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Effect of Exogenous Melatonin on The Duodenum of Wistar Rats' Offspring Submitted to Early Weaning

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ABSTRACT

Introduction: the small intestine is the main portion of the digestive tract responsible for the process of digestion and absorption of food. Early weaning directly affects the intestinal mucosa through atrophy of the intestinal crypts, decreased villi. Melatonin is a hormone produced by the pineal gland and is present in breast milk. It has antioxidant activity, being used as therapy against several pathologies of the digestive tract, such as esophagitis, peptic ulcer, ulcerative colitis, intestinal ischemia/reperfusion and liver cirrhosis. The main objective of this study was to investigate the short-term effect of exogenous melatonin on the duodenum of the offspring of Wistar rats submitted to early weaning.

Methods: for this purpose, Wistar rats were divided into four groups (10 animals each): GI- breastfed (AM), GII- breastfed plus melatonin (AM+M), GIII- early weaning (PD), GIV- early weaning plus melatonin (PD+ M). Gut was collected at day 21 postnatal life. The weights of the animals were analyzed. Histopathological evaluation, and morphometry of the duodenum, were performed by Hematoxylin and Eosin (HE) staining. Fractal dimension, lacunarity were and histochemistry of the duodenum were evaluated.

Results: the PD group showed lower body mass compared to the other groups. Through stained histological preparations we observed that melatonin was able to reverse, histomorphometrically, fractally and histochemically, some damages of early weaning, such as the presence of pyknotic nuclei, villus areas and crypt areas significantly showing its importance in breast milk.

Conclusion: we conclude that melatonin attenuates some of the damage caused by early weaning.

Keywords: Early Weaning; Duodenum; Melatonin; Fractal; Histochemistry

Introduction

Breastfeeding is a practice that provides benefits for both mother and child and should be exclusive in the first six months of life according to the World Health Organization (WHO)[1] This strategy aims to reduce neonate morbidity and mortality[2,3]. Breastfeeding is a critical and extremely important period for morpho-physiological maturation of the body, impacting on infant growth and development[2].

Breast milk is considered a complete food that meets the needs of the developing organism, containing in its composition carbohydrates, proteins, lipids, growth factors and others. The abrupt discontinuation of exclusive breastfeeding is called early weaning and is an issue of worldwide concern. Early weaning can be reflected in multiple systems, including the digestive system, which is responsible for the breakdown and absorption of proteins, lipids, vitamins, and minerals, with the intestine as the main organ in this process[2-4].

The small intestine is the main portion of the digestive tract responsible for the process of digestion and absorption of food[5]. Early weaning directly affects the intestinal mucosa through atrophy of the intestinal crypts, decreased villi or by increasing the activity of enzymes such as ornithine decarboxylase, reflecting on absorption, since the absence of it will lead to morphophysiological changes as well as the growth of the organ[6,7,8].

The gastrointestinal tract (GIT), in mammals, is known to be responsible for producing a variety of substances and is also an extra pineal source of melatonin hormone production[9].

Melatonin is a hormone produced by the pineal gland and is present in breast milk. It is produced in greater quantity at night, since the presence of light inhibits its production[10,11,12]. Because it has an amphiphilic characteristic, it easily passes through the cell membrane and has antioxidant activity, being used as therapy against numerous pathologies of the digestive tract such as esophagitis, peptic ulcer, ulcerative colitis, intestinal ischemia/reperfusion, and liver cirrhosis[13,14,15].

However, understanding the action of melatonin in individuals experiencing eating disorders during growth is necessary. Thus, taking into consideration few reports in the literature on the influence of

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melatonin on the consequences of eating disorders, the present work aimed to investigate the short-term effect of exogenous melatonin against the duodenum of the offspring of Wistar rats submitted to early weaning.

Materials and Methods

Ethical Considerations

All experimental procedures were conducted in accordance with the principles for the Guide for the Care and Use of Laboratory Animals (8th ed., 2011), national instructions (Laws 6638/79, 9605/98, Decree 24665/34) and had been approved by the Ethics Committee on Animal Use - CEUA of the Federal University of Pernambuco - UFPE under number 23/2020.

Animals

Eight albino rats (200-250g body weight) of the Wistar strain from the Breeding Animal Facility of the Nutrition Department of the Federal University of Pernambuco were used. The rats were mated in the ratio of two females (n=8) to one male (n=4). Pregnancy was diagnosed by the presence of spermatozoa in the vaginal smear and confirmed by body weight gain[16]. After the diagnosis, the pregnant rats were transferred to individual cages and during gestation and lactation received standard diets (Labina, Presence®). After the birth of the pups, sexing was performed for the formation of litters with 8 pups per mother. More than one mating was performed to obtain 10 animals per group. Throughout the experiment, the animals were kept under standard vivarium conditions (22°C ±2°C, under a 12-hour light/dark cycle, light off at 18h), receiving feed and water ad libitum.

Early Weaning

The day of birth of the litter was considered day zero. On the tenth postnatal day, the animals were sexed with the objective of keeping 8 male pups in each litter (the litters that did not obtain this number will be complemented by females). The experimental groups were formed according to the weaning period of each litter: GI (n=10); GII (n=10); GIII (n=10); GIV (n=10). The litters belonging to the groups (GI; GII) were weaned on the 15th postnatal day and the groups (GIII; GIV) were weaned on the 21st postnatal day which is considered the natural weaning period. The dams of the animals in the groups (GI; GII) were separated from their pups who received feed and water through disposable Pasteur pipettes and were massaged to facilitate the excretion of urine and feces[17].

