Histological Assessment of the Glandular Stomach in Cockatiels (Nymphicus hollandicus) Fed Different Dietary Regimens

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***Abstract*—** The purpose of the present study was to evaluate the effects of different dietary regimens on potential histological alterations of proventricular tissue in the adult cockatiel (Nymphicus hollandicus). Eighteen clinically healthy birds (6-8 months old) with body weights between 70-100 g were randomly divided into three groups (n=6). Group 1 (G1) was fed fruits and vegetables, Group 2 (G2) was fed abalanced diet (grains, fruits and vegetables) and Group 3 (G3) was fed sunflower seeds only. The animals were kept for two months under controlled environmental conditions (24-28 °C; 10-12 h light/day) with free access to feed and water. All birds were clinically healthy throughout the experimental period, and no mortality or abnormal behavior was recorded.

The birds were euthanized and placed in dorsal recumbency and the proventriculus was carefully excised, fixed in 10% neutral buffered formalin and processed for histological examination using standard techniques.Then, tissue sections were stained with Harris hematoxylin and eosin (H&E) and Periodic Acid–Schiff (PAS) stain also Masson's trichrome stain. The most important observations indicated a structural difference in the proventriculus among the groups, depending on the composition of the diet, with the fruits and vegetables group presenting a relatively thin muscle layer, small glandular diameter and fine septa between glands. In the balanced diet group, moderate development of the muscularis externa, diameter of the glands as well as thickness of the septa was observed, while in the sunflower seed group, increased thickness of the muscularis externa, enlarged glandular structures and widened septa were recorded. The morphometric measurements supported these findings, with significant differences in proventricular gland diameter and septal thickness between these groups. In conclusion, the findings suggest that the dietary composition is the main element affecting the metabolic activity and the structural integrity of the visceral organs, and is associated with visible structural variation in the proventriculus as a glandular stomach. Furthermore, these findings emphasize the significance of balanced nutrition for the normal physiological status and suggest that unbalanced diets may predispose birds to structural and functional modifications.

Keywords **—** Histology, Glandular stomach, Proventriculus, Cockatiels, Different Dietary Regimens

INTRODUCTION

The human-animal relationship has gained increasing scientific attention because of its important psychological, social and physiological benefits. Companion animals have been shown to reduce feelings of loneliness, alleviate depression and improve quality of life, especially for those in institutional settings. In addition, animal-assisted interactions improve social behavior and emotional well-being and are thus an important part of supportive care programs (1). Birds are an important group of companion animals that are used to provide emotional consolation and companionship. The ownership of birds has been demonstrated to be helpful for psychological and physical well-being, even resulting in decreased blood pressure and depression among pet owners . Scientific investigations have been based on restricted samples, due to methodological constraints (2,3,4).

The cockatiel (Nymphicus hollandicus) is a small Australian bird of the psittacine family Psittacidae and subfamily Cacatuinae. Budgerigars are recognized for their gentle disposition, flexibility and social behavior, making them one of the most popular companion birds in the world.They are native to open forests, grasslands and urban areas and may survive much longer in captivity with better nutrition and care techniques (5,6,7,8). In the wild, cockatiels are granivorous birds, consuming mostly seeds, fruit, berries and the occasional small insect . Their foraging behaviour has an ecological significance in seed distribution and ecosystem stability. For their health, proper management in captivity is particularly necessary because the environment and diet are of tremendous relevance for their longevity and physiological status (9).

Birds have no teeth and their digestive system is incredibly adapted to compensate for this.It is made up of a range of organs suitable for the absorption, storage and digestion of food.The stomach has two primary segments. The proventriculus (glandular stomach) plays a role in chemical digestion, releasing hydrochloric acid and enzymes. The ventriculus (gizzard) is responsible for mechanical digestion.(10,11). The proventriculus is structurally designed for secretion and plays a vital role in the commencement of digestion. Histology It has superficial mucous glands and deep stomach glands which release mucus, hydrochloric acid and pepsinogen. Dietary composition influences to a large extent the structure and function of the digestive tract, influencing the digestive efficiency and nutrient availability (12,13). Thus, the objective of this work was to evaluate histological alterations of the glandular stomach of cockatiels submitted to different feeding regimens, aiming to elucidate the effect of diet on organ integrity and functional health.

