DOI: 10.5212/Publ.Biologicas.v.29.0001

GROWTH OF INTESTINAL VILLI AND CRYPTS OF CHICKENS AND RATS DURING EMBRYONIC AND POSTNATAL DEVELOPMENT. A COMPARATIVE STUDY USING MORPHOMETRIC PARAMETERS

CRESCIMENTO DAS VILOSIDADES E CRIPTAS INTESTINAIS DE GALINHAS E RATOS DURANTE O DESENVOLVIMENTO EMBRIONÁRIO E PÓS-NATAL. UM ESTUDO COMPARATIVO USANDO PARÂMETROS MORFOMÉTRICOS

Araújo, Gillize Aparecida Telles[1], Camargo, Kamila Caroline[2], Gomes, José Rosa[4], Loddi, Maria Marta[3], Soares, Maria Albertina de Miranda[4]

[1]Graduate in Biological Science - University of Ponta Grossa (UEPG), Ponta Grossa, Paraná, Brasil.

[2]Postgraduate in Evolutionary Biology - University of Ponta Grossa (UEPG), Ponta Grossa, Paraná, Brasil.

[3]Department of Zootecny - University of Ponta Grossa (UEPG), Ponta Grossa, Paraná, Brasil.

[4]Department of Structural Biology, Molecular and Genetic - University of Ponta Grossa (UEPG), Ponta Grossa, Paraná, Brasil.

Corresponding author: Maria Albertina de Miranda Soares. University of Ponta Grossa (UEPG), Ponta Grossa, Paraná, Brasil. Avenida Carlos Cavalcanti, 4748 – Campus Uvaranas - Uvaranas- Ponta Grossa-PR, Brasil, CEP 84030-900. Phone: (55) 42 3220-3131.Email: tina@uepg.br

The authors declare no conflict of interest.

HIGHLIGHTS

The emergence and growth of the villi occur earlier in chickens than rats during the embryonic period and firs weeks of life.

The emergence and growth of the crypts occur earlier in chickens than rats during the first weeks of life.

The rapid growth of the intestinal mucosa in chickens correlates temporally with changes in diet soon after hatching.

ABSTRACT

The development of the villi and crypts determines the functionality of the small intestinal mucosa in birds and mammals. This work aimed to carry out a comparative study of the growth of the villi and crypts between chickens and rats during development. The measurements of the villus height and crypt depth were carried out using the Image J software. The results demonstrated that the emergence and growth of the villi occurred earlier in chickens than in rats. The villi were well-formed on the 17th day in chicken embryos and reached the pattern size of 600 µm on the 14th day after hatching. The villi emergence occurred later in the embryonic period of rats, and their growth was gradual, reaching a maximum size of 500 µm on the 25th postnatal day. The emergence and growth of the crypts occurred equally earlier in chickens than in rats. The chicken crypts were already differentiated at hatch, and their growth occurred until the 10th posthatch day. However, rats exhibited crypts with greater depth than chickens in adulthood. Chickens on the third posthatch day had approximately 92% of the villus height and 42% of the crypt depth

above 250 µm and 90 µm, respectively. In contrast, rats on the third postnatal day had about 13% of the villus height and 4% of the crypt depth above 250 µm and 60 µm, respectively. These differences in the emergence and growth of the villi and crypts between chickens and rats may be related to distinct nutritional requirements soon after birth.

Keywords: small intestine, villus height, crypt depth, development.

RESUMO

O desenvolvimento das vilosidades e criptas estabelece a funcionalidade da mucosa do intestino delgado em aves e mamíferos. Este trabalho teve como objetivo realizar um estudo comparativo do crescimento das vilosidades e criptas entre galinhas e ratos durante o desenvolvimento. As medidas das alturas das vilosidades e das profundidades das criptas foram realizadas usando o programa Image J. Os resultados demonstraram que a formação e crescimento das vilosidades ocorreram mais cedo em galinhas que em ratos. As vilosidades estavam bem formadas em embriões de galinhas no 17th dia e atingiram o tamanho padrão de 600 µm no 14th dia após a eclosão. A formação das vilosidades ocorreu mais tarde em ratos, durante o período embrionário, e o crescimento delas foi gradual, atingindo um tamanho máximo de 500 µm no 25th dia após o nascimento. A formação e crescimento das criptas ocorreram igualmente mais cedo em galinhas que em ratos. As criptas de galinhas já estavam diferenciadas no dia da eclosão e o crescimento delas ocorreu até o 10th dia após a eclosão. Entretanto, ratos apresentaram criptas com maior profundidade que galinhas na idade adulta. Galinhas no terceiro dia após a eclosão tinham aproximadamente 92% das vilosidades e 42% das criptas com altura e profundidade acima de 250 µm e 90 µm, respectivamente. Diferentemente, ratos no terceiro dia de vida pós-natal tinham 13% das vilosidades e 4% das criptas com altura e profundidade acima de 250 µm e 60 µm, respectivamente. Essas diferenças na formação e crescimento das vilosidades e criptas entre galinhas e ratos podem estar relacionadas a distintas necessidades nutricionais logo após o nascimento.

Palavras-chave: intestino delgado, altura das vilosidades, profundidade das criptas, desenvolvimento

INTRODUCTION

The gastrointestinal tube is one of the first organs to emerge from the endoderm layer in the vertebrate embryos, posteriorly to the formation of the notochord and the neural tube, which develop from the ectoderm layer. As a result of the rapid growth of the neural tube in the cranial region, the flat embryo folds in the cranial-caudal direction (anterior-posterior axis) and ventrally (dorsal-ventral axis). This process causes the embryo to take a cylindrical shape. During the ventral folding, the yolk sac is absorbed, originating a primordial gastrointestinal tube lined by the endoderm layer surrounded by the mesoderm. The gut tube is regionalized along its anterior-posterior axis by reciprocal signaling between endoderm epithelium and surrounding mesenchyme, creating three primordial compartments known as foregut, midgut, and hindgut. The endoderm epithelium, particularly in the midgut, undergoes morphological modifications as the intestine grows, originating the villi and crypts, which are structures responsible by absorption of nutrients and replenishment of epithelial cells, respectively (KOSTOUROS et al., 2020).

In some experimental animal models, such as rodents and chickens, villi and crypts are structures well characterized morphologically because of their relevant function in the small intestine. The villi are mucosal projections that increase the surface of the small intestine, optimizing food digestion and absorption of nutrients, ions, and water. The crypts are invaginations located in the base of the villi whose proliferative activity is responsible for producing new cells that migrate and differentiate to replace those sloughed off at the villus tips, maintaining the absorptive and neuroendocrine functions of the small intestine (ENSARI, MARSH, 2018; LEBLOND, STEVENS, 1948).