Experimental Group

Groups weaned on day 15

(GI), n= 10 - animals weaned on the 15th postnatal day;

(GII), n= 10 - animals weaned on the 15th postnatal day and treated with melatonin;

After early weaning, animals belonging to both groups were separated from the dam in individual cages until postnatal day 21.

Groups weaned on day 21

(GIII), n= 10 - animals weaned on the 21st postnatal day;

(GIV), n= 10 - animals weaned on the 21st postnatal day and treated with melatonin;

Melatonin Treatment

The treatment was performed according to the melatonin supplementation model (Sigma, St. Louis, MO, USA) already established in the literature by Prata Lima; Baracat; Simões[18]. Animals received 200 μg of melatonin per 100g of animal body weight intraperitoneally (i.p) in the evening period (6:00 pm) for 6 days (15th to 21st) in groups GII and GIV. Melatonin was dissolved in a volume of ethanol (0.02 mL) and diluted in saline solution (NaCl 0.9%). Animals in group GI and GIII received 0.9% NaCl solution and 0.02 mL of ethanol (placebo), respectively.

Obtaining Samples From the Duodenum

On the 21st day of life, the rats were submitted to dissociative anesthesia using ketamine hydrochloride (C) + xylazine (X) at a dose of 60-95mg/Kg (C) + 5-10 mg/Kg (X) (i.p) for surgical anesthesia. Afterwards the animals were positioned in ventral decubitus with tape immobilization, trichotomy and disinfection of the abdominal region were performed, followed by surgical intervention, exposure of the duodenum and removal of the organ. After the duodenum was removed (region after the pyloric sphincter) a small fragment was immersed in 10% buffered formalin fixative solution for 24 hours for the preparation of histological slides. At the end of the experiment the animals were euthanized. The body mass of the animals was weighed between the 15th and 21st day using a commercial scale.

Histological, Histochemical and Morphometric Analysis

Samples of the duodenum from the experimental groups were cleaved, dehydrated in ethyl alcohol solutions with increasing concentrations of 70, 80, 90, and 100%, diaphanized by xylol, impregnated by liquid paraffin in an oven regulated at a temperature of 60 °C, and embedded in histological purified paraffin (PF 56-58°C). Then, the blocks were cut in microtome, adjusted to 3 μm. The obtained sections were placed on slides previously greased with Mayer's Albumin and kept in an incubator regulated at 37°C for 24 hours for drying and sticking. Then they were submitted to hematoxylin and eosin staining (HE) for histopathological analysis. Ten slides were made for each group.

Histochemical evaluation was performed using

the following staining methods: PAS (Schiff's Periodic Acid), Alcian Blue, Mallory's Trichrome and Orcein.

For morphometric analysis, histological images were captured by the digital camera "Moticam 2300 3.0M Pixel USB 2.0" coupled to the optical microscope "Olympus CX22" with 400X magnification. The photomicrographs were evaluated using ImageJ software version 1.44 (Research Services Branch, U.S. National Institutes of Health, Bethesda, MD, USA), where the length, area of the intestinal villi and the area of the intestinal gland were analysed. Measurements of 10 fields per slide were taken, for a total of 1000 fields per group.

Method of Fractal Dimension

Microscopic images of the duodenum were used to calculate fractal dimension by the box counting method (Dbox) and dimension by information entropy (Dinf) according to Tenorio *et al.*[19]. These images were analysed using Benoit 1.3 software (Fractal Analysis System, Trusoft, St. Petersburg, USA). In summary, the fractal dimension obtained by the box counting method was calculated by covering the image with N(r) boxes, where N is the number of boxes and r is the length of a side of the box containing at least one point of the analysed structure. This process will be repeated with boxes of different sizes and plotted on a double log plot of N(r) as a function of r. The slope of this ratio between the reversed signs is the fractal dimension per box counting and ε is the smallest variation in the size of the boxes. This can be formally described by formula 1:

$$D_{bc} = -\lim_{\varepsilon \to 0}\left[\frac{\log N(r+\varepsilon) - \log N(r)}{\log(r+\varepsilon) - \log r}\right]$$

In the fractal dimension by information entropy (Dinf) the image is also covered by boxes, but this method is related to the frequency in which each box is occupied by the analysed structure; formally described by formulas 2 and 3:

$$D_{inf} = \lim_{\varepsilon \to 0}\left[\frac{(\mathrm{S}(r+\varepsilon) - S(r))}{\log(r+\varepsilon) - \log r}\right]$$

$$S(r) = -lim_{N\to\infty}\sum_{i=1}^{N(r)} m_i \log(m_i)$$

Where, S(r) is the Kolmogorov entropy, mi=Mi/M (Mi is the number of points in box i, and M is the number of points in the analysed structure).

Measurements of 10 fields per slide were performed, for a total of 1000 fields per group.

