

Histological Observations on the Proventriculus and Duodenum of African Ostrich (*Struthio Camelus*) in Relation to Dietary Vitamin A.

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Abstract: Research problem: The fine structure of the gut in different avian species in relation to dietary vitamins status have widely been studied with the exception of ostriches. **Objectives:** To study the histological structure of the African ostrich proventriculus and duodenum in relation to two levels of vitamin A (7500 and 9000) IU/kg diet using light microscope. **Methods:** Twenty male African ostriches with average age 65-67 weeks and apparently healthy were used. They were divided into two equal groups, the first one received diet adjusted to supply 7500 IU/kg diet vitamin A. The second group fed diet formulated to furnish 9000 IU/Kg diet vitamin A. Both diets were isonitrogenous and isocaloric. Body weight gain, feed intake and feed conversion rate were calculated. At the end of four weeks, pieces from the different parts of proventriculus and duodenum were taken for light microscopic examinations. **Result:** Body weight gain and feed conversion rate were improved in group received 9000IU of vitamin A comparable to group fed 7500IU. Histological structure of proventriculus of birds receiving 7500 IU/Kg vitamin A showing vascular congestion, thinning connective tissue and sloughing of the columnar epithelia lining the central collecting duct. The duodenum sinus showing shrinking of muscular layer with bleeding in the core layer and widening of the blood vessels in the outer layer. **Conclusion:** microscopic structure of proventriculus and duodenum indicated that there was a tendency for improvement in histomorphometry, as the level of vitamin A supplement increased from 7500IU to 9000IU. Except for few abnormality recorded in group fed high level of vitamin A, we suggested that 9000IU used in the present study was not yet enough to cover African ostriches requirement and excess vitamin is needed for that species.

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Key words: Histology, Proventriculus, Duodenum, African Ostrich, *Struthio Camelus*, Vitamin A.

1. Introduction

Ostrich, *Camelius struthious* is the sole species of the family Struthionidae and is the largest living bird. There is increasing demand for ostrich meat and hides, worldwide. Between 1996 and 2001, there was a six-fold increase in the consumption of ostrich meat. The rising demand is attributed to the low content of energy, total lipids, cholesterol and saturated fat, and the high content of protein and iron relative to that of beef, veal, pork, lamb, poultry, rabbit and horse meat (Fasone and Adamo, 2001). Recently, research has been conducted to define the nutritional requirements of this species, but there is still a lack of information. . Some researchers have incorrectly assumed that poultry diets are useful for ostriches(Miao *et al.*, 2003 and Jji, 2005) but the vitamin and mineral requirements of these birds are unique and their diets should never be substituted with poultry or other livestock feeds (Cooper and Horbanczuk, 2004).

In the speciality literatures regarding the microstructure of the duodenum in birds are relatively rare and refer, especially to the *Gallus*

domesticus species. Transposing the histological data from a species to another appears to be inadequate, being known that the structure and function of some organs presents particularities of species (King and Lelland, 1984). Recently, authors study the histology of many organs of ostrich, oesophagus (Attia *et al.*, 2011) tongue (Guimarães *et al.*, 2009) oviduct (Saber *et al.*, 2009) but little is known about ostrich gut. The avian stomach is formed of two distinct parts; the glandular or true stomach proventriculus and the muscular portion or gizzard. The proventriculus secretes HCL and pepsin which are needed for protein digestion. Then duodenum is connected with the gizzard at gizzard- duodenal junction (Maya and Lucy, 2000).

A study by (Iji *et al.*, 2003) concluded that unlike broiler chickens, the mucosa at the duodenum is relatively smooth, suggesting the presence of shorter villi in this region of the ostrich's gut. However, (Iji *et al.*, 2001) found that the protein content of the duodenal mucosa was greater than that of the jejunum or ileum at three days of age but not at subsequent periods of assessment.