MATERIALS AND MTHODS

The present study utilized eighteen (18) clinically healthy adult cockatiels (Nymphicus hollandicus) between the ages of 6 and 8 months. Birds were obtained from local bird markets and private breeders in Iraq during the experimental period (August 2025 to the October 2025). All birds were thoroughly clinically examined and only apparently healthy individuals without any clinical signs were included in the study. The initial body weight at the beginning of study was 70 g to 110g.Cockatiels were randomly divided into three experimental groups (n = 6 per group) based on dietary regimen . The first group (G1) was fed with fruits and vegetables, the second group (G2) with a balanced diet of grains, fruits and vegetables, and the third group (G3) only with sunflower seeds. Feed and sterilized drinking water were given ad libitum and changed twice daily. The feeding schedule was kept the same throughout the experiment. The main experimental factor was the diet composition due to its effect on the metabolic activity and histological structure of the visceral organs, especially the glandular stomach (proventriculus). All birds were housed in the animal house for a two month period (including an acclimatization period prior to sampling). To minimize the effects of non-dietary variables, all experimental groups were exposed to standardized and controlled environmental and management conditions. On the other hand the birds were housed in metal cages of size approximately 120 × 90 × 90 cm with six birds per cage providing adequate space, proper ventilation and animal welfare standards. The ambient temperature was kept at 24–28 °C and photoperiod was controlled at 10–12 hours per day. A mechanical ventilation system was used to ensure adequate air circulation daily from 1:00 PM to 3:00 PM. All birds were observed daily for general health status and behavior, and no signs of disease, abnormal behavior or mortality were recorded during the study period.

At the end of the experimental period, the birds were euthanized and placed in dorsal recumbency. The abdominal cavity was entered through a midline incision and the glandular stomach (proventriculus) was carefully excised without disturbing the anatomical integrity of the organ. The excised organ was gently washed in normal saline to remove blood and debris and fixed immediately by immersion in 10% neutral buffered formalin. The tissue samples were placed in labeled containers and sent for subsequent histological processing and light microscopic examination using Hematoxylin & Eosin (H&E) and Periodic Acid–Schiff (PAS) stains. Then samples were examined under light microscope and microphotographs were taken with the digital camera integrated in the microscope and the following dimensions were recorded of proventriculus; measuring thickness of inner circular layer of the muscularis externa (µm), measuring thickness of outer circular layer of the muscularis externa (µm), measuring thickness septa of proventricular glands (µm) and finally measuring proventricular glands diameter (µm).

Ethical approval , Under the reference number UOK.VET.AN.2025.139, this research was carried out in the anatomical laboratory of the College of Veterinary Medicine at the University of Kerbala – Iraq.

STATISTICAL ANALYSIS

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups in study parameters. Least significant difference-LSD was used to significant compare between means (ANOVA/ One way) in this study.

RESULT

The current research revealed in fruits and vegetables-fed group, the muscularis externa was characterized by relatively thin layer especially the inner circular smooth muscle fibers (Fig. 1) (Table. 1). The mucosa exhibited extensive folding and was lined with a simple columnar epithelium. Also the lamina propria was densely packed with a branched tubular proventricular glands, which were organized into a distinct lobular configuration and partitioned by delicate connective tissue septa (Fig. 2). Furthermore, the secretory cells of the proventricular glands demonstrated marked hyper-eosinophilia (Fig. 3). A marginal slightly increase in the density of PAS-positive mucous cells was observed within the epithelial lining, accompanied by the presence of attenuated septa (Fig. 4). These interglandular septa appeared notably thin throughout the proventricular structure (Fig. 5) (Table.1). Moreover the histological examination of the proventriculus glands revealed small in dimeter (Table. 1). Furthermore the initial weight was decreased from 74–78 g at the beginning of the experiment, until reached to the 48–55 g at the end of experiment.

Although in the group which received a balanced diet consisting of grains, fruits and vegetables, the proventricular glandular cells exhibited a moderate staining intensity (Fig. 6). Additionally, a notable proliferation in the population of PAS-positive mucous cells were observed within the epithelial lining (Fig. 7). There was also a distinct expansion of the interglandular septa, which appeared significantly thickened (Fig. 8) (Table.1). However our observations regarded an increase in thickness of muscularis externa with a relatively slight expansion in the diameter of the glands (Table. 1). In addition, this group had an initial weight of 74–80 g, which increased at the end of experiment to 86–94 g.

