

# **HISTOMORPHOLOGY OF PROVENTRICULUS, GASTRIC ISTHMUS AND GIZZARD OF ASEEL CHICKEN**

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SINDH AGRICULTURE UNIVERSITY  
TANDOJAM, SINDH, PAKISTAN**

**2021**

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# HISTOMORPHOLOGY OF PROVENTRICULUS, GASTRIC ISTHMUS AND GIZZARD OF ASEEL CHICKEN



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## LIST OF ABBREVIATIONS

Acronym / Abbreviation / Symbol	Description / Derivative
ANOVA	Analysis of Variance
LSD	Least Significant Difference
p-value	Probability Value
ANOVA	Analysis of Variance
LSD	Least Significant Difference
p-value	Probability Value
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LSD	Least Significant Difference
p-value	Probability Value

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## ABSTRACT OF THESIS

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Gross anatomical and histological observations were performed on proventriculus, gizzard and the intervening isthmus of healthy, adult male and female Aseel chicken ( $n=20$ , 10 birds of each sex). Gross morphological parameters were recorded before and after the collection of specimens. The tissue specimens were stained with Hematoxylin and Eosin stain for light microscopy. The mean values for the weight of proventriculus of adult male and female Aseel chicken were  $3.63 \pm 0.25$  g and  $2.16 \pm 0.26$  g, respectively ( $P<0.078$ ). The length of proventriculus of adult male and female Aseel chicken were  $0.70 \pm 0.13$  cm and  $0.86 \pm 0.10$  cm, respectively ( $P<0.004$ ). The mean values for the circumference of proventriculus of adult male and female Aseel chicken were  $0.70 \pm 0.13$  cm and  $0.86 \pm 0.10$  cm, respectively ( $P<0.004$ ). The weight of isthmus of was recorded as  $0.42 \pm 0.05$  g, and  $0.39 \pm 0.03$  g in male and female, respectively ( $P<0.613$ ). The length of isthmus was  $1.15 \pm 0.08$  cm, and  $0.88 \pm 0.10$  cm in male and female, respectively ( $P<0.051$ ). The mean values for the circumference of the isthmus of adult male and female of Aseel chicken were  $0.87 \pm 0.10$  cm, and  $0.54 \pm 0.10$  cm, respectively ( $P<0.035$ ). The weight of gizzard of adult male and female Aseel chicken were recorded as  $32.84 \pm 1.91$  g, and  $26.99 \pm 1.31$  g, respectively ( $P<0.021$ ). The length of gizzard of male and female were recorded as  $4.09 \pm 0.40$  cm, and  $3.92 \pm 0.34$  cm, respectively ( $P<0.743$ ). The mean values for the circumference of gizzard of adult male and female Aseel layers, viz. mucosa, submucosa, muscularis and serosa. The mucosa was lined with simple columnar epithelium and thrown into papillae. Submucosa comprised of large conical / triangular lobules of simple branched tubular glands. Each lobule was separated by an organized into bundles, and outer longitudinal layer of smooth muscle. Isthmus was identified as a narrow intervening portion between the proventriculus and gizzard, serving as a conduit was divisible into two layers, namely inner longitudinal layer and outer thick circular layers. The serosa comprised of simple squamous epithelium with interspersed adipose tissue. The gizzard of Aseel chicken was lined with simple columnar epithelium covered externally with thick cuticle. The mucosal glands were simple branched tubular type. The submucosa was filled with loose connective tissue. The muscularis layer was by far the thickest layer and occupied  $\frac{3}{4}$ <sup>th</sup> of the wall thickness. It comprised of smooth muscle and was arranged into heavy bundles. Conclusively, the digestive anatomy of Aseel chicken predicts better production potential which warrants further research for improved growth performance of this breed.