Wang *et al.* (2007) compare between the lining epithelium of chicken and ostrich, and observed certain differences were observed. The superficial proventricular glands were simple, branched tubular glands, while the deep proventricular glands were restricted to a slipper-shaped area and extended into the muscularis mucosae. The gizzard had a variably developed muscularis mucosae, a feature that seems to be unique to the ostrich. The villi of the small intestine were long and branched profusely, forming a labyrinthine surface. No Paneth cells were observed. The mucosa of the ceca and the first part of the rectum was thrown in large circular folds, forming a compressed spiral. Numerous melanocytes were seen in the sub mucosa and the connective tissue around the blood vessels of the muscle layers at the tips of the ceca. A well developed subserosa was present throughout the gastro-intestinal tract. Proventriculus and duodenum were of particular interest for histomorphological study in the current work because the literature regarding the microstructure of the duodenum in birds are relatively rare and refer, especially to the *Gallus domesticus* species (Predoi *et al.*, 2008).

Vitamin A is involved in the growth and repair of epithelial cells. Its deficiency might affect the functioning of digestive system, disrupting the mucosal surfaces and potentially preventing proper nutrient absorption. The health of the digestive tract lining is important in protecting against ulcers by safeguarding surrounding tissues against corrosive effects of digestive acids and abrasion and bacteria (Tremblay, 2011). The loss of epithelial cells leads to problems in digestion and absorbing of nutrients (Montagne *et al.*, 2004). Abnormal intake of dietary vitamin A causes keratinization and drying of the epithelial in the gastrointestinal tract, respiratory tract and ocular surface (Tei *et al.*, 2000). Vitamin A deficiency in experimental animals leads to depression of synthesis in the intestinal mucosa of glycoproteins, activation of the enzymes of their catabolism, a decrease in the number of goblet cells and lengthening of the cell cycle of the epithelium of the crypts (Troen *et al.*, 1999). Deficiency of vitamin A produces a loss of normal stimuli for cellular growth and differentiation. Thus vitamin A supplementation to be monitored on the gastrointestinal tract (McCullough *et al.*, 1999).

There is conflict in vitamin A requirement of ostrich, 5000 IU/kg diet depending on turkey requirement (NRC 1984), 7500 IU/kg diet (Farrell *et al.*, 2000), 8000 IU/kg diet (Ullrey and Allen, 1996 and Merck Sharp & Dohme Corp., USA, 2011) and 9000IU/kg diet (Miao *et al.*, 2003 and Aganga *et al.*, 2003).

Although, the histological structure of the digestive tract in different avian species has widely been studied by many authors, the available literatures on the histomorphology of the of ostrich gut are scanty. Beside vitamin A requirement of ostrich is conflicted: 5000 IU, 7500 IU, 8000 IU and 9000IU as recommended in many literatures. The present work was target to study the effect of two levels of vitamin A (7500 IU and 9000IU) depending upon histomorphology observations of the African ostrich proventriculus and duodenum in relation to their dietary vitamin A status using light microscope. Hoping that, this paper will contribute with further researches in future to assist nutritionists to ensure profitable production by formulating diets that are scientifically and economically.

2. Material & methods

Birds and feeding: Twenty male African ostriches with average age 65±2 weeks were used in the current study. They were reared in Tabuk farm (north western Saudi Arabia) during autumn season 2011. They were divided into two equal groups ten per each: The first group received formulated diet which adjusted to contain 7500 IU/kg diet vitamin A. The second group fed diet formulated to furnish 9000 IU/Kg diet vitamin A (Table 1). Dietary vitamin A content was controlled by manipulation of mineral mixture composition used in diet formation. The experimental diets were isonitrogenous and isocaloric, consisted of 1- protein mixture (ground yellow corn, wheat, gelatin, eggshell, dicalcium phosphate and amino acids) 2- minerals mixture (Ca, P, Mn, Zn, and Cu) and 3- vitamins mixture (A, D3, E, B1, B2, B6, and B12). The experiment was conducted for four weeks in which water and feed were provided *ad libitum*. All the birds were maintained in a heated room with slatted plastic flooring.

