. The non-statistical difference found for these parasites in relation to the other factors can be attributed to the climatic conditions, which were within the range for sporulation of these oocysts for both variables. According to Pinheiro et al. (2014), these ideal ranges for air temperature and relative humidity are 25 to 30 °C and 70 to 80%, respectively. Similar conditions were found in the region in all months of the study. Therefore, the difference detected by the statistical test in the rainy season can be attributed to slight changes in air humidity and temperature during this period.

In addition to *Ascaridia*, the most common parasites in poultry productions are H. gallinarum, Capillaria spp., and Eimeria spp. (Quadros et al., 2015; Silva et al., 2016; Siqueira, 2016; Vita et al., 2014). Eimeria spp. are among the main concerns for poultry production because their causes coccidiosis, which is an enteric parasitic disease that causes tissue damage, intestinal disorders, poor nutrient absorption, dehydration, blood loss, and increase of susceptibility to other pathogenic agents (Kaboudi et al., 2016; Cardozo & Yamamura, 2004).

No significant difference was found for Capillaria sp. in relation to the climatic conditions. Nevertheless, this endoparasite was present in all evaluations. Quadros et al. (2014), evaluated the presence of intestinal parasites in free-range chickens reared in the highland region of the state of Santa Catarina, Brazil, and reported the presence of eight species of intestinal parasites, predominantly *Capillaria* sp., *Ascaridia galli*, and oocysts of the genus *Eimeria*. However, Brandão et al. (2008) evaluated free-range chickens in the state of Paraíba, Brazil, and found only *Ascaridia galli* in 68.1% of the properties evaluated. Thus, considering the climatic differences between the cited studies, not only the health influences the parasite load, but climatic parameters contribute to the presence of endoparasites in the chickens.

Some study limitations were found, such as: the use of a convenience non-probabilistic sampling; and the possibility of bias regarding the collection and management of the environment, in which the application of a technical-sanitary questionnaire would enable a greater basis for the results obtained. Therefore, the findings can´t be generalized.

4. Final Considerations

The findings suggest that the food safety of the eggs is satisfatory, despite the lack of

investiment by the producers in their farms, which are considered as very rustic. Therefore, future implementation of a qualification network, aiming a greater technification and further development, is needed. The adequate physical quality and absence of contamination in the eggs evaluated show that the free-range chicken production in the study region has a high development potential

Regarding the endoparasites found, proper use of antiparasitic medication is important, mainly in months when their occurrence is favored by the climatic conditions of the region, since the prevalence of parasites can negatively affect the production rates.

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