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TOXICITY IMPACT OF CADMIUM NITRATE ON THE HISTOPATHOLOGICAL ALTERNATION IN STOMACH AND INTESTINE OF FRESHWATER FISH *CHANNA PUNCTATUS* FROM NANDED DISTRICT.

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Abstract: In the present investigation acute toxicity bioassay tests were conducted at various concentrations of Cadmium nitrate ranging from 0.6 to 1.2 mg/l freshwater fish, *Channa Puncatatus* for 24, 48, 72 and 96 h. The acute toxicity values were determined as 0.90, 0.77, 0.57 and 0.52 ppm respectively under laboratory conditions. These LC_{50} values showed that the chemical is highly toxic to the fish. Then the fish were exposed to sub lethal concentrations 96 h LC_{50} 0.52 ppm and studied the significant changes histopathoogical changes in stomach and intestine of freshwater fish *Channa puntatus*. The intestine shows normal structure in control fish but in exposed fish degenerative effect is evident in the mucosal lining and villi of the intestine, rupture of villi at the tip and cracked clay appearance, degeneration of Circular muscle layer swelling in lamina propria were noticed. In the exposed fish stomach shows columnar epithelial cells, degeneration on mucosa, degeneration of villi, Vacuoles in muscular layer, swelling of lamina propria reduction and fusion of stomach microvilli degenerate of the sub mucosal zone. The microvilli lost their normal appearance and become highly folded and vacuole formations, shrinkage of villi were noticed.

Key Word: LC_{50}, Stomach, intestine, *Channa punctatus*, Cadmium nitrate.

INTRODUCTION

Histology is an important tool for determining the action of any toxicant at tissue level, providing data concerning tissue damage (Sprague, 1964). Histopathology deals with the study of pathological changes induced in the microscopical structure of body tissue. Any peculiar alteration of cells may indicate the presence of disease or the effect of toxic substance. Environmental pollution caused by the development of industries, urbanization and informal settlements does though threaten many freshwater ecosystems. Environmental pollution causes a decrease in water quality and afterward affects all living organisms in that system. It is necessary identify and manage these pollution sources, but also to monitor their effects on the aquatic ecosystems. Histological analysis is a one of the technique to assess the sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Datta, 1996). Meyers and Hendricks (1982) studied on the heavy metals substances are harmful to fish at

very low concentration. Hence, in the present investigation to see Histopathological changes due to exposed of Cadmium nitrate on the Stomach and intestine of freshwater fish *Channa punctatus.*

MATERIALS AND METHODS

Fresh water fish *channa punctatus* were collected from Godawari river Nanded and brought to laboratory and similar size of fishes were used for the experiment i.e it measuring about 12 ±2 cm and weighing 14± 2 gms. The fishes were maintain in laboratory by following methods mention in chapter no 1st the acclimatized fishes were separated in to batches A and B each batch ten fishes are taken. "A" batch maintain as a control and "B" batch considered as experimental group the fishes were exposed to lethal concentration of metal Cadmium nitrate (96 hr LC_{50} value) at the end of experiment i.e 96 hr the survival fishes were taken for histopathological examination. They were decapitated and immediately removed tissue like gill, liver, kidney, intestine and stomach from control and experimental fishes. The tissue removed from the test fish was rinsed in 0.90% saline solution for two times for remove blood or debris attached on external surface. The tissues were then cut into small pieces of approximately 3-5mm, keeping in view the orientation of the tissue. In the present study the tissues were fixed in Bouine's fluid. The tissue was kept in fixative for 6-24 hrs depending upon the size of the tissue.(Drury and Wallington, 1980). Dehydration was carried after washes the tissues were directly transferred from xylene to the molten paraffin wax (56-58C) for overnight in oven (Drury and Wallington, 1980). After a making blocks with the help of microtome machine 5 µm thicknesses of sections were selected in the form of ribbons of 10-15cm in length. They were stained with Haematoxylin for 15-20mins. Then stainedthe slide in 1% aqueous Eosin for 1-3mins. In the last slide was dipped into 90% and 100% alcohol for a few minutes. Candabalsam was used on the slide and cover slip was placed. For drying and preservation kept the slides at room temp for 3-4days. After that take microphotographs used the Labomed LX-400 binocular microscope with attached camera ivu 5100 Labomed. All the slides were observed under low and high resolution for their histological findings.