Body weight, feed intake and feed conversion rate: Ostriches were weighed at the beginning and at the end of experiment, body gain; feed intake and feed conversion rate were calculated.

Tissue Sampling: At the end of experiment, ostriches from each group were slaughtered, the abdomen was cut open, proventriculus and the entire small intestine, from the pylorus to the ileo-cecal sphincter were removed. Samples taken from the proventriculus and duodenum (approximately 4 cm) were treated for histological examination using both light and electronic microscope.

Light microscopy examination: The samples were gently flushed with 0.85% normal saline to remove the intestinal content, and post fixed for more than 24 h with the same fixative solution (4%

paraformaldehyde PBS). Samples were dehydrated, cleared, and embedded in paraffin. Serial sections (5 μ m) were cut on a Leica microtome (Nussloch GmbH, Nussloch, Germany), mounted on slides, and stained as following: 1- Hematoxylin and eosin to study the general morphology of cells. 2- Crossmon trichrome to study collagen fibers and connective tissue (Crossmon, 1937). 3- Periodic acid Schiff technique (PAS) dye to study mucin (American Forces Institute of Pathology, 1992). The slides were deparaffinized, rehydrated, incubated with 5 g/L of periodic acid solution for 15 min, washed, and finally incubated with Schiff's reagent (1 g of basic fuchsin, 200 mL of distilled water, 20 mL of 1 mol/L of HCl, 6 g of sodium pyrosulfite) for 30 min. The sections were then photographed under a Nikon microscope (Nikon Corp., Tokyo, Japan).

Statistical Analysis:

The data were analyzed using SPSS program version 16. The analysis of covariance (one way ANOVA) was used to detect the differences in the means between the control and treated group

Table (1) Composition of diets of experimental African ostrich .

Diet Groups	Low vitamin A	High vitamin A
Protein %	14	14
ME/kg diet	2300	2300
*Vitamin A IU	7500	9000

* Dietary vitamin A content was controlled by manipulation of mineral mixture composition used in diet formation.

Table (2) Overall performance of African ostriches during experiment.

Parameters	Low vitamin A group	High vitamin A group
Initial body weight/ kg	92.0 \pm 2.2	^a 91.0 \pm 2.0
Final body weight /kg	97.7 \pm 2.0	^a 103.0 \pm 1.9
Body gain / kg	5.7	12
Daily gain /g	190	400
Daily feed /kg	2.5	3.6
Feed conversion rate	13.15	9

Same letters in the same column are significant different.

3.Results

Body weight gain and feed conversion rate were improved in group received 9000IU of vitamin A comparable to group fed 7500IU of vitamin A (Table 2).

Histological structure of proventriculus of male African ostriches receiving diet furnished 7500 IU/Kg vitamin A (Fig.1) showing vascular congestion and thinning of the connective tissue that form barriers between the lobules (A). Sloughing of the columnar epithelia lining the central collecting sinus (B). The cells lining the follicles of glandular stomach showing many vacuoles in the lower portion of the cytoplasm. There was also severe corrosion in the side membrane of these cells resulting in increased appearance and more invagination of the cells in the direction of the lumen. The nuclei of these cells appeared regular and spherical with clear nucleolus (C). The apical part of cells lining the folds of gland was sloughed with the emergence of many cavities in the cytoplasm of the upper part (D). Widening of the central collecting sinus of gastric gland and sloughing of the columnar cells were observed (E). It appeared that the central collecting sinus opened directly into the core layer with graduated mucous secretions to the outside in the gastric lumen, in addition to the erosion and sloughing of the cells lining the tubules (T).