Whereas in the group fed exclusively on sunflower seeds, noticeable the proventricular glandular cells appeared noticeably paler in staining (Fig. 9), suggesting a variation in their cytoplasmic content. Within the epithelium, an intensive distribution of PAS-positive mucous cells was clearly evident (Fig. 10) and high increase in the proventricular glands diameter (Table. 1). Furthermore, the muscularis externa exhibited marked thickening (Fig. 11) (Table. 1). This structural reinforcement was accompanied by a significant increase in the width of the septa partitioning the proventricular glands (Fig. 12) (Table. 1). Moreover, this group had an initial weight of 74–77 g, which increased at the end of experiment to 100–110 g.

Table 1. Comparison between different groups in parameters of proventriculus

Groups	Spta thickness (µm)	Gland Diameter (µm)	Longitudinal muscle Thickness (µm)	Circular muscle Thickness (µm)
Fruits and vegetables diet	0.460 ± 0.09 b	0.50 ± 0.06 c	0.226 ± 0.03	3.46 ± 0.90
Balanced diet	1.46 ± 0.19 a	1.02 ± 0.11 b	0.240 ± 0.06	3.66 ± 0.25
Sunflower diet	1.46±0.19 a	1.98 ± 0.18 a	0.310 ± 0.05	3.84 ± 0.21
LSD	0.5110**	0.3807**	1.707 NS	0.1594 NS
P-value	0.0013	0.0001	0.8900	0.4902
Means having different letters in same column indicated significantly different (P<0.01, n=18)				

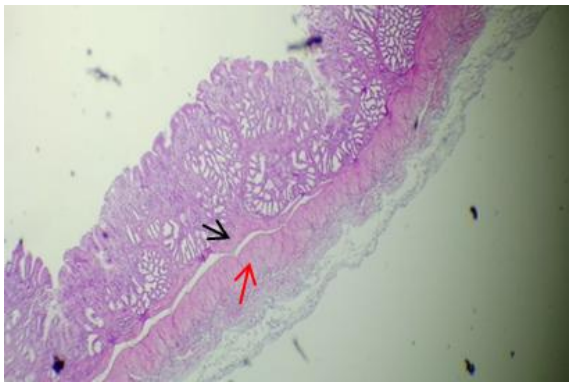

Figure 1. longitudinal section of proventriculus of group**1** showed muscularis externa with thin inner circular (⟶) and outer longitudinal (⟶) layers, (H&E 40x).

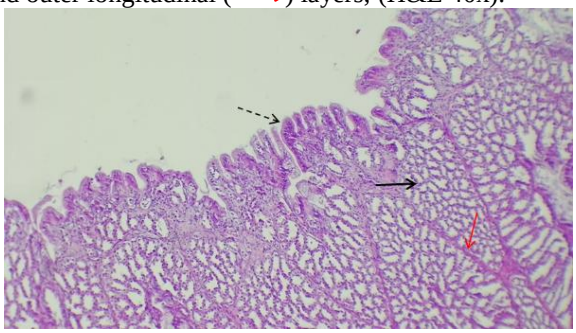

Figure 2. cross section of proventriculus of group**1** showed highly folded mucosa lined by simple columnar epithelium (⇢), lamina propria was densely packed with branched tubular proventricular glands (⟶) arranged in a characteristic lobular pattern separated by thin connective tissue septa (⟶), (H&E 40x).

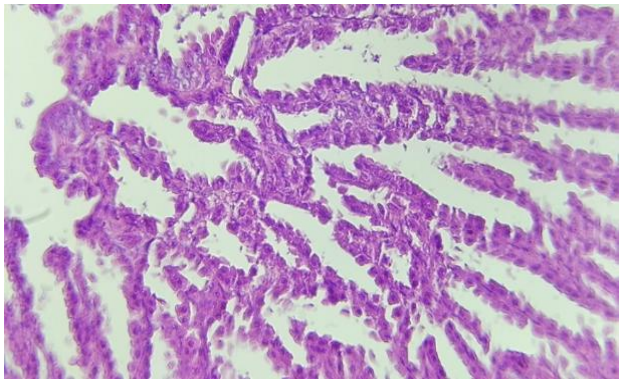

Figure 3. cross section of proventriculus of group**1** showed proventricular glandular cells with hyper-eosinophilia, (H&E 100x).