Although chickens and rats have the same time of embryonic development and similar morphology of the small intestinal mucosa, the initial mechanisms of the villus morphogenesis show significant differences between these animals (CAMARGO et al., 2016; CHIN et al., 2017; SHYER et al., 2013; SPENCE, LAUF, SHROYER, 2011; WALTON et al., 2016a).

The villus morphogenesis in chickens begins by the eighth day of embryonic development with the formation of projections of the small intestinal mucosa known as longitudinal ridges (previllus). From the 12th to the 14th embryonic days, the longitudinal ridges acquire a zigzag pattern (BURGESS, 1975; COULOMBRE, COULOMBRE, 1958; GREY, 1972; HUYCKE, TABIN, 2018; SHYER et al., 2013). Cell proliferation is a requirement for the folding of the longitudinal ridges into zigzags (PAIVA et al., 2019). On the 16th day of embryonic development, the formation of individual finger-like villi requires additional compression of the regular zigzags by the inner longitudinal muscle layer and spatial changes in endodermal and mesenchymal proliferation (SHYER et al., 2013).

In mammals, the villus morphogenesis is initiated by a flat pseudostratified intestinal epithelium. Firstly, there is the formation of condensed cluster through mesenchymal cells aggregation under the intestinal epithelium. Then, the intestinal epithelium passes to morphological changes, culminating to the villi emergence. During the villi lengthen and the intestine growth, there is the formation of news mesenchymal clusters under the intervillus epithelium, which will give origin another villi (KNOW, HAN, SON, 2020; WALTON et al., 2012, WALTON et al., 2016b). In rats, villus morphogenesis occurs between the E16 and the E19 stage of embryonic development (MADARA, NEUTRA, TRIER, 1981; MATHAN, MOXEY, TRIER, 1976).

It seems relevant to highlight that the small intestinal mucosa from chickens must undergo physiological alterations during the embryonic period to replace a liquid feed based on yolk with a protein-rich solid diet soon after hatching (NOY, SKLAN, 2001). Moreover, the small intestine of chickens goes through drastic kinetic changes that accelerate its growth until the 7th day after hatching (GEYRA, UNI, SKLAN, 2001).

In contrast, rats soon after birth replace the colostrum with milk, which will be their principal food source during the suckling period (from birth until the 16th postnatal day). In the weaning phase (from the 17th postnatal day), a gradual replacement of milk for a carbohydrate-rich diet (solid) occurs. The maturation of the small intestinal mucosa takes place during the first three weeks of postnatal life, when morphological and physiological alterations will improve their digestive and absorptive functions (GOMES et al., 2017).

Even though rats and chickens have the same time of embryonic development (21 days), no reports comparing the growth of the villi and crypts between these species have been found to date. Consequently, the purpose of our current study was to compare the growth of crypts and villi between rats and chickens during embryonic period and the first weeks of postnatal life. Moreover,

the present study aimed to verify if the villi lengthen, and the crypt deepen in both animals correlated to changes in feedings habits soon after birth or hatching.

2. MATERIALS AND METHODS

2.1. ANIMALS

Wistar rats (*Rattus novergicus*) were obtained from the bioterium of the State University of Ponta Grossa. The animals were maintained under conventional conditions with a 12-hour light/dark cycle (lights on at 06:30h am/lights off at 6:30h pm) at 25°C and received a balanced ration and water *ad libitum*.

Chicken eggs (*Gallus gallus domesticus*) on the 10th post-incubation day were obtained from Matrizeiro Industrial Idôneo (Carambeí, Paraná, Brazil). After sanitizing with commercial sodium hypochlorite, eggs were maintained in an incubator with an automatic egg turning system (IP 70 Premium Ecológica; Belo Horizonte, Brazil) at constant temperature (37.8°C) and humidity (60%).

Chickens were maintained in metal cages under conventional conditions with a 12-hour light/dark cycle (lights on at 06:30h am/lights off at 6:30h pm) at 25°C and received a balanced ration and water *ad libitum*.

All experiments were approved by the Animal Ethics Committee of the State University of Ponta Grossa (Number CEUA – 01/2012)

2.2. DEVELOPMENTAL TIMES

The developmental times of chickens and rats were chosen based on the morphological aspects of the small intestinal mucosa in the embryonic and postnatal periods (DAUÇA et al., 1990; DUNN, 1967; HIRAMATSU, YASUGI, 2004; PENKOVA et al., 2010; VAGNEROVÁ, KUČERA, 2004).

The rat embryos were sampled at the 17th and 19th (named E17, and E19, respectively). The rat pups were sampled at birth, 3rd, 10th, and 25th days (named P0, P3, P10, and P25, respectively). Females and rat pups were briefly anesthetized with an intraperitoneal ketamine (Laboratório Cristália, Itapira, Brazil) and Xylazine (Rompun, Bayer, São Paulo, Brazil) injections at a dose of 100mg/kg and 10 mg/kg, respectively, and sacrificed by cervical dislocation.

The chicken embryos were sampled at 17th and 21st days (named E'17 and E'21, respectively). The chickens were sampled at hatch, 3rd, 10th, and 14th days (named P'0, P'3, P'10, and P'14, respectively). The chicken embryos were killed by opening the egg's shells. The chickens were briefly anesthetized with halothane (Sigma, USA) and sacrificed by cervical dislocation.

2.3. HISTOLOGICAL PROCEDURES

The fragments of the small intestine from chickens and rats were fixed in 2% paraformaldehyde (Synth, USA)/0.1M phosphate buffer, pH 7.4, for 48h. The fragments of the small intestine were dehydrated in alcohol and carefully embedded in paraffin (Synth, USA) to obtain semi-serial sections of 5 µm, which were stained with hematoxylin (Merck, Germany).

2.3. MICROSCOPIC ANALYSIS

Sections of the small intestine were analyzed and photographed using a BX41 microscope with a Camera DP72 coupled (Olympus, Japan). The images were acquired using the CellSens Imaging software (Olympus, Japan).

2.4. SAMPLE SIZE CALCULATION

The calculation of the sample size was based on the pilot experiment that measured 30 villus height of the small intestine from chicken embryos on the 16th and 17th days (PAIVA et al., 2019). Using the Image J software (public domain), the measurements of the villus height were performed and expressed in µm after pixel calibration from a bar of 20 µm. Then, the values were imported into the Bioestat 5.3 software (public domain.). The Shapiro-Wilk test was used to determine whether the measurements come from a normal distribution before their conversion to square roots. The values of descriptive statistics were obtained and tested using the two-sample Z test to provide the means and standard deviations. This information was then subjected to independent sample testing to determine the sample size for each developmental time. Considering the means and standard deviations obtained, the bilaterality of the test, the relationship between the samples of 1:1, the test power of 80% and the alpha level of 0.05 reliability, the sample size obtained was three embryos for each developmental time per specie to be evaluated.