Histological structure of proventriculus of male African ostriches receiving diet contain 9000 IU/kg vitamin A showed a communication between the lining epithelium of the central collecting sinus of gastric gland and lining tissue of the folds where these glands open. There was also, increase in the thickness of the outer muscular layer which contains blood vessels, nerves and lymphatic vessels (Fig.2).

Histological structure of the cells lining the duodenum of male African ostriches receiving diet furnished 7500 IU/Kg vitamin A (Fig.3) presented a contraction of mucous muscle which appeared as thin packages separated from the shrinking muscle layer (A). Bleeding in the core layer and widening of the blood vessels in the outer layer was noticed (B). The goblet cells in the Crypts of Leiberkein were numerous and interact positively with blue stain (C). There were many invaginations and lymphocytes in tissue lining the villi with many cracks in the connective tissue and core layers (D).

Histological structure of the cells lining the duodenum of male African ostriches receiving diet contain 9000 IU/kg vitamin A (Fig.4) showing the emergence of some leucocytes and goblet cells between chief cells (A). The goblet cells interact positively with blue dye (B). Connective tissue was appeared in the middle of villi with scattered white milky, smooth muscle, and lymphocytes (C). Also, short villi of small intestine widen in their base. One to two Crypts of Leiberkein from them (D) open between them.