Lacunarity Method

Lacunarity analysis was performed using the procedures previously described in Tenorio *et al*[19]. The distribution of the lacunae in the analysed structure was described by the lacunarity value obtained using Image J software (National Institutes of Health -NIH, USA) with the FracLac plug-in (A. Karperien - Charles Sturt University, Australia). The image was covered by a series of grids, each grid containing a number of boxes of different sizes (ε) and orientations (g). The average lacunarity (Λ) will be obtained by formula 4:

$$\Lambda = \left[\sum_{g}\sum_{i}(1 + (\sigma|\mu)^2)\right]\Big/ n$$

Where σ is the standard deviation; μ is average pixel value per box of side ε; n is the number of the size of the boxes in an orientation g.

Measurements of 10 fields per slide were performed, for a total of 1000 fields per group.

Statistical Analysis

Statistical analysis was performed in a computer program GraphicPad Prism 5, where data were evaluated using Kruskal Wallis non-parametric tests with Dunn's post-hoc. A p value ≤ 0.05 was considered statistically significant.

Results

Weight Evaluation

The weight of the animals in the DP group showed a significant difference when compared to the other groups, showing lower mean weights (Table 1).

Table 1. Weight of animals in the experimental groups. DP group with statistical difference when compared to the other groups.

Experimental Groups (N=10)	Weights Of Animals (G)
AM	37,9 ± 4,20 a
AM+M	39,6 ± 6,89 a
DP	28,3 ± 4,69 *
DP+M	34,1 ± 7,12 a
K	0.0007

Kruskal Wallis test with Dunn's post hoc, significance level p ≤0.05

Histological Analysis

The duodens of the rats in the AM and AM+M groups presented the villi with a leaf-like morphology typical of the duodenum, with well-preserved mucosa, submucosa, and muscle. The mucosa presented simple cylindrical epithelium with a brush border, composed of enterocytes, calyceal cells, and mast cells. In addition to the thick lamina propria, rich in loose connective tissue, blood vessels, and defense cells such as lymphocytes. The submucosal layer was

present rich in duodenal glands such as Brünner's gland and the muscular layer showed two layers an inner circular and an outer longitudinal layer covered by a connective tissue serosa covered by simple flat epithelium, mesothelium. Myenteric intestinal plexus and Paneth cells were also observed (Figures 1).

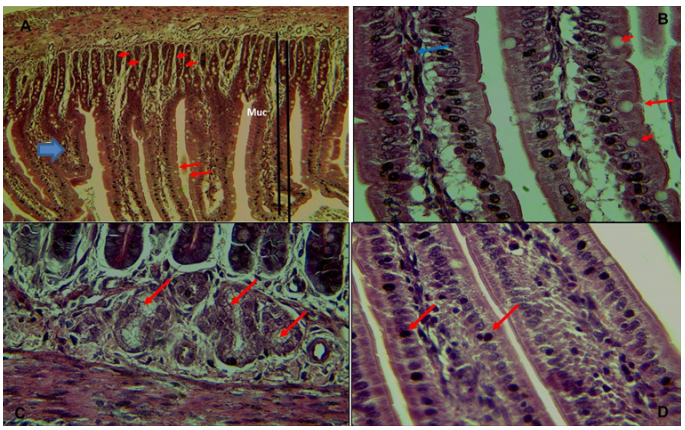

Figure 1. Photomicrographs of the duodenum of animals in the experimental groups. A: Duodenum of the AM group. Mucosa (Muc) with villi lined by simple columnar epithelium (long arrow); lamina propria (blue arrow). Intestinal glands (short arrows); Submucosa (Sb); Muscular (Mus); AU 100X; H.E. staining. B: Duodenum from the DP group. Villi lined by simple columnar epithelium with brush border (Long arrow); presence of calyceal cells (short arrow); Mast cell (blue arrow); AU 400X; H.E. staining. C: Duodenum from Group AM+M. Brünner's glands in the duodenal submucosa. AU 400X; H.E. staining D: Duodenum of Group DP+M. Mast cells (arrows) AU 400X; H.E. staining.

The duodenum of the DP and DP+M groups showed morphological characteristics similar to the AM and AM+M groups. However, the DP group presented some cells with pyknotic nuclei. In the DP+M group this characteristic was reduced (Figure 2).

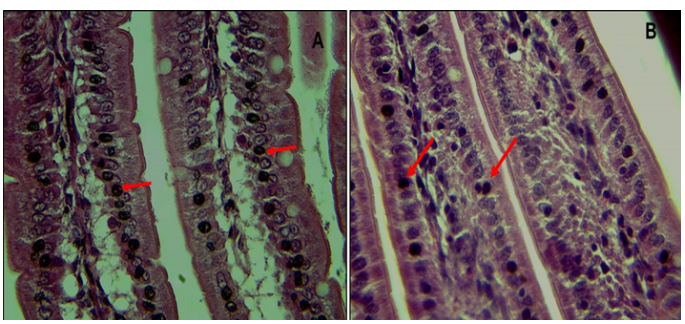

Figure 2. Photomicrographs of the duodenum of the animals in the experimental groups. A: Duodenum of the DP group. Large amount of cells with pyknotic nuclei (red arrows). AU 100X; H.E. staining B: Duodenum of the DP+M group. Reduced number of cells with pyknotic nuclei (red arrows).