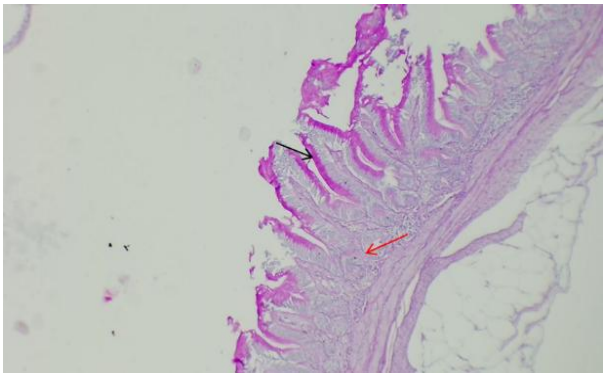

Figure 4. longitudinal section of proventriculus of group**1** showed slightly increase number of PAS-positive mucous cells in epithelia (→) with thin septa (⟶), (PAS 40x).

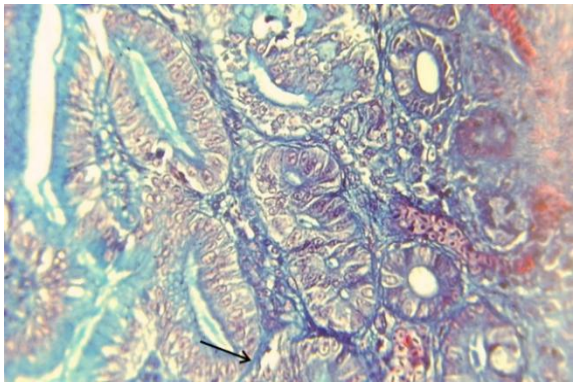

Figure 5. cross section of proventriculus of group**1** showed very thin septa (⟶), (Masson's trichrome staining 100x).

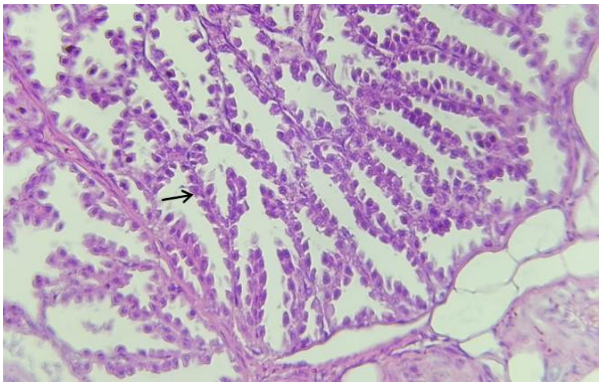

Figure 6. cross section of proventriculus of group**2** showed moderate staining intensity of proventricular glandular cells (⟶). (H&E 400x).

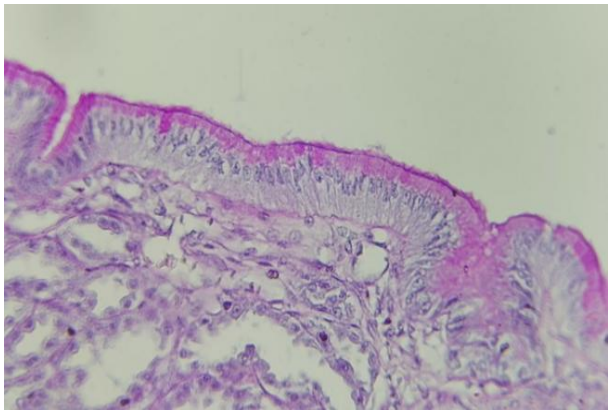

Figure 7. cross section of proventriculus of group**2** showed increase number of PAS-positive mucous cells in epithelia (⟶), (PAS 100x).