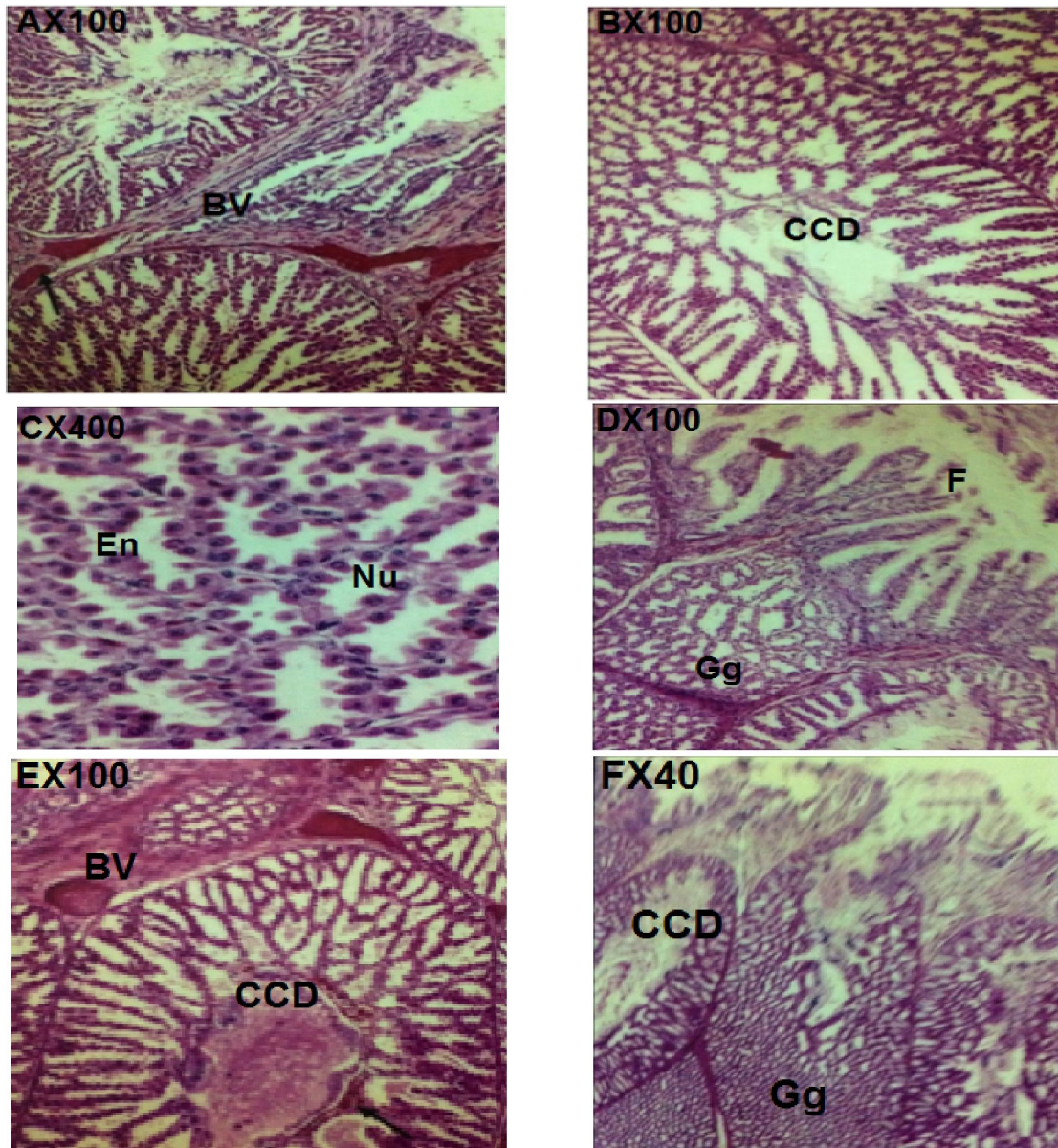


Fig.(1): Histological structure of proventriculus of male African Ostriches receiving vitamin A 7500IU/kg diet showing vascular congestion (BV) and thinning of the connective tissue that form the barriers between the lobules (A). Observed sloughing of the columnar epithelia lining of central collecting sinus (CCS), (B). The cells lining the tubules of glandular stomach showing many vacuoles in the lower portion of the cytoplasm. Severe corrosion in the side membrane of these cells resulting in increased appearance more envagination (En) of the cells in the direction of the lumen. The nuclei (Nu) of these cells appeared regular and spherical with clear nucleolus (C). The apical part of cells lining the folds of gland (F) was sloughed with the emergence of many cavities in the cytoplasm of the upper part of these cells (D). Widening of the central collecting sinus (CCS) of gastric gland (Gg) with sloughing of their columnar epithelium (E). It appeared that the central collecting sinus (CCS) opened directly into the core layer with graduated mucous secretions to the outside in the gastric lumen together with erosion and sloughing of the cells lining the of tubules (T).

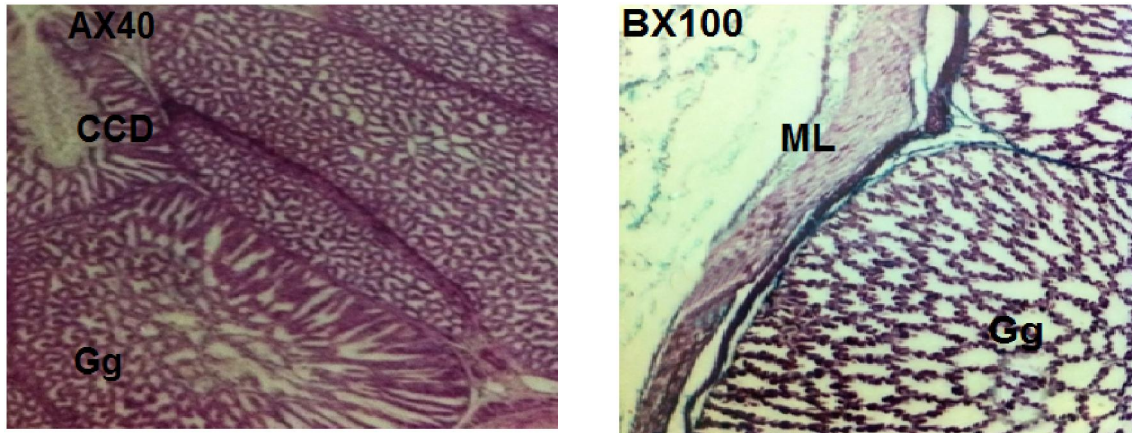


Fig.(2): Histological structure of proventriculus of male African ostriches receiving vitamin A 9000IU/kg diet showing a communication between the lining epithelium of the central collecting duct (CCD) of gastric gland (Gg) and lining tissue of the folds where these glands open (A). An increase in the thickness of the outer muscular layer (ML) with blood vessels, and nerves (B).