Histochemical Analysis

In the histochemical analysis it was found that mucin expression increased progressively in the DP group, as the number of Alcian Blue and PAS positive calyceal cells increased (figure 3 and 4).

Regarding histochemistry by orcein, there were no significant differences in the increase in the number of orcein-positive fibers among the animals in the experimental groups (Figure 5).

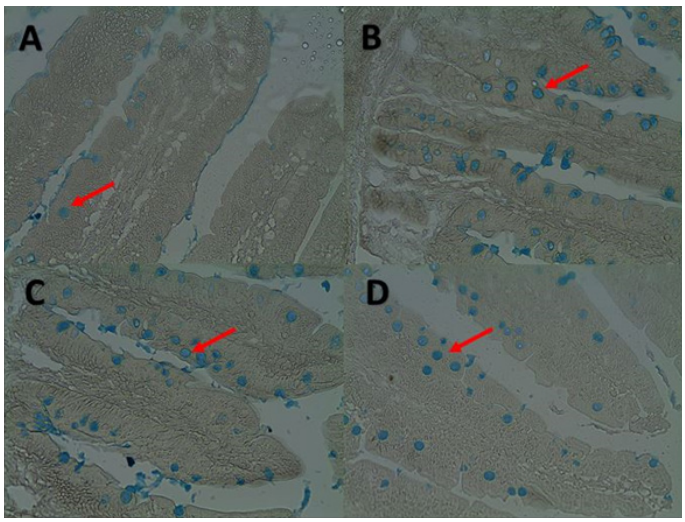

Figure 3. Photomicrographs of the duodenum of animals in the experimental groups. A, B, C and D: observe Alcian Blue positive calyceal cells (red arrows); AU 100x. Alcian Blue staining.

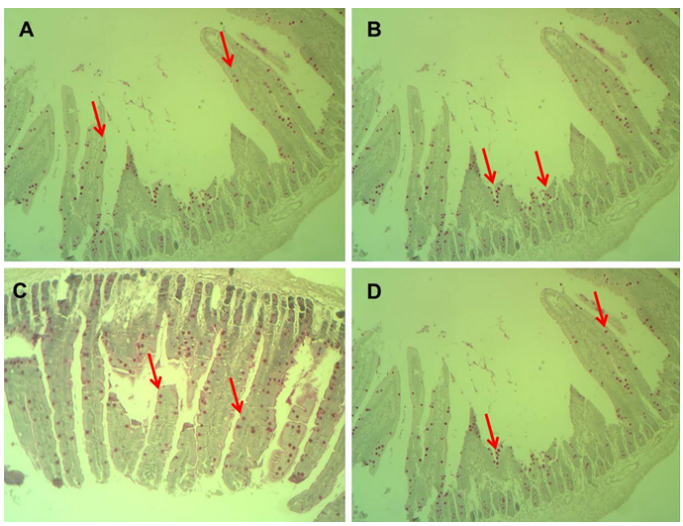

Figure 4. Photomicrographs of the duodenum of the animals in the experimental groups. A, B, C and D: observe PAS-positive calyceal cells (red arrows); AU 100x. PAS staining.

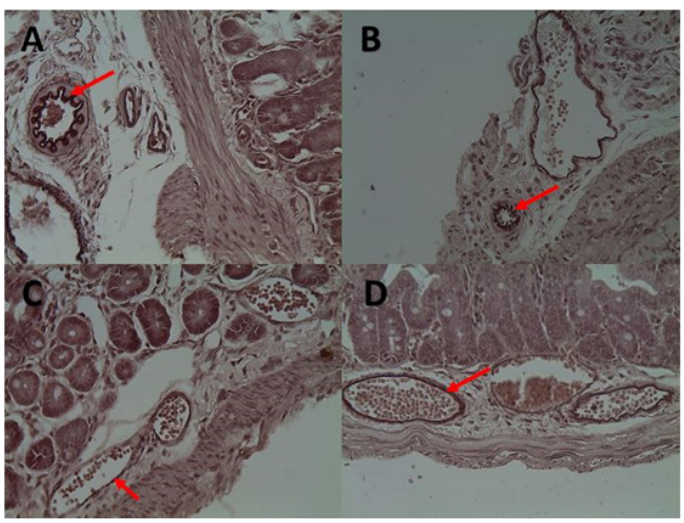

FIGURE 5: Photomicrograph of the duodenum of the experimental animals. A, B, C and D: observe positive orcein (purple staining) structures (red arrows); AU 100x. Orcein staining.

Regarding the count of PAS and Alcian Blue positive cells, there was a greater increase in the DP group when compared to the other groups. The individual area and the total area of PAS and Alcian Blue positive cells did not show significant differences (Table 2).

the lumen, perimeter of the lumen, circularity of the lumen, ellipse of the lumen, and feret of the lumen (maximum diameter) showed no statistical differences (Table 3).

Table 2 Morphometry of the calyceal cell count, individual area and total area of the PAS and Alcian Blue positive cells.