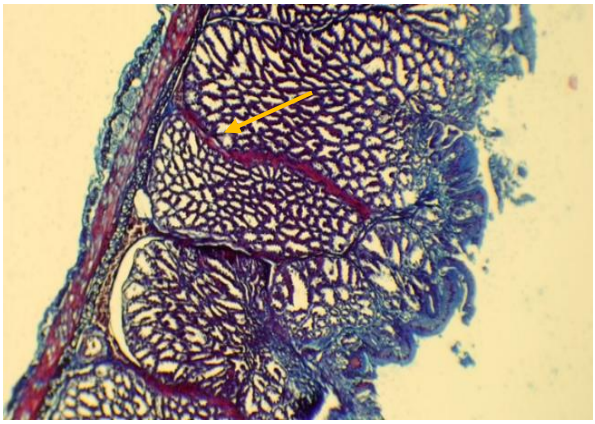

Figure 8. cross section of proventriculus of group**2** showed increase thickening of septa (⟶), (Masson's trichrome staining 100x).

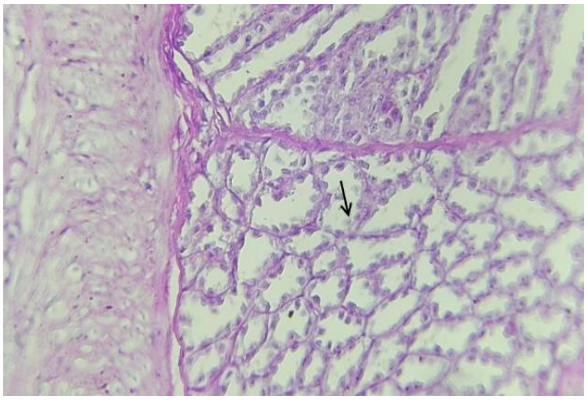

Figure 9. cross section of proventriculus of group**3** showed paler proventricular glandular cells (⟶), (H&E 100x).

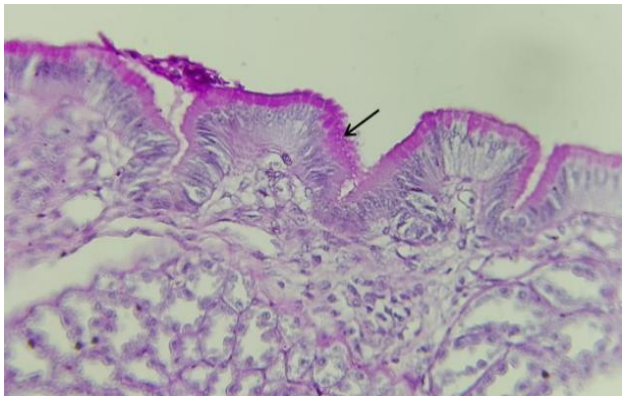

Figure 10. cross section of proventriculus of group**3** showed intensive number of PAS-positive mucous cells in epithelia (⟶), (PAS 100x).

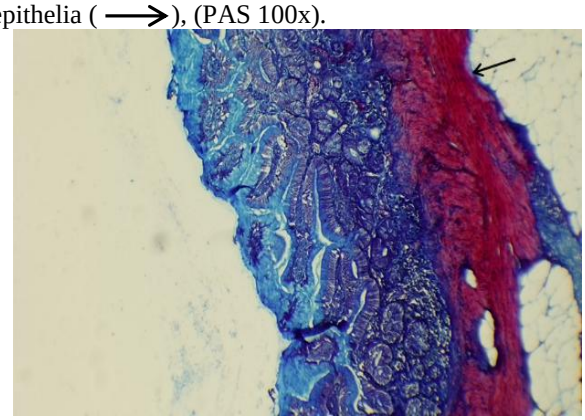

Figure 11. longitudinal section of proventriculus of group**3** showed thick muscularis externa (⟶), (Masson's trichrome staining 40x).

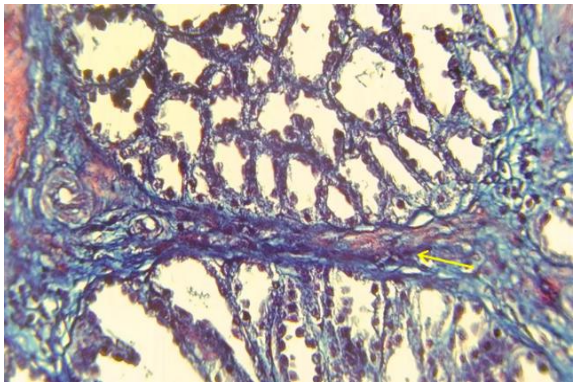

Figure 12. cross section of proventriculus of group**3** showed increased thickness of septa between proventricular glands (⟶), (Masson's trichrome staining 400x)