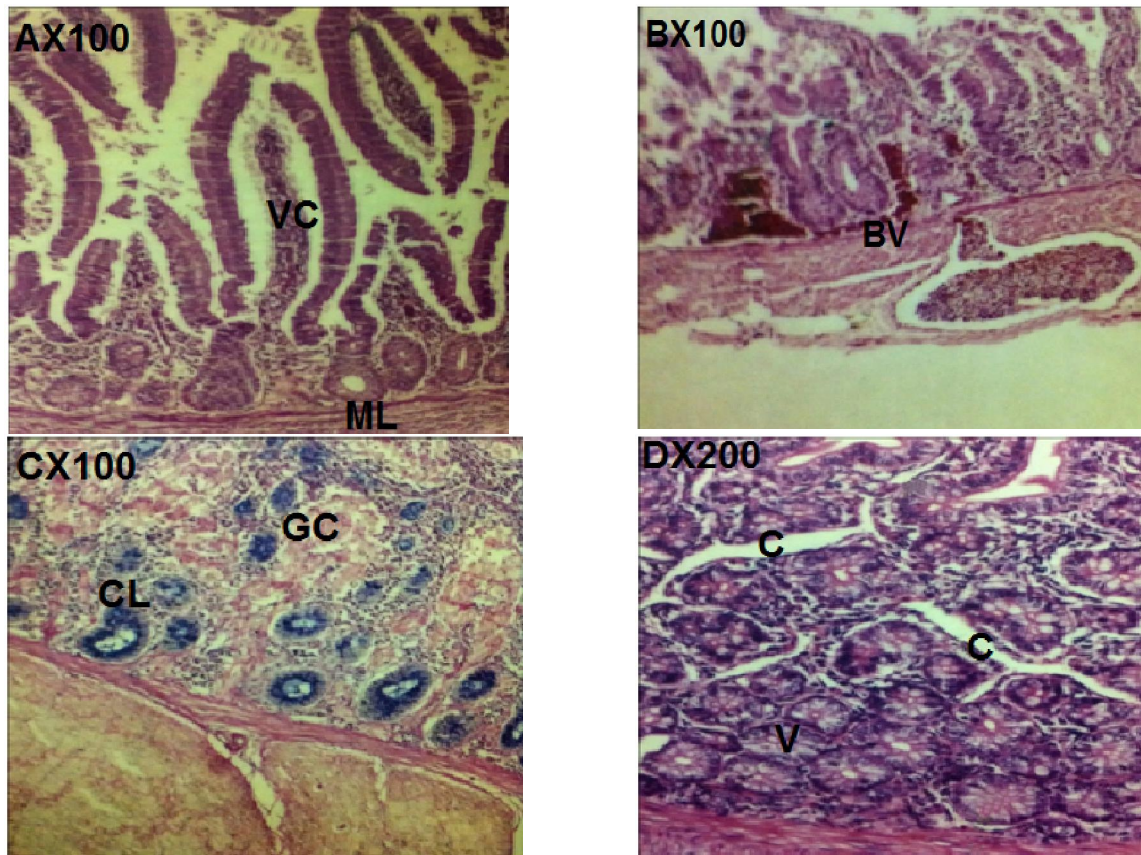


Fig.(3): Histological structure of the cells lining the duodenum of male African ostriches receiving vitamin A 7500IU/kg diet showing contraction of mucous muscle which appeared in the form of thin packages separated from the muscle layer. Shrinking of muscular layer (A). Bleeding in the core layer and the widening of the blood vessels (BV) in the outer layer (B). Increase the number of goblet cells (GC) in the Crypts of Leiberkein (CL) which interact positively with blue stain (C). Many invaginations and lymphocytes in tissue lining the villi (V) with many cracks (C) in the connective tissue and core layers (D)