RESULTS

In the present investigation, histopatholigocal studies of Intestine and stomach of *Channa punctatus* were done in fishes exposed to 96 hours Lc_{50} of cadmium nitrate. The histological changes were noticed in the intestine and stomach. The histological changes in the tissues of exposed fishes were compared with that of the control fish.

Histologically stomach is important organ for the storage of food, stomach assumes different shapes according to the availability of space in the body cavities of different fishes. In the present study the control stomach shows gastric pits, mucosa, muscularis, submucosa and gastric glands were observed in (Fig. no. 1A). In the exposed fish stomach shows columnar epithelial cells, degeneration on mucosa, degeneration of villi, Vacuoles in muscular layer, swelling of lamina propria reduction and fusion of stomach microvilli degenerate of the sub mucosal zone. The microvilli lost their normal appearance and become highly folded and vacuole formations, shrinkage of villi were noticed (Fig. no. 1B).

In the present investigation the control intestine shows columnar epithelium, circular muscle layer, lamina propria, Serosa, Submucosa and longitudinal muscle layer were observed (Fig. no. 2 A). But in exposed fish degenerative effect is marked in the mucosal lining and villi of the intestine. The villi is to

become flattened, sloughing off the mucosal lining, Hypertrophy of epithelial cells swelling, fusion of villi due to extensive hypertrophies leading to rupture of villi at the tip and cracked clay appearance, degeneration of Circular muscle layer swelling in lamina propria hypertrophied epithelial cell and Swelling of Lamina propria were noticed (Fig. no. 2 B).