Experimental Groups (n=10)	AM	AM+M	DP	DP+P	K
Number of cells	159,8±49,2a	166,0±32,2a	220,4±16,4b	159,5±15,8a	0.0024
Individual Area	39,6±9,5a	40,7±5,7a	49,6±6,9a	46,4±1,2a	0.1044
Total Area	6681,3±3299,1a	6940,4±2373,3a	11146,4±2292,8a	7438,9±656,5a	0.0728

Kruskal Wallis test with Dunn's post hoc, significance level p ≤0.05

Histomorphometric Analyses

The histomorphometric results showed that the DP and DP+M groups had the lowest mean villus lengths compared to the experimental groups AM and AM+M, being statistically different (p<0.005) (Figure 6).

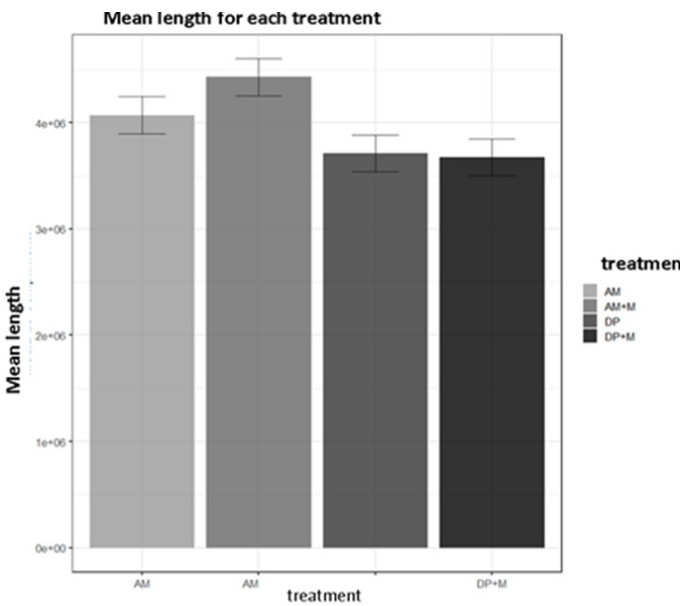

Figure 6. Villus length of the experimental groups. The AM+M group showed greater mean length compared to the DP and DP+M groups. (p<0,005).

In the villus area, the highest mean was in the AM group, statistically different from the AM+M and DP groups, which had the lowest means (Figure 7). The DP+M group had a larger villus area than the DP group and the AM+M group.

Regarding the area of the crypts, the DP group showed the lowest mean when compared to the other experimental groups, showing a significant difference. The groups AM, AM+M and DP+M showed no significant differences (Figure 8).

Fractal And Lacunarity Analysis

Binarized image analysis (Figure 9) of the fractal method and lacunarity of the duodenum of animals in the experimental groups showed no statistical differences. In addition, the area of

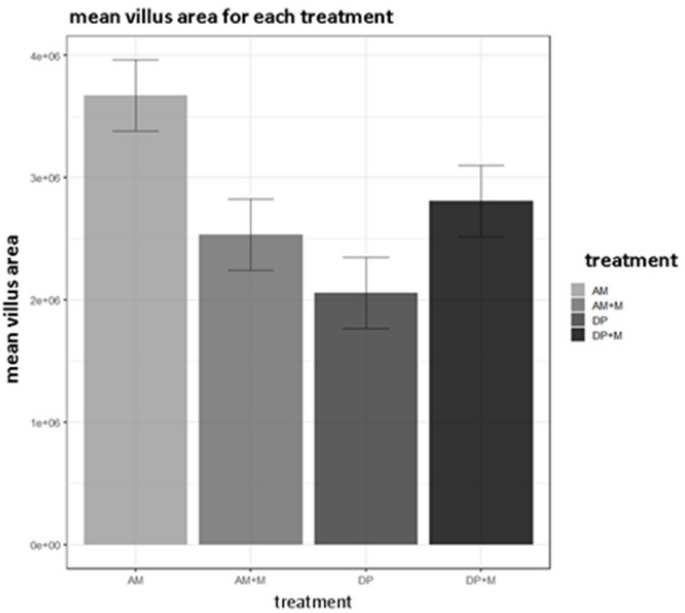

Figure 7. Mean villus area. The AM group presented a larger villus area compared to the other groups. The groups AM+M and DP showed the lowest averages (p<0.005).

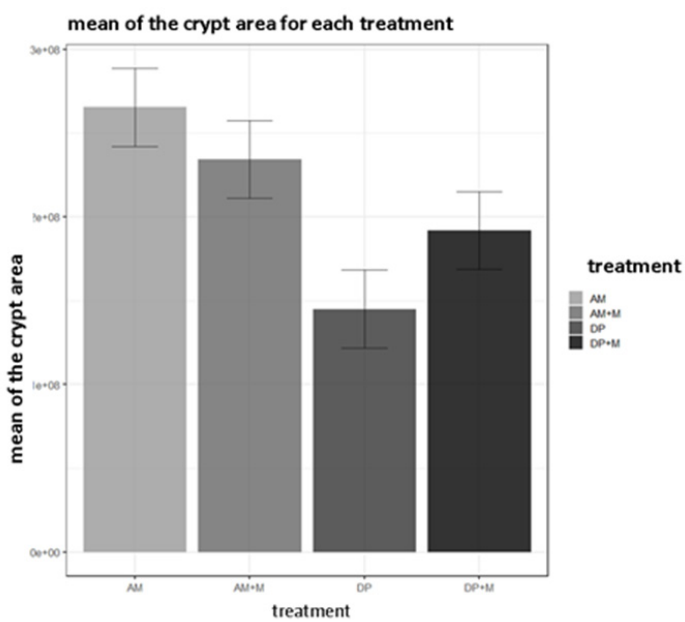

Figure 8. Crypt area. The DP Group showed a significant difference when compared to the other experimental groups. (p<0,005).