2.5. MORPHOMETRIC MEASUREMENTS AND GRAPHIC CONSTRUCTION

The height of 30 villi and the depth of 30 crypts per animal at each point of the development were obtained in the areas of the sections well-oriented, allowing the observation of their entire structure. The measurements of the villus height were carried out considering the distance the villus tip until the villus-crypt junction. The measurements of the crypt depth were carried out considering the distance the villus-crypt junction until crypt bottom (PIRES, SILVERIA, SILVA, 2003). The measurements were taken as described for sample size calculation. Firstly, the measurements of villus height and crypt depth carried out for every animal at each developmental point were grouped in ascending order. From the ordination of the data, it was possible to identify the villus and crypt in distinct stages of development. To better represent the data, these were organized by size ranges for the villus: 0-100 µm, 100-150 µm, 150-200 µm, 200-250 µm, and >250 µm; and for the crypt: 0-60 µm, 60-90 µm, 90-120 µm, 120-150 µm, and >150 µm. The measurements were represented in percentage after the dataset was determined and reviewed for each developmental point. To create comparisons between the temporal dataset and between chickens and rats, the total number of villi and crypts in each category was calculated and taken as 100%. The data were expressed in graphics using the GraphPad Prism® 5.01 software.

3. RESULTS

3.1. VILLUS HEIGHT DURING EMBRYONIC DEVELOPMENT OF CHICKENS AND RATS

The chicken embryos on the 17th day (E'17) exhibited well-developed villi with a maximum height of 150 µm. The rat embryos at the same developmental time had villi in the initial phase of their morphogenesis. Therefore, it was not possible to perform their measurements. The chicken

embryos on the 21st day (E'21) exhibited about 62% of the villi with a height between 150–250 µm, and about 12% were longer than 250 µm, with few reaching 300 µm. The rat embryos on the 19th day (E19) had about 17% of the villi with a height between 150-250 µm, and only about 4% were longer than 250 µm (Figure 1).

3.2. VILLUS HEIGHT DURING POSTNATAL DEVELOPMENT OF CHICKENS AND RATS

At hatching (P'0), the chickens had approximately 87% of the villi longer than 250 µm, and some reached 700 µm. At birth (P0), the rats exhibited about 67% of the villi with a height between 0-150 µm, and only about 7% were longer than 250 µm, with few reaching 360 µm. The chickens on the third posthatch day (P'3) had approximately 92% of the villi longer than 250 µm, with few achieving 900 µm. The rats on the third postnatal day (P3) exhibited approximately 72% of the villi with a maximum height of 200 µm, and only about 13% were longer than 250 µm, with few reaching 400 µm (Figure 2).

The chickens on the 10th posthatch day (P'10) had approximately 80% of the villi longer than 250 µm, and some reached 1000 µm. A small percentage of the villi had a height between 100-150 µm. The rats on the 10th postnatal day (P10) exhibited approximately 95% of the villi with a maximum height of 250 µm. The chickens on the 14th posthatch day (P'14) had about 86% of the villi longer than 250 µm, and many reached 600 µm. However, villi in the 100-150 µm range were observed in this period. Rats on the 25th postnatal day (P25) exhibited approximately 77% of the villi longer than 250 µm, and many reached 500 µm. No villus from P25 was shorter than 150 µm (Figure 3).

FIGURE 1. Changes in the villus height of the small intestinal mucosa of chickens and rats during the embryonic period. A. Semi-serial sections of 5 µm stained with hematoxylin. E'17 had well-developed villi. E17 had villi in the initial phase of their morphogenesis. E'21 had many long villi. E19 had few long villi. B. Morphometrics measurements of villus height. E'17 had villi with a maximum height of 150 µm. E'21 had about 62% of the villi with a height between 150 – 250 µm, and about 12% were longer than 250 µm. E19 had about 17% of the villi with a height between 150-250 µm, and only about 4% were longer than 250 µm. E'17 and E'21 = 17 and 21 days of chicken embryonic development, respectively. E17 and E19 = 17 and 19 days of rat prenatal development, respectively. L = lumen.