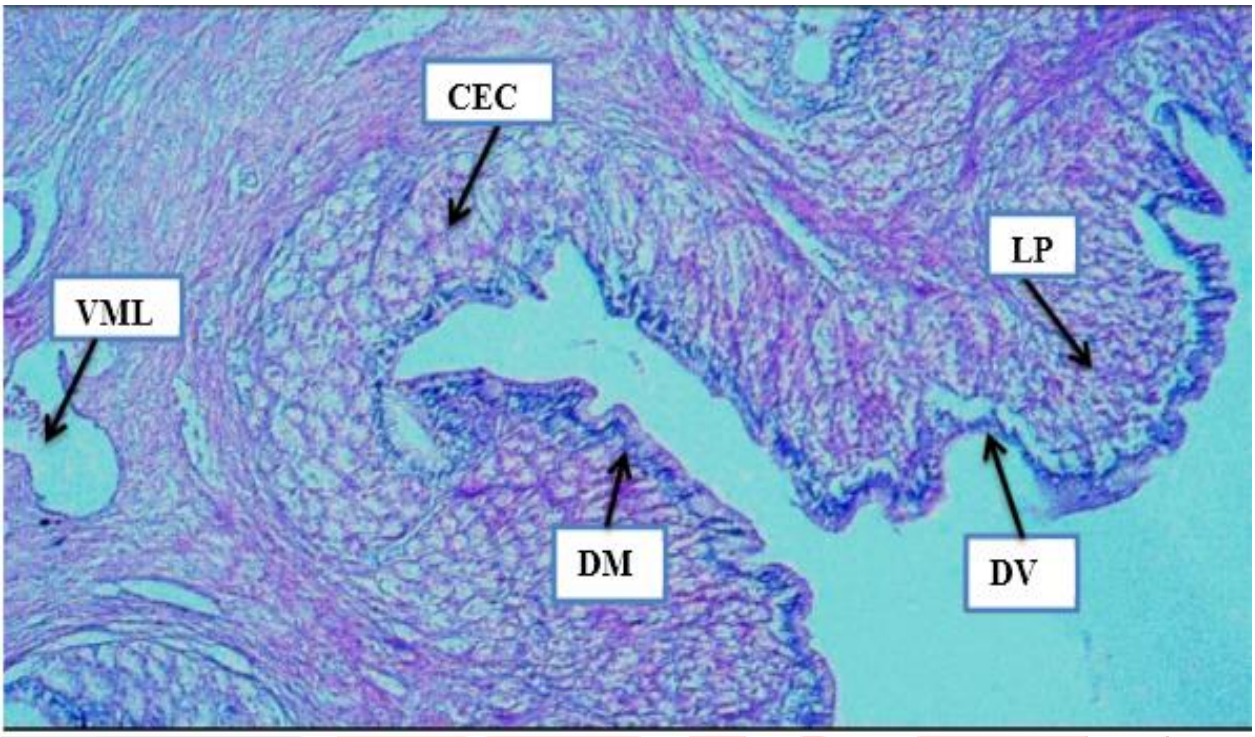

Fig.1.(A) Photomicrograph of *Channa punctatus* control stomach shows Gastric pits (GP), Mucosa (M), Muscularis (ML), Submucosa (SM) and Gastric glands (GG)Control stomach H/E- 100X.

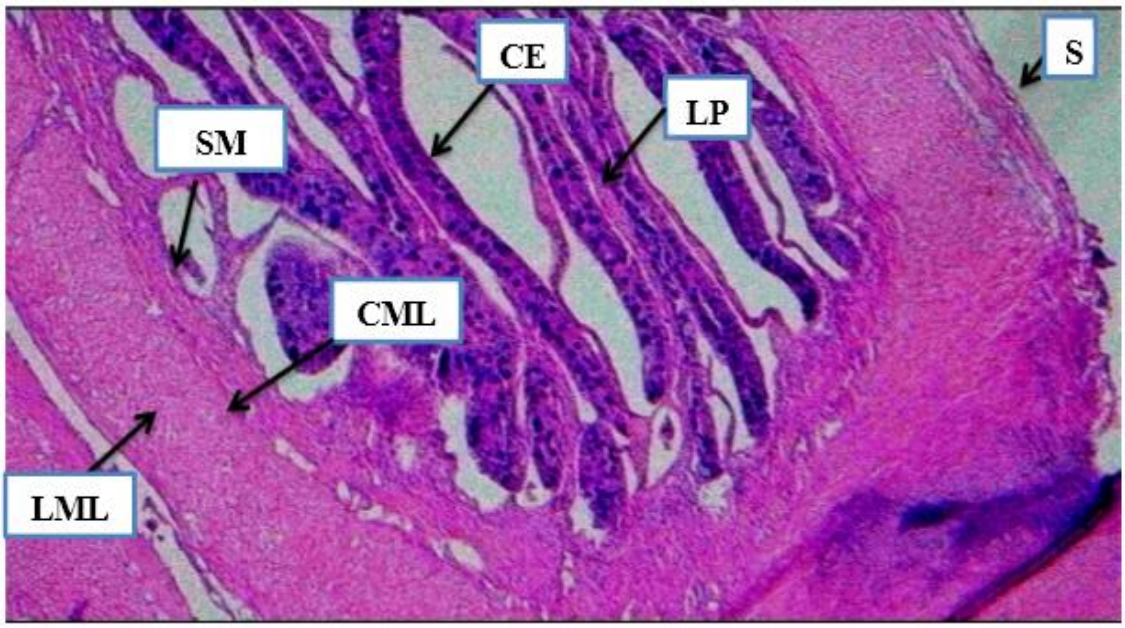

Fig.1.(B) Photomicrograph of *Channa punctatus* stomach after exposed to cadmium nitrate at 96 hours shows columnar epithelial cells (CEC), Degeneration on Mucosa (DM), Degeneration of Villi (DV), Vacuoles in Muscular Layer (VML) and Swelling of lamina propria (LP).