DISCUSSION

Cockatiels consuming fruits and vegetables exhibited comparatively thin inner circular muscle of proventriculus which was significant decrease in thickness (3.46 ±0.90) due to attributed to the soft texture and high water content of their diet easily digestible, these results agreed with (14,15,16), who revealed that birds consuming soft, water-saturated food had less muscle mass and smaller glandular structures due to decreased mechanical resistance and lower physiological burden. On other hand the proventriculus of group (1) showed proventricular glandular cells with hyper-eosinophilia and slightly increase number of PAS-positive mucous cells in epithelia due to the soft, high-moisture nature of food which does not require complex digestion, and increase in mucopolysaccharides due to the high sugar content of the fruit, this agreed with (17,18), who reported that diets rich in plant-drived components and non-starch polysaccharides stimulate goblet cell activity and increase mucin secretion within the mucosa, representing a physiological protective response of the mucosa, but disagreed with (19,20) who suggested that increased eosinophilic infiltration within the mucosa may be associated with dietary antigenic stimulation and hypersensitivity reactions rather than a simple physiological adaptation. Although the anterior stomach region had a very intricate mucosa, enveloped by the most basic form of columnar epithelium, with a lamina propria populated by branched tubular glands grouped into lobules, separated by slender connective tissue partitions, this configuration was regarded as an adaptation that augmented the secretory surface area and improved functional efficiency in a confined area, the results concurred with (21,22).

A notable reduction in gland diameter (0.50 ± 0.06 µm), due to the fruit and vegetables soft food with high level of water and easily digested, this finding was corroborated by (23, 22), who reported similar histological patterns of maintained glandular function despite reduced structural dimensions, this conclusion disagreed with (24, 25) that suggested the alterations in glandular morphology,

including reduced glandular diameter, may also be associated with changes in intestinal microflora and mild mucosal stress induced by imbalanced diets, which they reported that diets high in certain plant components may lead to suboptimal nutrient availability and altered epithelial turnover, so can result in reduced glandular development or mild atrophy not solely explained by decreased functional demand.

A reduction in the weight of birds consuming fruits and vegetables was noted, from 74-78 grams to 48-55 grams, this was ascribed to insufficient food energy, resulting in a negative energy balance and the consumption of bodily reserves, which agreed with (26, 10) who reported that inadequate dietary energy leads to negative energy balance, forcing birds to utilize body reserves, ultimately resulting in reduced body weight.

In addition the mild staining noted in the gastric gland cells of group (2), which provided a balanced diet of seeds, fruits, and vegetables, signified a balanced composition and stable secretory activity within the gland epithelium, due to a diverse diet that delivers adequate energy, proteins, and micronutrients supports the natural control of cells, preventing both excessive accumulation and lack of secretory products, thereby showing tissue equilibrium within the primary gastric glands, therefore we agreed with findings of (27), who suggested that the balanced meals in hens facilitate the natural development and maintenance of digestive tissues while keeping the stable tissue structure suggestive of physiological equilibrium.

The histological study noticed that the increase in PAS-positive mucus cells in the epithelium signifies heightened glycopolysaccharide production and improved protective capability of the mucous membrane. A balanced diet provides the necessary nutrients for correct epithelial differentiation and glycoprotein synthesis which contribute to the integrity of the mucosal barrier and lubrication of the epithelium. Therefore, the large number of PAS-positive cells indicated a structural modification indicative of increased mucosal membrane integrity and increased carbohydrate-rich secretory activity within the epithelium, These results were in agreement with (23) who stated that the histological study showed a relation between the increase in PAS-positive epithelial cells and high mucus concentration but were not in agreement with (28) who explained that the PAS staining results are affected by technical factors such as the quality of the fixation, carbohydrate preservation, and staining techniques.

Also cockatiels fed a balanced diet, showed increased septal thickness (1.46 ±0.19µm) that may reflect enhanced structural development and functional activity associated with adequate nutrient intake, this agreed with the findings of (29), who indicated that adequate nutrition enhances tissue development and structural integrity, while disagreed with (30), who indicated that balanced diets generally maintain normal histological structure without marked septal thickening. Likewise, (31) observed that increased connective tissue deposition was commonly associated with stress or pathological situations instead of normal nutritional status.