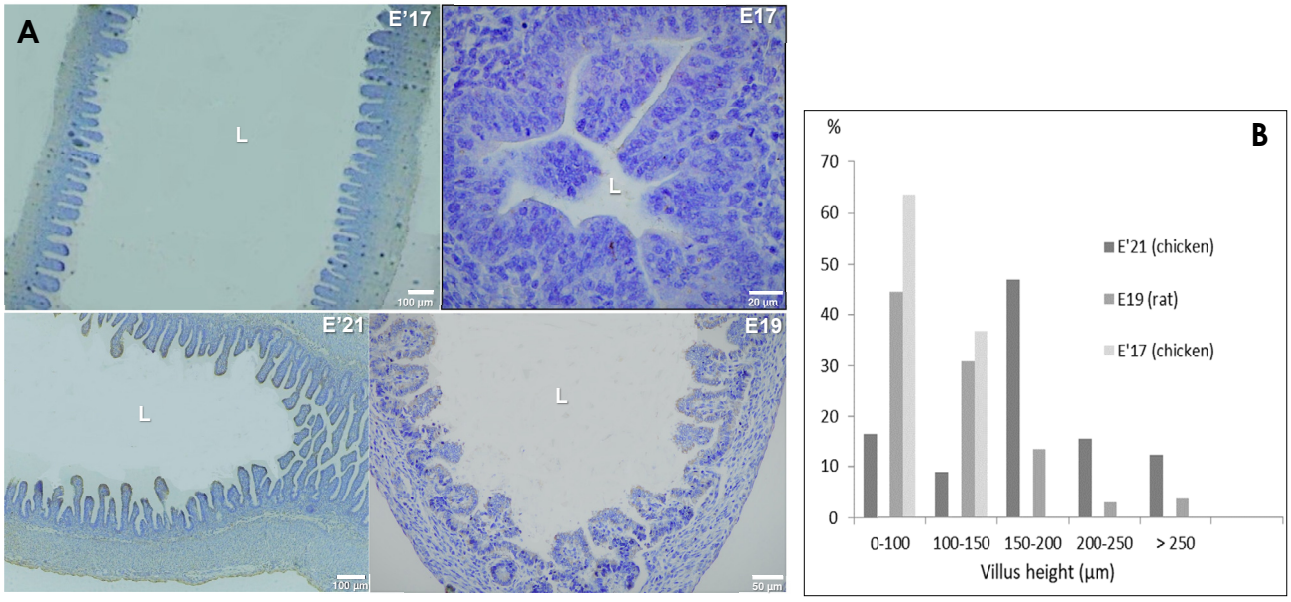


FIGURE 2. Changes in the villus height of the small intestinal mucosa of chickens and rats during the first week of the postnatal period. A. Semi-serial sections of 5 µm stained with hematoxylin. P'3 had most of villi high. P3 had few high villi. B. Morphometrics measurements of villus height. P'0 had approximately 87% of the villi longer than 250 µm. P0 exhibited approximately 67% of the villi with a height between 0-150 µm, and only about 7% were longer than 250 µm. P'3 had approximately 92% of the villi longer than 250 µm. P3 exhibited approximately 72% of the villi with a maximum height of 200 µm, and only about 13% were longer than 250 µm. P'0, P'3 = zero (hatching) and three days of chicken posthatching life, respectively. P0, P3 = zero (birth) and three days of rat postnatal life, respectively. L = lumen.

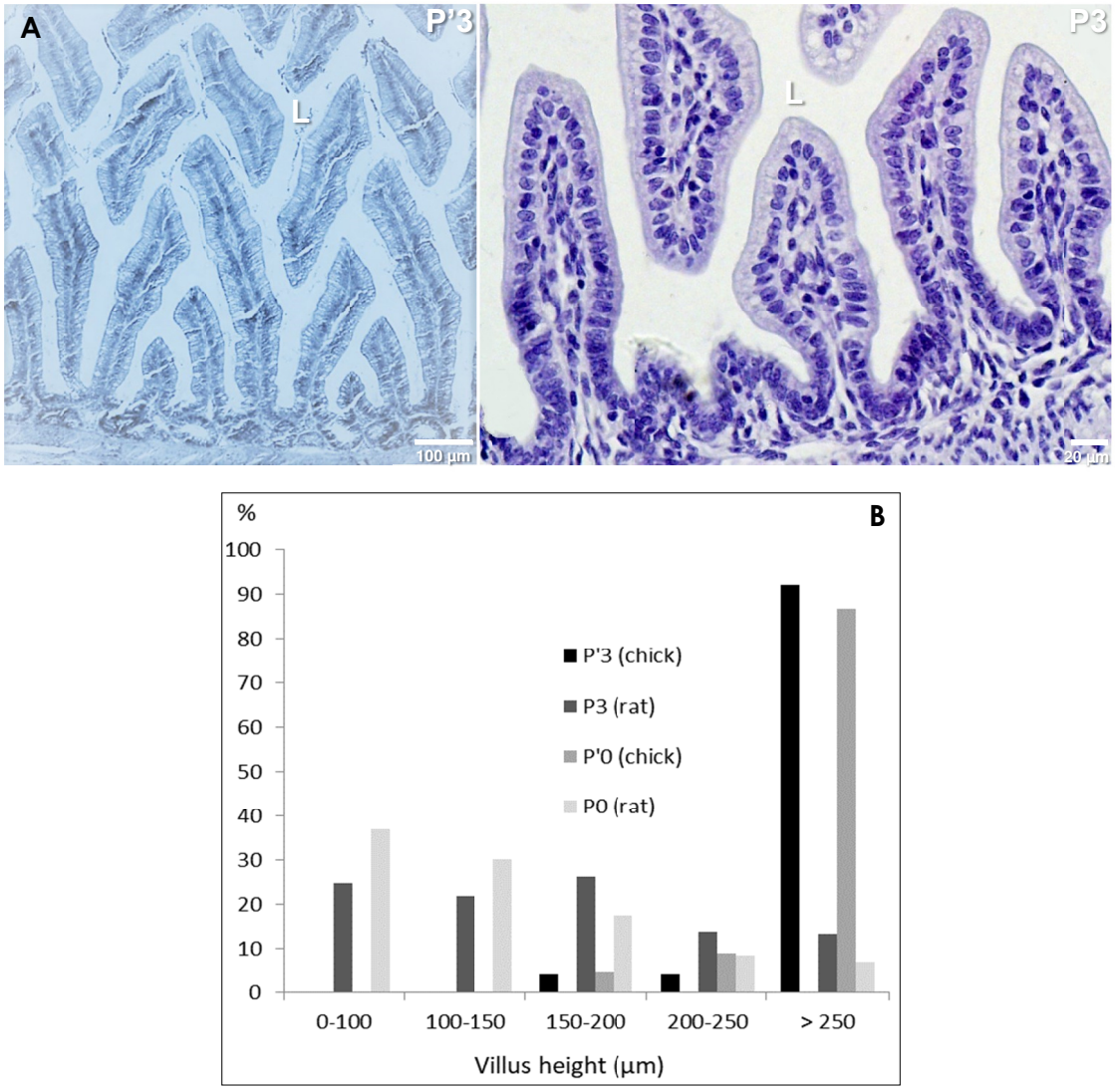
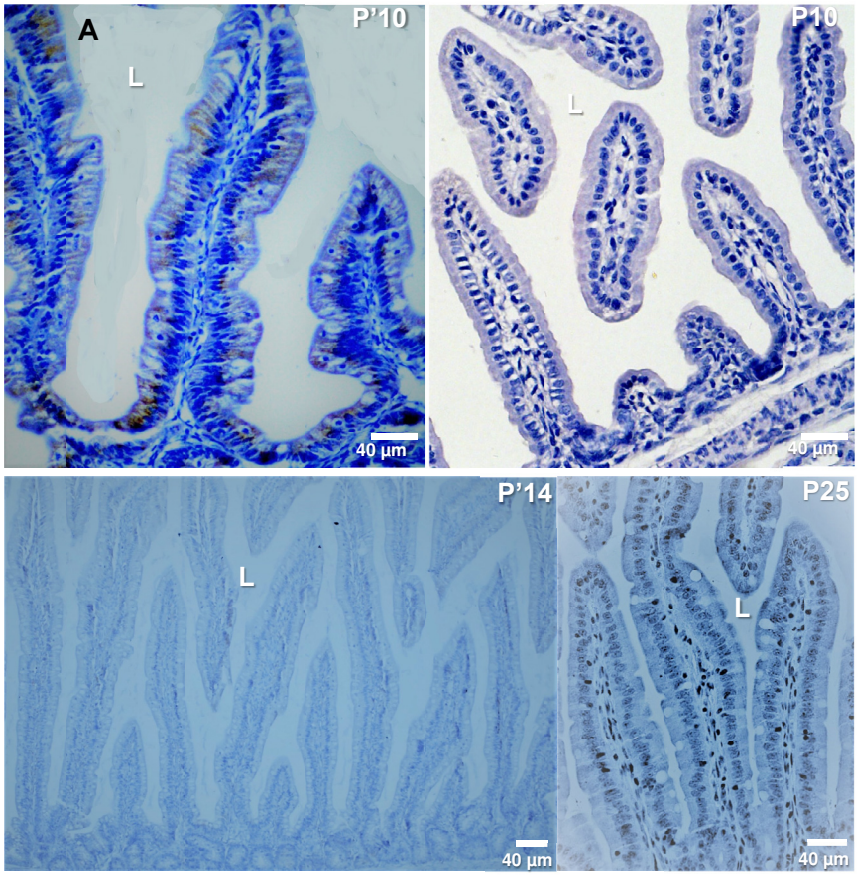
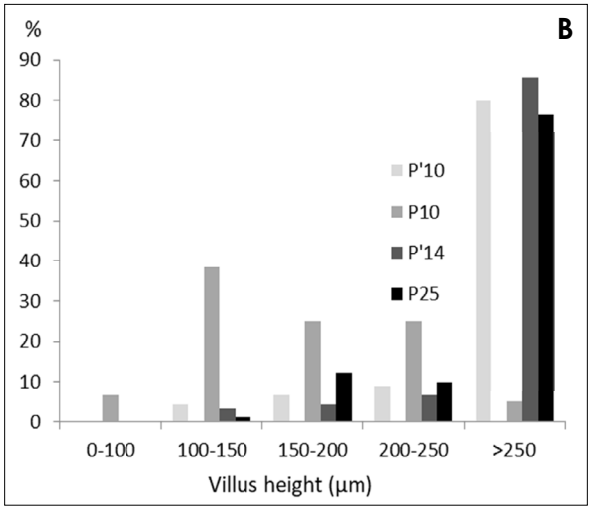