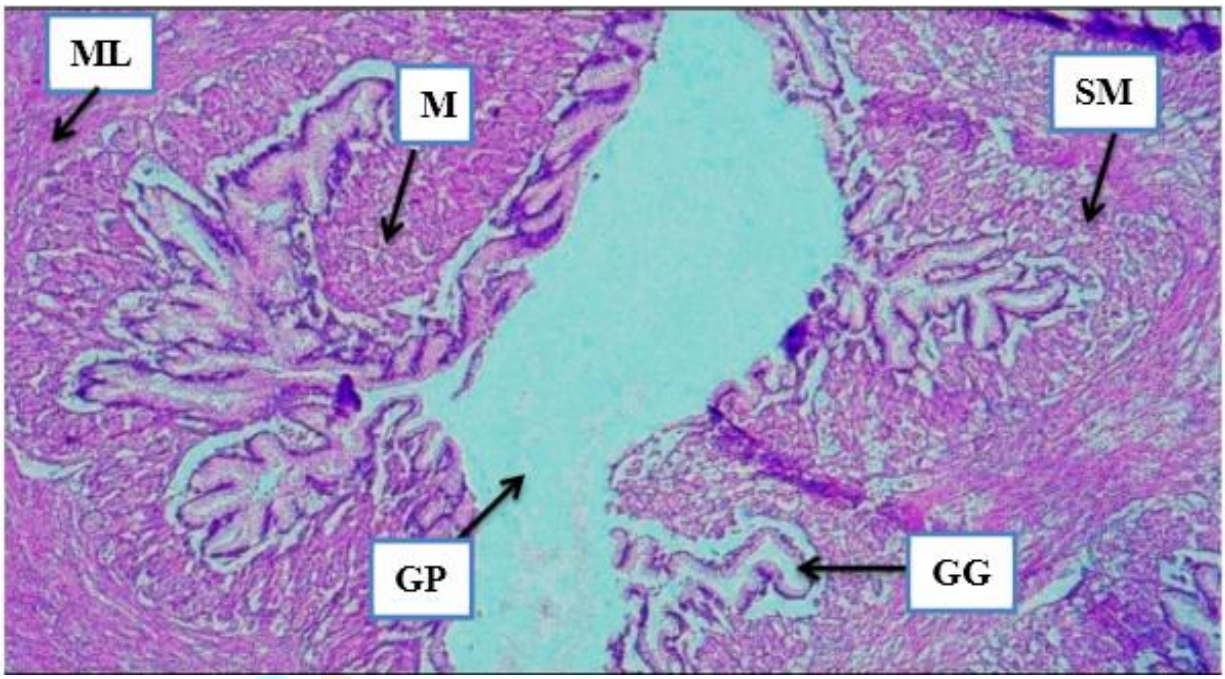

Fig.2(A) Photomicrograph of *Channa punctatus* control intestine shows Columnar epithelium (CE), Circular muscle Layer(CML), Lamina propria (LP), Serosa (S), Submucosa (SM) and Longitudinal muscle layer (LML) Control Intestine H/E- 100X.