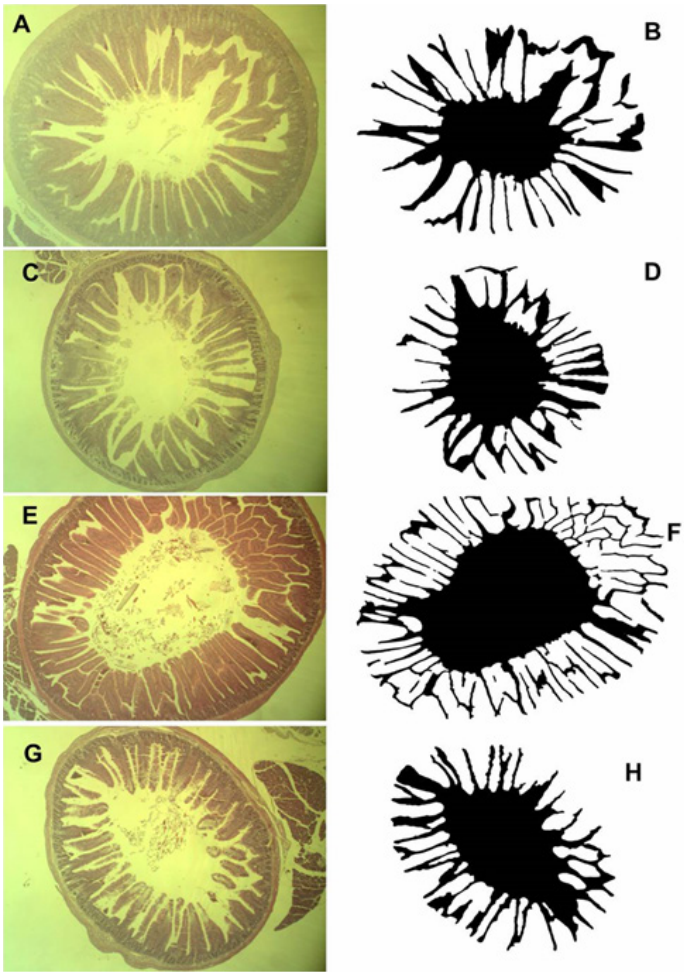

Figure 9. Images A, C, E and G- Photomicrographs of the duodenum intestine of the Experimental groups: AM, AM+M, DP, DP+M respectively. B, D, F, H- binarized segmentation image of the duodenum intestine lumen of the experimental groups: AM, AM+M, DP, DP+M respectively.

Discussion

In this study, a difference was found in the weight of animals in the DP group compared to the other experimental groups. Such findings are in disagreement with Tavares *et al.*[20] where no differences in body mass were found between early and regular weaning in male rats, but are in agreement with Pietrobon *et al.*[3] in which at day 21, males submitted to early weaning showed lower body mass compared to the control group. Teles Silva *et al.*[17] found reduced body mass on day 18 of the weaned group compared to the control (breastfed) group but this difference was reduced on day 60, leaving their respective body masses similar. Thus, treatment with melatonin may have interfered with the body weight of animals, since the animals in the DP+M group had body mass similar to the control group, reversing the process.

Baptista *et al*[21] found a large amount of thymocytes in apoptosis in young rats subjected to protein malnutrition, corroborating the present findings. In histochemistry regarding acidic (stained by Alcian Blue) and neutral (stained by PAS) mucin-producing calyceal cells Rosa *et al*[22] observed considerable difference in the population of calyceal cells with diet restriction when compared to the control group. Melatonin supplementation is known to have an effect on the mRNA expression of caliciform cell-derived mucin2 (Muc2) and trefoil factor 3 (Tff3) in the ileum, as well as on the number of caliciform cells in the villi of the ileum[8]. However, Ren *et al.*,[23], observed that melatonin did not alter the number of calyceal cells in the villi of the ileum in newly weaned mice, which is in agreement with the present research where it is suggested that

Table 3. Analysis of the duodenum of the animals in the experimental groups: fractal, lacunarity, lumen area, lumen perimeter, lumen circular, lumen ellipse, and lumen feret.