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# CHAPTER 1

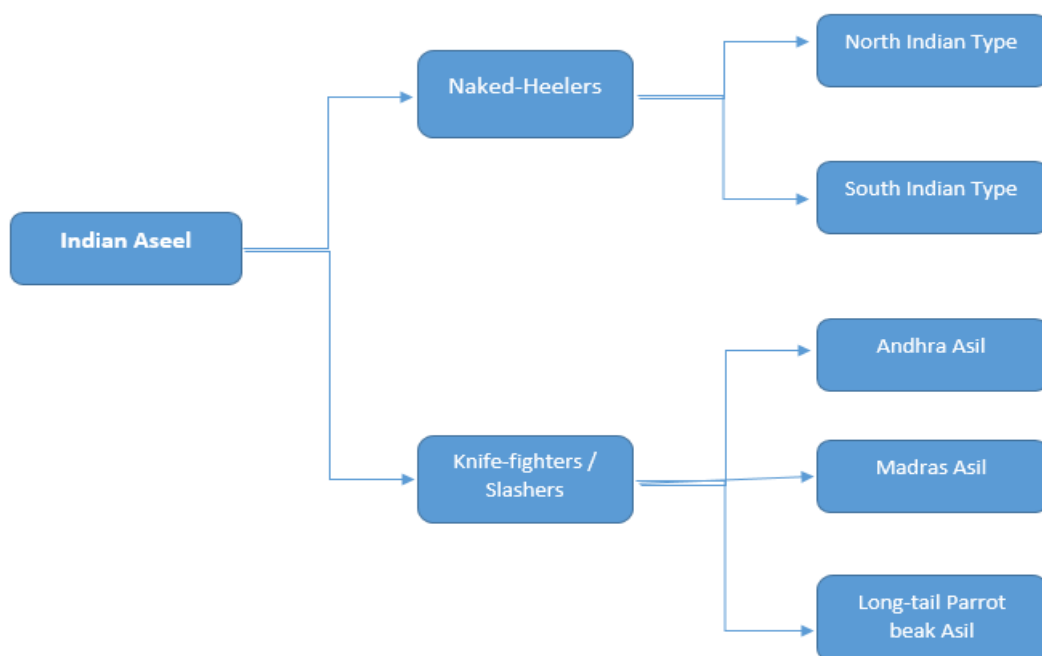
## INTRODUCTION

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The digestive tract of the chicken presents special features throughout its length. It starts from the mouth and connects with the pharynx rostrally, which in turn continues via esophagus into a series of tubular structures. The chicken stomach comprises of two chambers; the proventriculus is glandular (*pars glandularis*) and the gizzard is muscular (*pars muscularis*) chamber. The proventriculus is mammalian counterpart, a fusiform organ and the site of acid secretion. It is located oral to the gizzard or muscular stomach, which is the site of glandular digestion and gastric proteolysis. The pyloric region connects the gizzard to duodenum. The proventriculus and gizzard are separated by an isthmus the zona intermedia gastric. In ostriches, the crop is absent and the proventriculus is especially large (Sturkie, 2000).

The gastric isthmus is considered an important regulator of the food transfer rate into the muscular stomach and the gastric motility. In chicken with more efficient digestive system and higher digestive capacity, the isthmic lumen occupies 11% of the entire size and appears oval and more sinuous, higher mucosa relative surface, and the muscularis mucosa is more developed, as compared to 24% in the birds with less efficient digestion (Rideau et al., 2013).



**Plate 1.1**      **Classification of Indian Aseel chicken (“Asil chicken,” 2021)**

Page numbers in English numerals



Despite of the significance of Aseel, this variety has been overlooked by the native researchers. Much of the work has been done on the classification of Indian Aseel variety as shown in Plate 1 (“Asil chicken,” 2021). Attempts can be made to exploit the characteristics of this variety for increasing meat and egg production. Moreover, the key to fast growing modern broiler strains is better feed conversion ratio. Production from poultry is directly proportional to the feed consumption and feed efficiency, which; in turn necessitates an efficient digestive system. However, the details on the morphology of the gastric isthmus of chicken are also not well documented. This study is therefore designed to conduct the gross morphological and histological observations on the proventriculus, isthmus and gizzard in the adult male and female of the Aseel chicken.