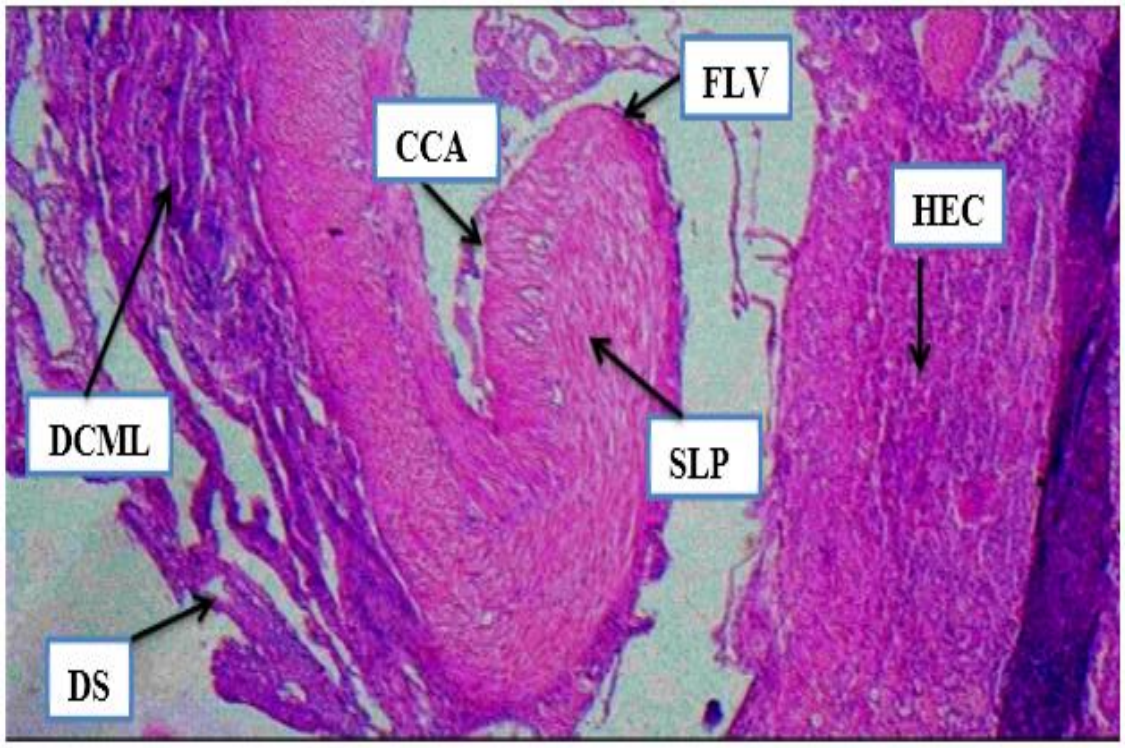

Fig 2.(B) Photomicrograph of *Channa punctatus* intestine after exposed to cadmium nitrate at 96 hours shows Cracked clay appearance(CCA), Degeneration of Circular muscle layer (DCML), Flattened villi (FLV), Swelling in lamina propria (SLP), Degeneration of Serosa (DS), Hypertrophied epithelial cell (HEC) and Swelling of Lamina propria(SLP) H/E- 100X.

DISCUSSION

Senapati ,*et. al.* (2013) reported that exposure of Almix 20WP herbicide to the stomach of *anabas testudineus* (cuvier) and showed the degeneration and vacuolation in the basal region of the gastric epithelium, columnar epithelial cells were degenerated in some areas and damage of gastric gland. The similar finding noted on investigation the stomach of *Channa punctatus* exposed to the cadmium nitrate for 96 hrs LC_{50}. Amminikutty and Rage (1977) working on stomach of *Gymnocorymbus ternitzi* after chronic

exposure of endosulfan and methyl ethyl mercurial and reported that the swelling, distortion and vacuolation with a tendency to necrotization in the mucosal epithelial cells of stomach. Ghanbahadur and Ghanbahadur (2012) reported vacuolization in the submucosa, shrinkage of mucosal folds in the stomach of larvivorous fish *Rasboradaniconius*due the toxic effect of endosulfan. Saeeda Fatima, *et.al.* (2014) studies on effect of hydrolyzed leather shavings to the fish rohu (*Labeo rohita*) fingerlings and reported that pyknosis, necrosis, disruption in the outer lining of longitudinal muscles, degeneration and development of fibrosis, degeneration of epithelial lining, gaps appeared between the gastric glands and vacuolization was also apparent in the gastric glands epithelium in the stomach.

Joycelyn C. Jumawan (2015) studies on histopathological effects of sub-acute lead chloride on the stomach of catfish *Pterygoplichthyspardalis*and observed that the stomach wall and destruction of the mucosa, respiratory cells exhibited pyknosis while a portion of granular cells appears necrotic. He stated that there was also a collapse of the epithelial lining of the stomach in some samples. Chhaya Bhatnagar, *et.al.* (2007) Studied on fluoride-induced histopathological changes in intestine of fresh water teleost, *labeorohita* and reported that degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to become flattened, and there is sloughing off of the mucosal lining.

Juan B. Ortiz, *et.al.*(2003) histopathological changes induced by lindane (y YCH) in organs of fishes and reported in exposed group a degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to become flattened, and there is sloughing off of the mucosal lining. Flattening of microvilli and a cracked clay appearance of the tissue are likewise apparent. Shanta Satyanarayan, *et.al.* (2012) studied on histopathological changes due to some chlorinated hydrocarbon pesticides to *cyprinus carpio* and reported that the intestine the flattening of intestinal folds, fusion with each other, shrinkage of cells and acute epithelial necrosis, more flattening of intestinal folds. In 30 days exposed tissue the intestinal folds were completely flattened thereby reducing the surface area, necrosis was also very defined, vacuolation and acute necrosis were observed.