FIGURE 3. Changes in the villus height of the small intestinal mucosa of chickens and rats after the first week of life. A. Semi-serial sections of 5 µm stained with hematoxylin. P'10 had most of the villi high. P10 had still short villi. P'14 had most of the villi high. P25 had most of the villi high. B. Morphometrics measurements of villus height. P'10 had approximately 80% of the villi longer than 250 µm. A small percentage of the villi had a height between 100-150 µm. P10 exhibited approximately 95% of the villi with a maximum height of 250 µm. P'14 had approximately 86% of the villi longer than 250 µm. P25 exhibited approximately 77% of the villi longer than 250 µm. No villus from P25 was shorter than 150 µm. P'10, P'14 = 10 and 14 days of chicken posthatching life, respectively. P10 e P25 = 10 and 25 days of rat postnatal life, respectively. L = lumen.





3.3. CRYPT DEPTH DURING POSTNATAL DEVELOPMENT OF CHICKENS AND RATS

At hatching (P'0), the chickens had approximately 87% of the crypts with a depth between 0-90 µm. The rats in the same developmental time showed non-differentiated crypts. Therefore, it was not possible to perform their measurements. The chicks on the third posthatch day (P'3) exhibited approximately 91% of the crypts with a maximum depth of 120 µm, and about 9% equal to or >120 µm. The rats on the third postnatal day (P3) showed about 95% of the crypts with a maximum depth of 60 µm (Figure 4).

The chickens on the 10th posthatch day (P'10) had about 94% of the crypts with a maximum depth of 120 µm, and about 6% equal to or >120 µm. The rats on the 10th postnatal day (P10) exhibited about 98% of the crypts with a maximum depth of 60 µm. The chickens on the 14th posthatch day (P'14) had about 88% of the crypts with a depth between 60-120 µm and about 5% equal to or >120 µm. The rats on the 25th postnatal day (P25) exhibited about 47% of the crypt with a depth between 60-120 µm, about 37% with a depth between 120-150 µm, and about 14% equal to or >150 µm (Figure 5).

FIGURE 4. Changes in the crypt depth of the small intestinal mucosa of chickens and rats during the first week of the postnatal period. A. Semi-serial sections of 5 µm stained with hematoxylin. P'0 had well-developed crypts. P0 showed non-differentiated crypts. P3 had shallow crypts. B. Morphometrics measurements of crypts. P'0 had approximately 87% of the crypts with a depth between 0-90 µm. P'3 exhibited approximately 91% of the crypts with a maximum depth of 120 µm, and about 9% equal to or >120 µm. P3 exhibited about 95% of the crypts with a maximum depth of 60 µm. P'0, P'3 = zero (hatching) and three days of chicken posthatching life, respectively. P0, P3 = zero (birth) and three days of rat postnatal life, respectively. L = lumen.