Experimental Groups (n=10)	AM	AM+M	DP	DP+M	p
Fractal	1,7713± 0,023a	1,7735± 0,022a	1,7953± 0,029a	1,7713± 0,016a	0,6019
Lacunarity	0,4437± 0,100a	0,4417± 0,076a	0,4742± 0,048a	0,4363± 0,060a	0,7651
Lumen Area	1469255,28± 1512461,34a	1281575,07± 1254643,16a	1931626,09± 345513,10a	1917341,78± 458409,46a	0,61
Lumen Perimetro	31748,35± 6448,05a	27083,85± 10356,18a	38875,79± 5867,98a	25643,73± 2603,23a	0,08
Lumen Circular	0,02± 0,02a	0,02± 0,01a	0,02± 0,01a	0,04± 0,01a	0,090
Lumen Ellipse	1,33± 0,18a	1,31± 0,23a	1,41± 0,15a	1,44± 0,25a	0,32
Feret of Lumen	2154,14± 671,13a	1947,49± 518,71a	2511,47± 260,16a	2331,44± 93,66a	0,14

Kruskal Wallis test with Dunn's post hoc, significance level p ≤0.05

melatonin treatment may have caused an alteration to normality where the DP+M group resembles in this histochemical parameter to the AM group. Regarding Orcein staining, which is specific for elastic fibers, Brito *et al*[24] observed the presence of more mature elastic fibers in adult rats than in older ones.

Corroborating with our results, Da Costa *et al.*[8] evaluated the same parameter (height/length) of villi from early weaned rats and it was smaller in the weaned groups compared to the rats that were breastfed. Previous studies where melatonin supplementation was performed (for two weeks after day 21) had an effect on the villus/crypt ratio in the duodenum, although melatonin decreased villus length and crypt depth in the duodenum[23]. Therefore, at the histomorphometric level there was no interference of melatonin facing villus length on the early weaned (DP) group.

In the study by Crispel *et al.*,[25], where it was found that early weaning resulted in deeper crypts, lower villous to crypt ratio, and smaller villous area at day 21. This result persisted until adulthood of the animals, when assessing at day 90, animals that underwent early weaning remained with the smallest villous area[25]. As for the DP+M group, which had a larger villous area than the DP group, this corroborates the findings of Ren *et al.*,[23], in which the melatonin-treated group showed a high villous/crypt area in the ileum. Therefore, melatonin reversed the damage of early weaning on the villus area.

Barbosa *et al.*,[26] reported in their study that early weaning caused atrophy of the crypts. Lemos *et al.*[2] stated that the animals in the (PD) group showed a reduction in the depth of the intestinal crypts. This corroborates other studies, which showed a decrease in the villus/crypt ratio, in which there were more villi than intestinal crypts[25-27].

Therefore, melatonin reversed the atrophy of the intestinal crypts since the MP+M group showed a significant increase in relation to the DP group regarding the area of the crypts. Regarding the lumen area through the methods of fractal and lacunarity, it was found in this study no significant difference between the groups, suggesting that even with the difference in length in the AM+M group with the other groups, the fractal analysis and lacunarity of the lumen showed no changes.

Conclusion

Thus, we conclude that early weaning can promote morphometric changes in the duodenum, as well as decrease in body mass, and that exogenous melatonin is able to attenuate some damage caused by this process. We suggest that these results should be used in preventive measures and development of public policies regarding breastfeeding.

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References

1. Brasil. (2015). Saúde da criança: aleitamento materno e alimentação complementar. Ministério da Saúde.
Lemos, G. T. D. L. (2019). Avaliação histomorfométrica do intestino delgado de ratos wistar adultos submetidos ao desmame precoce (Bachelor's thesis).
2.Pietrobon, C. B., Miranda, R. A., Bertasso, I. M., Mathias, P. C. De F., Bonfleur, M. L., Balbo, S. L., Reis, M. A. De B., Latorraca, M. Q., Arantes, V. C., Oliveira, E., Lisboa, P. C., & Moura, E. G. (2020). Early weaning induces short- and long-term effects on pancreatic islets in Wistar rats of both sexes. The Journal of Physiology, 598(3), 489–502. Https://doi.org/10.1113/JP278833.
3. da Costa, M. A. S., dos Santos Silva, R. R., da Silva, M. E., da Silva Linhares, L. H., Nascimento, T. O., de Oliveira, M. L. F., ... & Tenorio, F. C. A. M. (2020). Efeito da melatonina exógena frente ao trato digestório da prole de camundongos submetidos ao desmame precoce: uma revisão sistemática. Research, Society and Development, 9(10), e5009108869-e5009108869.
4. Ross, M. H., & Wojciech, P. (2016). Histologia. Grupo Gen-Guanabara Koogan.
5. Lin, C.-H., Correia, L., Tolia, K., Gesell, M. S., Tolia, V., Lee, P.-C., & Luk, G. D. (1998). Early Weaning Induces Jejunal Ornithine Decarboxylase and Cell Proliferation in Neonatal Rats. The Journal of Nutrition, 128(10), 1636–1642. Https://doi.org/10.1093/jn/128.10.1636.
6. Barbosa, Mércia Maria Bezerra. Avaliação histomorfométrica da mucosa gástrica e intestinal em ratos Wistar submetidos ao desmame precoce. 2019. Dissertação de Mestrado. Universidade Federal de Pernambuco.
7. Da Costa, A. V., Rattes, I. C., Silva, K. M., Rigonati, C. A. M., & Gama, P. (2019). Effect of early weaning on proliferation and differentiation of intestinal mucosa. The FASEB Journal, 33(1_supplement), 762-1.
Tolosa de Talamoni, Nori (2017). Gastrointestinal Tissue || Melatonin, Gastrointestinal Protection, and Oxidative Stress. , (), 317–325.
8. Amaral, F. G. D., & Cipolla-Neto, J. (2018). A brief review about melatonin, a pineal hormone. Archives of endocrinology and metabolism, 62(4), 472-479.
9. Tan, D. X., Xu, B., Zhou, X., & Reiter, R. J. (2018). Pineal calcification, melatonin production, aging, associated health consequences and rejuvenation of the pineal gland. Molecules, 23(2), 301.
10. Moderie, C., Van der Maren, S., Paquet, J., & Dumont, M. (2020). Home versus laboratory assessments of melatonin production and melatonin onset in young adults complaining of a delayed sleep schedule. Journal of Sleep Research, 29(3), e12905.
11. de Talamoni, N. T., Areco, V., Rodríguez, V., Marchionatti, A., & Pérez, A. (2017). Melatonin, Gastrointestinal Protection, and Oxidative Stress. In Gastrointestinal Tissue (pp. 317-325). Academic Press.
12. Ferreira, R. A. P., de Sá Ramos, P., Salvador, A. A. A., da Silva, A. A. N., Rossetti, F. X., dos Anjos Tamasia, G., ... & de Brito Bello, S. R. (2019). O efeito do uso da melatonina no tratamento das doenças inflamatórias intestinais: uma revisão integrativa. Research, Society and Development, 8(9), 12.
13. Ma, N., Zhang, J., Reiter, R. J., & Ma, X. (2020). Melatonin mediates