Muley D. V.,*et.al. (*1996) worked on the intestine of *Channa punctatus* after the exposure to carbofuran and reported that degenerative changes and rupture in tip of villi, loss of structural integrity of mucosal folds and degeneration & necrosis of submucosa in are found in literature while working on endosulfan toxicity in the freshwater fish *Tilapia mossambica.* Sastry and Sharma S. K. (1979) Report on vacuolization in submucosa& circular muscles and dilation of columnar & goblet cells of mucosal folds while working on toxic effects of endrin on liver and kidney of a freshwater fish. Srivastava A. K. &Srivastava A. K. (1995) studies on histopathological changes in the liver associated with selenium exposure in the freshwater Indian catfish *Heteropneustes fossilis* and reported that the destruction of columnar epithelium, submucosa fused with muscles and serosa was found in broken condition after 10 days exposure to malathion. Ravanaiah and Narasimha Murthy (2010) studied on *Tilapia mossambica*exposed to industrial pollutants and reported that the vacuolizations, damage of villi and serosa layer, necrosed mucous epithelium, congested blood capillaries and hyperactivity of mucous cells in fish. Lokhande and Bais (2012) studies on effect of cadmium chloride n the freshwater fish *Ophiocephalus striatus* and observed that the intestine of fish degenerative changes in the tips of villi like hydropic degeneration, cloudy swelling and

necorosis and stated that changes is possibly due to the fulfillment of extra energy requirement under the toxicity of Cadmium Chloride

Ghanbahadur and Ghanbahadur (2012) studied on endosulfan toxicity *Rasbora daniconius* and reported that the destruction of mucosa and particularly the columnar epithelial cells in the intestine. Damage of brush border on the luminal surface of the intestinal villi could reduce the ability of absorption of various macromolecules from the intestinal lumen to tissue interior. Furthermore, the disruption of blood vessels in the submucosa might impair its ability of absorption (Ghosh, 1990). Velmurugan,*et. al*. (2007) when exposed *Cirrhinus mrigala* to sub lethal concentrations (0.3ppb and 0.6ppb) of lambda cyhalothrin (a synthetic pyrethroid pesticide) observed intestinal lesions, infiltration of eosinophils into lamina porpia and atrophy of epithelial cells. Flattening and cracked clay appearance was observed by Bhatnagar, *et. al.* (2007) after chronic exposure for 30, 60, 90 and 120 days of fingerlings of *Labeo rohita* to 15mg NaF/L.

Joycelyn C. Jumawan (2015)studies on histopathological effects of sub-acute lead chloride on the vital organs of the sucker mouth sailfin catfish *Pterygoplichthyspardalis*and observed that the small intestine of treated samples show the distinct occurrence of pyknotic epithelial cells in the intestinal mucosa. Sastry, K.V. and Gupta, P.K. (1978) reported that the heavy metal, lead, hypersecretion of pepsin, leading to the degradation of tissue protein and increased ammonia and urea excretion in freshwater fish *Channa punctutus.* Bais and Lokhande (2012) studied on cadmium toxicity and observed that the degenerative changes in the tips of villi like hydropic degeneration, cloudy swelling and necrosis of intestine of *Ophiocephalusstriatus*. Monica K. Kale (2015) working on admium chloride exposed to 110ppm induced histopathological changes in the intestine of *Rasbora- daniconius* for 24h and showed that the mucosal disruption at the tips of folds, appearance of gaps between mucosal and sub mucosal layer, intracellular vacuolation in the submucosa, columnar epithelium lining was destructed, absorptive cells and the mucous secreting cells were damaged, the free striated border was ruptured. Histopathological findings of intestine of some fish recorded degenerative and necrotic area of some lining mucosal epithelium, sloughing of the epithelium lining of tip of some villi by Amany Mohammed Jasim(2015).

CONCLUSION

In the present study intestine shows normal structure in control fish but in exposed fish degenerative effect is evident in the mucosal lining and villi of the intestine. The villi is to become flattened, sloughing off the mucosal lining, Hypertrophy of epithelial cells swelling, fusion of villi due to extensive hypertrophies leading to rupture of villi at the tip and cracked clay appearance, degeneration of Circular muscle layer swelling in lamina propria hypertrophied epithelial cell and swelling of Lamina propria were noticed. In the exposed fish stomach shows columnar epithelial cells, degeneration on mucosa, degeneration of villi, Vacuoles in muscular layer, swelling of lamina propria reduction and fusion of stomach microvilli degenerate of the sub mucosal zone. The microvilli lost their normal appearance and become highly folded and vacuole formations, shrinkage of villi were noticed.

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