This group gained weight from 74 to 80 grams (pre-experiment) to 86 to 94 grams (post-experiment), ascribed to improved energy balance in the diet and the supply of sufficient nutrients. A balanced diet including seeds, fruits and vegetables provides useable energy, proteins, vitamins and minerals, thus promoting biosynthesis and tissue development. Therefore, continuous food intake resulted in a positive energy balance and increased weight gain, which was also observed by (32) who confirmed these results, showing that balanced diets improve growth performance and body weight gain. This is however in contrast to (33) who stated that changes in body weight are regulated not only by dietary composition but also by digestive efficiency, metabolic flexibility and physiological differences.

The current findings are related to the nutritious character of sunflower seeds, which raised the need for the functional activity of the proventriculus. Pale glandular cells may represent higher secretory activity with ongoing ejection of intracellular material and a resulting decrease in staining intensity. The increase in PAS positive mucous cells was also indicative of an adaptive response of the epithelium to increased requirements for mucosal protection and lubrication which agrees with (34) who reported that dietary composition can modulate secretory activity in avian digestive glands, where increased functional demand was associated with continuous secretion and changes in cellular staining characteristics. On the contrary, it is in disagreement with (35) that states that the paler glandular cells usually indicate a decrease in secretory activity due to the depletion of intracellular protein content, not an increase in function, and also (36) stated that decreased staining intensity of the digestive gland cells is usually associated with a decrease in secretory and metabolic activity, which represents a reduction of function and not an increase. The sunflower seed group showed a thick muscularis externa, with increased thickness of both the outer longitudinal muscle (0.310 ± 0.05 µm) and the inner circular muscle (3.84 ± 0.21 µm), in addition to increased thickness of the septa between proventricular glands (1.46 ± 0.19 µm), these changes may be attributed to the physical and nutritional characteristics of sunflower seeds, which were relatively hard and energy-dense, thereby increasing the mechanical and functional demand on the proventriculus, then led to stronger muscular contractions to facilitate food processing, resulted in hypertrophy of the muscular layers, in addition, the increased functional activity of the glandular tissue may stimulate connective tissue proliferation, contributing to the thickening of the interglandular septa, these findings agreed with (37), who reported that the diets containing hard and energy-dense components increase the functional demand on the gastrointestinal tract, resulting in muscular hypertrophy as an adaptive response, while disagreed with (38), who reported that the dietary differences alone do not

necessarily induce marked hypertrophy of the gastrointestinal musculature, as structural changes were more closely related to long-term physiological regulation rather than short-term diet composition.

As a result a high increases in weight of the third group (from 74-77 gm to 100-110 gm) which resulted from heightened energy consumption and enhanced food assimilation. The sunflower seed-derived meal, distinguished by its high fat content and energy density, may have facilitated a sustained positive energy balance, resulted in the accumulation of body reserves and steady weight increase, therefore agreed with (26) whom observed that the high-energy meals in birds enhance weight gain efficiency by improving nutrient utilization and fat deposition, whereas (39) emphasized that energy and fat-dense meals promote anabolic processes and typically augment body mass, while disagreed with (33) that highlighted the body weight responses were additionally affected by physiological adaptability, digestive efficiency, and metabolic variability, suggested that development was not exclusively reliant on diet.

CONCLUSION

•The present investigation showed distinct histological variations of the glandular stomach (proventriculus) of cockatiels fed with different feeding regimens.

•The proventriculus showed evident changes in the organization of the mucosa and in the structure of the glands, indicating its sensitivity to the composition of food.

•The fruit- and vegetable-based diet group exhibited reduced proventricular activity, with signs of diminished efficiency or relative atrophy, and a significant reduction in body weight compared to other groups.

•The sunflower seed diet caused considerable histological and physiological alterations. This group showed a significant increase in body weight when compared with the others.

•Overall, the balanced diet was the greatest nutritional strategy to maintain normal organ structure and function and, hence, optimum bird health. This was evidenced by a steady and persistent growth in the body weight together with a well-preserved balanced tissue organization in the examined organs.

Acknowledgements

N/A

Conflict of Interest

The authors declare no conflict of interest.

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