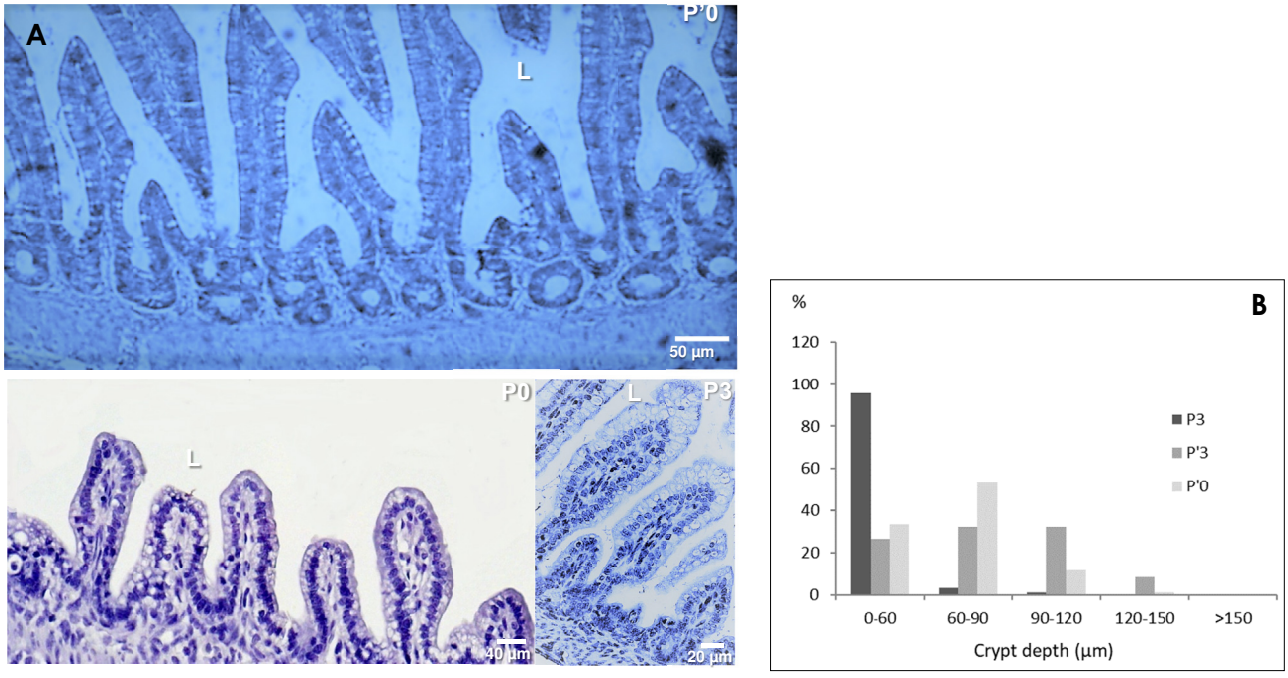
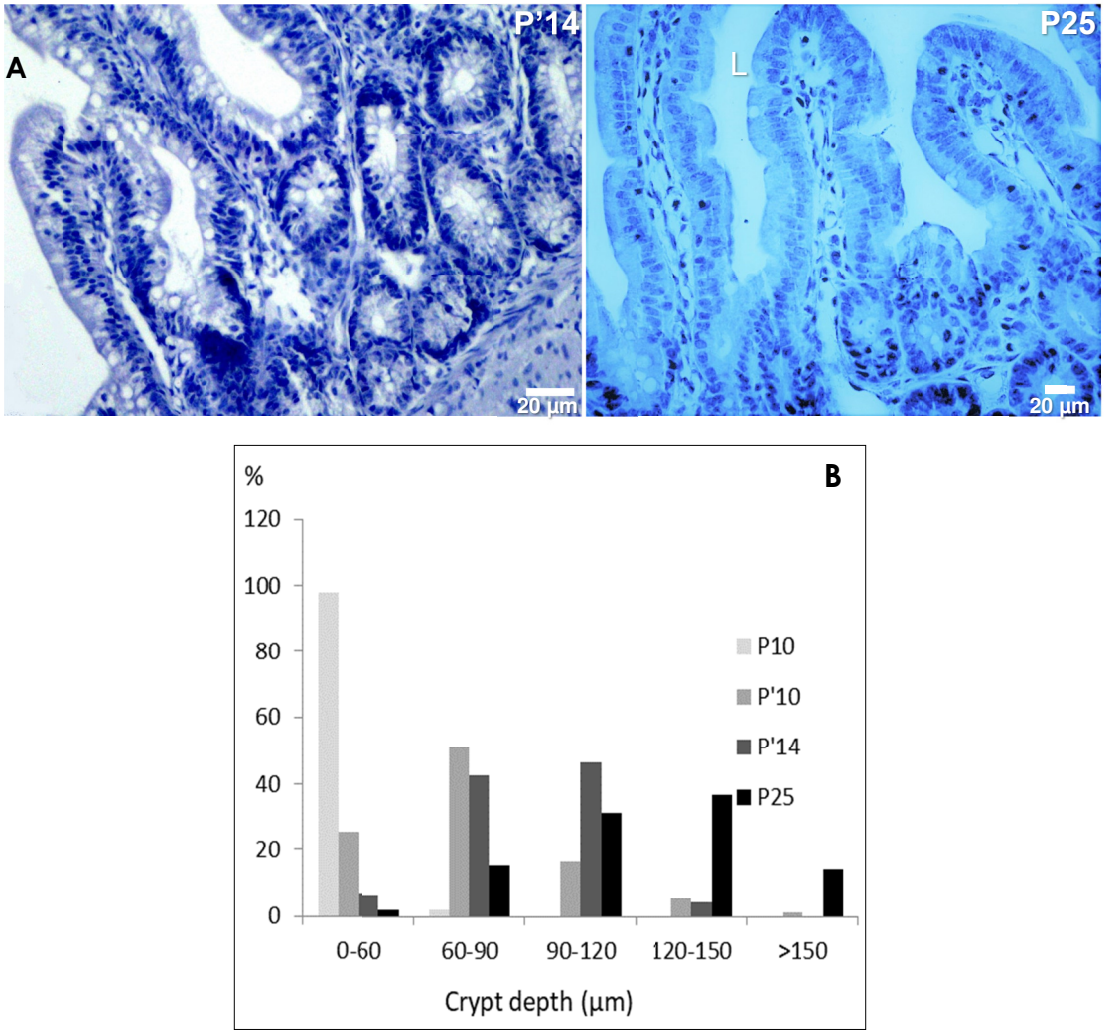


FIGURE 5. Changes in the crypt depth in the small intestinal mucosa of chickens and rats after the first week of life. A. Semi-serial sections of 5 µm stained with hematoxylin. P'14 had deep crypts. P'25 had deep crypts. B. Morphometrics measurements of crypts. P'10 had about 94% of the crypts with a maximum depth of 120 µm, and about 6% equal to or >120 µm. P10 had about 98% of the crypts with a maximum depth of 60 µm. P'14 exhibited about 88% of the crypts with a depth between 60-120 µm and about 5% equal to or >120 µm. P25 exhibited about 47% of the crypt with a depth between 60-120 µm, about 37% with a depth between 120-150 µm, and about 14% equal to or >150 µm. P'10, P'14 = 10 and 14 days of chicken posthatching life, respectively. P10, P25 = 10 and 25 days of rat postnatal, respectively. L= lumen



4. DISCUSSION

Considering that the time of embryonic development is the same in chickens and rats, we demonstrated that the emergence and growth of the villi occur earlier in former compared to latter. On the 17th day (P'17), the chicken embryos exhibited well-formed villi with a maximum height of 150 µm, and the rat embryos at the same developmental time had non-individualized villi. Chicken embryos on the 21st day (P'21) and at hatching (P'0) had well-developed villi when compared to the rats at birth (P0). Chickens at hatching (P'0) had most of villi with a height >250 µm, while rats had a

high number of villi at birth (P0) with a maximum height of 150 μm. From these findings, it is plausible to suggest that a considerable growth of the small intestinal mucosa in chickens occurs during the embryonic period and, consequently, its functional maturation, so that the animal can respond to drastic diet changes soon after hatching.