mucosal immune cells, microbial metabolism, and rhythm crosstalk: a therapeutic target to reduce intestinal inflammation. Medicinal research reviews, 40(2), 606-632.
14. DAMASCENO, Débora Cristina *et al*. Efeito do ácido acetilsalicílico na performance reprodutiva e na prole de ratas Wistar. Revista da Associação Médica Brasileira, v. 48, p. 312-316, 2002.
15. Teles Silva, M., Mesquita da Silva, K., Campos Rattes, I., Maria Agostini Zonta, G., Vasques da Costa, A., Galvão Figueredo Costa, R., Karen Cordeiro Nogueira, L., Ogias, D., & Gama, P. (2019). Immediate and Late Effects of Early Weaning on Rat Gastric Cell Differentiation. International Journal of Molecular Sciences, 21(1), 196. Https://doi.org/10.3390/ijms21010196.
16. PRATA LIMA, M. F.; BARACAT, Edmund Chada; SIMÕES, Manuel de Jesus. Effects of melatonin on the ovarian response to pinealectomy or continuous light in female rats: similarity with polycystic ovary syndrome. Brazilian Journal of Medical and Biological Research, v. 37, p. 987-995, 2004.
17. Tenorio, Bruno Mendes *et al*. Can fractal methods applied to video tracking detect the effects of deltamethrin pesticide or mercury on the locomotion behavior of shrimps?. Ecotoxicology and environmental safety, v. 142, p. 243-249, 2017.
18. Tavares, G. A., do Amaral Almeida, L. C., de Souza, J. A., de Farias, V. V., de Souza, F. L., de Andrade Silva, S. C., Lagranha, C. J., Kaeffer, B., & de Souza, S. L. (2020). Early weaning leads to disruption of homeostatic and hedonic eating behaviors and modulates serotonin (5HT) and dopamine (DA) systems in male adult rats. Behavioural Brain Research, 383, 112531. https://doi.org/10.1016/j.bbr.2020.112531
19. Baptista, J. D. S. (2008). Repercussões morfológicas no timo de ratos jovens submetidos à desnutrição proteíca e à renutrição precocemente corrigida (Doctoral dissertation, Universidade de São Paulo).
20. Rosa, a. C. S., Siervo, g. E., Fernandes, g. S. A., & Schneider, l. C. L. (2019). Análise quantitativa das células caliciformes e colágeno tipo ie iii no íleo de ratos expostos a restrição de sono.
21. Ren, W., Wang, P., Yan, J., Liu, G., Zeng, B., Hussain, T., ... & Zhu, G. (2018). Melatonin alleviates weanling stress in mice: involvement of intestinal microbiota. Journal of pineal research, 64(2), e12448.
Brito, M. F. G., Barbosa, A. C. S., Silveira, M. P., & Chopard, R. P. (2017). Morphological and morphometric study of collagen and elastic fibers of the gastroduodenal junction of adult and old Wistar rats. Journal of Morphological Sciences, 30(4), 0-0.
22. Crispel, Y., Shaoul, R., Khamaise, R., Sabo, E., & Hochberg, Z. (2019). Effect of weaning age on the small intestine mucosa of rats. Applied Physiology, Nutrition, and Metabolism, 44(9), 985–989. Https://doi.org/10.1139/apnm-2018-0454.
23. Barbosa, Mércia Maria Bezerra. Avaliação histomorfométrica da mucosa gástrica e intestinal em ratos Wistar submetidos ao desmame precoce. 2019. Dissertação de Mestrado. Universidade Federal de Pernambuco.
24. Rojas Castañeda, J. C., Vigueras Villaseñor, R. M., Vences Mejía, A., & Carmona Mancilla, A. (1995). [The effects of early and late weaning on the development of the small intestine in the rat: a light-microscopic morphometric study]. Revista de Gastroenterologia de Mexico, 60(2), 70–77. Http://www.ncbi.nlm.nih.gov/pubmed/7638535.

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