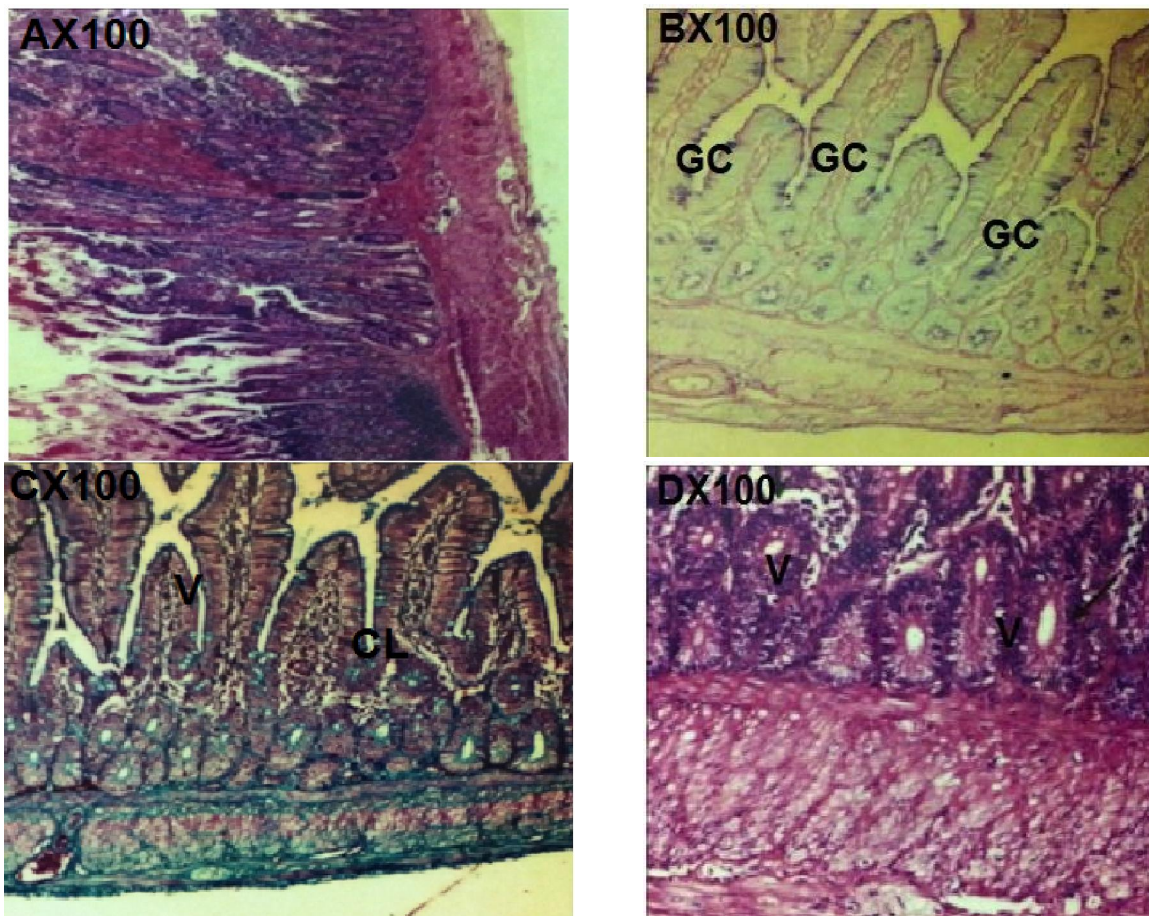


Fig.(4): Histological structure of the cells lining the duodenum of male African ostriches receiving vitamin A 9000IU/kg diet showing some white cells (leucocytes) and goblet cells between chief cells (A). The goblet cells (GC) interact positively with blue dye (B). Connective tissue was appeared in the middle of villi (V) with scattered white milky, smooth muscle, and lymphocytes (C). Short villi of small intestine widen in their base. One to two Crypts of Leiberkein (CL) open between them (D).