How does rapid emergence and growth of the small intestinal mucosa occur in chicken embryos? The development of intestinal villi is different between chickens and rats. In mammals, there is the formation of condensed cluster through mesenchymal cells aggregation under the intestinal epithelium. Then, the intestinal epithelium passes to morphological changes, culminating to the villi emergence (KNOW, HAN, SON, 2020; WALTON et al., 2012, WALTON et al., 2016b). In contrast, the villi in chickens emerge from projections of the small intestinal mucosa called longitudinal ridges (previllus) after acquiring the form of regular zigzags (BURGESS, 1975; COULOMBRE, COULOMBRE, 1958; GREY, 1972; HUYCKE, TABIN, 2018; SHYER et al., 2013). It is possible to suggest that the formation of the previllus constitutes a morphological adaptation in chickens to promote rapid emergence and growth of the villi before hatching.

On the third day after hatching (P'3), the chickens had approximately 92% of the villi longer than 250 μm, and some of them reached 900 μm. On the other hand, rats had most of the villi with height equal or below 200 μm on the third day after birth (P3), and some reached 400 μm. After hatching, the chicken small intestine is affected by the change in the type of ingested food, which is no longer liquid and lipid-rich (yolk) but becomes solid and composed of carbohydrates and proteins (NOY, SKLAN, 2001). Moreover, the small intestine of chickens goes through drastic kinetic changes that accelerate its growth until the 7th day after hatching (GEYRA, UNI, SKLAN, 2001). This rapid intestinal growth occurs due to an increase in the number of cells caused by an acceleration of enterocyte proliferation and migration (GEYRA, UNI, SKLAN, 2001; UNI, NOY, SKLAN, 1999). CAMARGO et al. (2016) described a strong MT1-MMP immunoreactivity in the chicken epithelium and crypts until the 7th posthatch day, suggesting that this may be related to an increase in kinetic processes such as cell proliferation.

In contrast, diet changes occur gradually during the weaning phase of rats, when the animals start to consume different types of food. In the same way, the morphological and functional alterations in the small intestine of rats occur gradually during the first weeks of postnatal life (DOS REIS, SOARES, GOMES, 2020; GOMES et al., 2017). Unlike chickens, the MT1-MMP immunoreactivity was absent in the crypts during the postnatal period (CAMARGO et al., 2016). These findings suggest that cell proliferation, differentiation, and migration are under different controls in these animals during the first weeks of life.

On the 10th day after hatching (P'10), the chicken villi reached a height of 1000 μm. However, villi with heights shorter than 150 μm were observed. These results indicate the second stage of growth of the small intestinal mucosa from chickens, well-marked temporally, leading to an increase in the number of villi. In contrast, rats on the 10th day after birth (P10) had most of the villi with a maximum height of 250 μm. The second wave of growth of the small intestinal mucosa in rats was not observed.

On the 14th day after hatching (P'14), the chicken villi reached the pattern size of 600 μm. According to UNI, NOY, SKLAN (1999), villus height in chickens increases rapidly after hatching and reaches a plateau after six days in the duodenum. It is possible to suggest that the small intestinal mucosa of chickens on the 14th posthatch day presents an adult pattern concerning their morphological and functional aspects.

On the 25th day after birth, rats had many villi with a height of 500 μm. Some reports already demonstrated that from 24th to 30th of rat postnatal life there is a stabilization of the villus height (CUMMINS et al., 1988; TRAHAIR, 1989). During the sucking and weaning phase, cell proliferation is not in a steady-state condition, i.e., the cell production rate exceeds the cell loss rate, allowing intestinal growth (WRIGHT et al., 1975). In contrast, the small intestinal epithelium of adult rats is in a steady-state condition, i.e., the proliferation of cells and migration along the villus occurs only to replace those sloughed off at the tip (LEBLOND, STEVENS, 1948). It is possible to suggest that the small intestinal mucosa of rats on the 25th of postnatal life presents an adult pattern concerning their morphological and functional aspects.

Our findings demonstrate that the emergence and growth of crypts occur earlier in chickens than in rats. Chicken crypts exhibited a maximum size of 90 μm soon after hatching, while rats showed non-differentiated crypts at birth. Some reports already described that chicken crypts contained few cells and incomplete invagination at the hatch (GEYRA, UNI, SKLAN, 2001; UNI et al., 2000). In mammals, crypts develop during the early postnatal period from a flat epithelium (CALVERT, POTHIER, 1990; HIRANO, KATAOKA, 1986).

Until the 10th posthatch day, chickens exhibited most of the crypts with a maximum depth of 120 μm, while rats in the same period had crypts with a maximum depth of 60 μm. The crypts in the intestinal mucosa of chickens are already well-formed 48 hours after hatching, but their enlargement continues until the 10th day (GEYRA, UNI, SKLAN, 2001; UNI et al., 2000). During the weaning phase in rats, crypt depth increases steadily. After this period, the enlargement of the crypts occurs faster (TRAHAIR, 1989).

As observed for the villus, the emergence and growth of the crypts occur earlier in chickens than in rats until the second week after hatching, indicating that the proliferative activity of this gland is important to the considerable growth of the small intestinal mucosa observed in this animal. This result agrees with the literature, which reports that there is a considerable growth of chicken small intestine until the 7th day after hatching (GEYRA, UNI, SKLAN, 2001)

Rats on the 25th day after birth had deeper crypts than chickens on the 14th day after hatching. According to CUMMINS et al. (1988) there is a stabilization in the crypt depth in rats until the 24th day after birth. From this time, a gradual increase in the crypt depth is observed until the 30th after birth. In chickens, cell proliferation is observed both in crypts and in villi in the third week after hatching, while, in rats, the cell proliferation is restricted to the crypts throughout life (UNI, PLATIN, SKLAN, 1998). These spatial differences in cell proliferation could also explain the morphological pattern of growth of the crypt and villi observed in the intestine of chickens compared to rats.

Finally, our findings show that, unlike rats, chicken villi and crypts emerge and grow during embryonic period, correlating temporally with critical alterations in food intake soon after hatching. However, research comparing functional characteristics of the small intestine mucosa between chickens and rats during embryonic and postnatal development is necessary to understand all the physiological adaptations.

5. CONCLUSION

We conclude that villi and crypts in the chickens emerge and grow earlier than in rats during development. These findings could be explained by the differences in their feeding patterns throughout the postnatal period.

6. ACKNOWLEDGEMENT

We thank the National Council for Scientific and Technological Development (CNPq) and Coordination for the improvement of Higher Education Personnel (CAPEs) by providing financial support through a grant to Araújo, GAT and Camargo, KC, respectively.

7. REFERENCES

BURGESS, D. R. Morphogenesis of intestinal villi II. Mechanism of formation of previllous ridges. **Journal of Embryology and Experimental Morphology**, v.34, n.3, p.723-740, 1975.

CALVERT, R., POTHIER, P. Migration of fetal intestinal intervillus cells in neonatal mice. **The Anatomical Record**, v.227, n.2., p.199–206, 1990.

CAMARGO, K. C. et al. MT1-MMP and its potential role in the vertebrate intestinal morphogenesis. **Acta Histochemica**, v.118, p.729-735, 2016.

CHIN A. M. et al. Morphogenesis and maturation of the embryonic and postnatal intestine. **Seminars in Cell & Development Biology**, v.66, p.81-93, 2017.

COULOMBRE, A. J.; COULOMBRE, J. L. Intestinal development I. Morphogenesis of the villi and musculature. **Journal of Embryology and Experimental Morphology**, v.6, n.3, p.403-411, 1958.

CUMMINS A. G. et al. Maturation of the rat small intestine at weaning: changes in epithelial cell kinetics, bacterial flora, and mucosal immune activity. **Gut**, v.29, n.12, p.1672-1679, 1988.

DAUÇA M. et al. Development of the vertebrate small intestine and mechanisms of cell differentiation. **The international Journal of Developmental Biology**, v.34, n.1., p.205–218, 1990.

DOS REIS, C. A.; SOARES, M. A. M.; GOMES, J. R. Expression of the matrix metalloproteinases 2 and 9 in the rat small intestine during intrauterine and postnatal life. **Anatomical Record (Hoboken)**, v. 303, n.11, 2839-2846, 2020.

DUNN, J. S. The fine structure of the absorptive epithelial cells of the developing small intestine of the rat. **Journal of Anatomy**, v.101, n.1, p.57-68, 1967.

ENSARI, A., MARSH, M. N. Exploring the villus. **Gastroenterol and Hepatology from Bed to Bench,** v. 11, n.3, p., 181-190, 2018.

GEYRA, A.; UNI, Z.; SKLAN, D. Enterocyte dynamics and mucosal development in the posthatch chick. **Poultry Science**, v.80, n.6, p.776–782, 2001.

GREY, R. D. Morphogenesis of intestinal villi I. Scanning electron microscopy of the duodenal epithelium of the developing chick embryo. **Journal of Morphology**, v.137, n.2, p.193-214, 1972.

GOMES, J. C. et al. Goblet cells and intestinal Alkaline phosphatase expression (IAP) during the development of the rat small intestine. **Acta Histochemica**, v.119, n.1., p.71-77, 2017.

HIRAMATSU, H.; YASUGI, S. Molecular analysis of the determination ofdevelopmental fate in the small intestinal epithelium in the chicken embryo. **The international Journal of Developmental Biology**, v.48, n.10, p.1141–1148, 2004.

HIRANO, S; KATAOKA, K. Histogenesis of the mouse jejuna mucosa, with special reference to proliferative cells and absorptive cells. **Archivum Histologicum Japonicum** v.49, n.3, p.333-348, 1986.

HUYCKE, T. R.; TABIN, C. J. Chick midgut morphogenesis. **The International Journal of Developmental Biology,** v.62, n.1-2-3, p.109-119, 2018.

KNOW, O.; HAN, T. S.; SON, M. Y. Intestinal morphogenesis in development, regeneration, and disease: the potential utility of intestinal organoids for studying compartmentalization of the crypt-villus structure. **Frontiers in Cell and Development Biology**, v. 8, p.1-14, 2020.

KOSTOUROS, A. et al. Large intestine embryogenesis: Molecular pathways and related disorders. **International Journal of Molecular Medicine**, v.46, n.1, p.27-57, 2020.

LEBLOND, C. P.; STEVENS, C. E. The constant renewal of the intestinal epithelium in the albino rat. **Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology**, v.100, n.3., p.357-371, 1948.

MADARA, J. L.; NEUTRA, M. R.; TRIER, J. S. Junctional complexes in fetal rat small intestine during morphogenesis. **Developmental Biology**, v .86, n.1, p.170–178, 1981.

MATHAN, M.; MOXEY, P. C.; TRIER, J. S. Morphogenesis of fetal rat duodenal villi. **The American Journal of Anatomy**, v.146, n.1, p.73–92, 1976.

NOY, Y., SKLAN, D. Yolk and exogenous feed utilization in the posthatch chick. *Poultry* **Science,** v.80, n. 10, p.1490–1495, 2001.

PAIVA, N. H. et al. Spatial and temporal changes in cell proliferation in the chick jejunum during the folding of the ridges into zigzags. **Acta Histochemica**, v.121, p.376-379, 2019.

PENKOVA, N. I. et al. Prenatal and postnatal differentiation of the small intestine in rat. **Folia Medica**, v.52, n.1., p.54–62, 2010.

PIRES, A. L. G.; SILVEIRA, T. R.; SILVA, V.D. Estudo morfométrico e estereológico digital da mucosa do intestino delgado de crianças eutróficas e desnutridas com diarréia persistente. **Journal de Pediatria**, *v.*79, n.4, p.329-336, 2003.

SHYER, A. E. et al. Villification: How the gut gets its villi. **Science,** v.342, n.6155, p.212-218, 2013.

SPENCE, J. R.; LAUF, R.; SHROYER, N. F. Vertebrate intestinal endoderm development. **Developmental Dynamics**, v.240, p.501–520, 2011.

TRAHAIR, J. F. Remodelling of the rat small intestine mucous during the suckling period. **Journal of Pediatric Gastroenteroly and Nutrition**, v.9, n.2, p.232-237, 1989.

UNI, Z.; PLATIN, R.; SKLAN D. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. **Journal of Comparative Physiology**, v.168, n.4., p.241-247, 1998.

UNI, Z.; NOY, Y.; SKLAN, D. Posthatch development of small intestinal functioning the in the poult. **Poultry Science**, v.78, n. 2, p.215–222, 1999.

UNI, Z. et al. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. **British Poultry Science**, v.41, n.5, p.544-551, 2000.

VAGNEROVÁ, R., KUČERA T. Rebuilding of rat intestinal mucous epithelium in prenatal ontogenesis. **Biomedical Papers of the Medical Faculty of the University Palacky**, v.148, n.2., p.253–254, 2004.

WALTON, K. D. et al. Hedgehog-responsive mesenchymal clusters direct patterning and emergence of intestinal villi. **Proceedings of the National Academy of Sciences**, v. 109, n.39, p.15817-15822, 2012.

WALTON, K. D. et al. Vilification in the mouse: Bmp signals control intestinal villus patterning. **Development**, v.143, n.3., p.427-436, 2016a.

WALTON, K. D. et al. Generation of intestinal surface: an absorbing tale. **Development,** v.143, n.13, p.2261-2272, 2016b.

WRIGHT, N. A. et al. Cell population kinetics in the rat jejunal crypt. **Cell and Tissue Kinetics,** v.8, n.4., p.361-368, 1975.