4. Discussion

Vitamin A requirement of ostrich is conflicted, 5000 IU depending on turkey recommendations (NRC 1984), 7500 IU (Farrell *et al.*, 2000), 8000 IU (Ullrey and Allen, 1996 and Merck Sharp & Dohme, USA, 2011), and 9000IU (Aganga *et al.*, 2003 and Miao *et al.*, 2003). No studies have compared between these different levels. In the current study, we try to compare between two levels depending upon histomorphology observations of proventriculus and duodenum using light microscope. Since alterations in the epithelial lining of vital organs occur early deficiency (McCullough *et al.*, 1999).

Body weight gain and feed conversion rate were improved in group received 9000IU of vitamin A comparable to group fed 7500IU (Table 2). Reduction of ostrich body weight may be due to impaired nutrient utilization as has been previously suggested by Zaiger *et al.* (2004). Lost epithelial cells leads to

problems in digestion and absorbing of nutrients (Montagne *et al.*, 2004).

Histological structure of proventriculus of male African ostriches receiving diets containing 7500 IU/kg and 9000 IU/Kg vitamin A showed that low level of vitamin A lead to vascular congestion, thinning of the connective tissue and sloughing of the columnar epithelia lining of central collecting sinus. In addition, severe corrosion in the side membrane of cells was observed. Mucous secretions appear outside in the gastric lumen with erosion and sloughing of the cells lining the tubules. These observations indicated that 7500 IU/kg of vitamin A is not enough to support epithelial integrity of ostrich proventriculus. Vitamin A is responsible for healthy mucosa in ostrich (Kwon *et al.*, 2004) and in infant (McCullough *et al.*, 1999). Cortes *et al.* (2006) found that vitamin A deficiency in turkey poults lead to squamous metaplasia of the mucosal epithelium of the oral mucosa, oesophagus,

sinuses, nasal glands, bronchi, proventriculus, and the bursa of Fabricius.

Histological structure of duodenum of male African ostriches receiving diets containing 7500 IU/kg vitamin A showed many changes in the lining epithelium, appearance of mucous as thin packages, shrinkage of muscular layer and bleeding in the core layer. Mucin serves a crucial role in protecting the gut from acidic chyme, digestive enzymes and pathogens. It is also involved in filtering nutrients in the gastrointestinal tract (GIT) and can influence nutrient digestion and absorption (Montagne, 2004). The invaginations and increased lymphocytes in tissue lining the villi indicated inflammation. Numerous goblet cells in the crypts of Lieberkühn and many cracks in the connective tissue of villi were recognized. The villi in group fed 9000IU vitamin A appeared short and intact. Moghaddam *et al.* (2010) concluded that villi of jejunum were improved in broiler chickens by increasing dietary vitamin A.

The obtained findings confirm the work of Moghaddam (2010). Experimental vitamin A deficiency in turkeys is characterized by squamous metaplasia in the esophagus, oropharynx, paraocular glands, sinuses, proventriculus, and bursa of Fabricius (Cortes *et al.*, 2006).

General observations of the proventriculus and duodenum obtained from either light or transmission electron microscope indicated that there was a tendency for improvement the histomorphometry as the level of vitamin A supplement increased from 7500IU to 9000IU. Vitamin A has an important role in the integrity and functionality of the proventriculus and intestine of ostriches similarly as reported by Tremblay (2011). Except for few abnormalities of lining epithelium of proventriculus and duodenum recorded in group fed high level of vitamin A suggested that 9000IU used in the present study not completely enough to cover African ostriches requirement and excess vitamin is needed for that species so further studies should be done for that concept.

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