### **Objectives**

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1. Gross morphology and peripheral relations of the proventriculus, isthmus and gizzard of Aseel chicken
2. Microscopic anatomy of proventriculus, isthmus and gizzard of Aseel chicken

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## CHAPTER 2

### REVIEW OF LITERATURE

The Aseel chicken breed is famous as a game bird. It is the native chicken of Pakistan and found especially in areas of Punjab and Sindh. It is held as few birds or in small flocks in rural areas as backyard poultry farming (Abedullah & Bukhsh, 2007). In Asia, the Indus Valley civilization is believed to be the first where Aryan people started the domestication of chicken during 2500 – 2000 BC (Crawford, 1995; West & Zhou, 1988).

#### 2.1 Aseel chicken

The Aseel chicken breed is famous as a game bird. It is the native chicken of Pakistan and found especially in areas of Punjab and Sindh. It is held as few birds or in small flocks in rural areas as backyard poultry farming (Abedullah & Bukhsh, 2007). West and Zhou (1988) In Asia, the Indus Valley civilization is believed to be the first where Aryan people started the domestication of chicken during 2500 – 2000 BC (Crawford, 1995; West & Zhou, 1988). Aseel is especially bred in rural areas because of its unique aggressive behavior and meat value. The live body weight of mature male and female birds is 2570 and 1870 g respectively. The hens may lay 49 eggs per year with an average egg weight of 45 g. The age of sexual maturity in males is around 8 months, while females start laying eggs at 9 months. The males are used for fighting at 16 to 18 months of age. In addition, the Aseel is resistant to many diseases and can also withstand to high ambient temperatures (Babar et al., 2012). Aseel chicken are large breed with beautiful coloring on the males and females, with distinct features of color in either sex (Figure 2.1).

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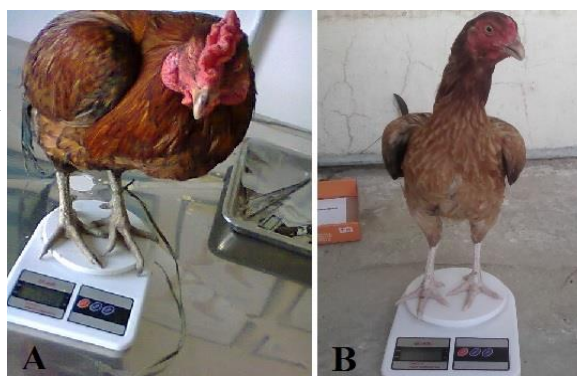
#### 2.2 The digestive cycle of the chicken

The digestive cycle of the chicken is a bit complex but interesting. In a bird with empty crop and stomach, the feed goes directly to the stomach due to the closure of crop sphincter; this movement is aided by the faster peristalsis in the esophagus. When the gizzard is full, it is the esophageal peristaltic waves that stop, causing relaxation and opening of the crop sphincter and thus much food can be stored in the crop. Thus, a double capacity for food storage is provided, a property seemingly essential owing to the life style and habitat of birds. The crop then supplies food when the stomach is empty, thus it has a regulatory role.

#### 2.3 Phases and patterns of motility of foregut in poultry

Dziuk and Duke (1972) characterized the the sequence of the passage of ingesta in chicken. The ingesta leave the proventriculus shortly after they enter in it, and flow into the

muscular stomach. This movement is aided by the contraction of thin muscles of the proventriculus. The contraction of thick muscle layer of gizzard causes the reflux of ingesta from the muscular to the glandular stomach. The ejection of ingesta into duodenum occurs during the latter phase of contraction of the two thin muscles. The gastric motility in great horned owls was characterized into three phases; namely, the mechanical digestion phase, chemical digestion phase, and pellet formation and egestion phase (Kostuch & Duke, 1975).



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**Figure 2.1 Adult male and female Aseel chicken**

A. male, B. female

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## **2.4 Gross morphology of proventriculus, isthmus and gizzard**

The proventriculus of falcon showed no papillae, however, in the proventriculus and gizzard, the mucosa was folded and lined with simple columnar epithelium. There was no isthmus region between the glandular and non-glandular regions of the stomach. Cuticle was present over the gizzard epithelium to which it was closely adhered (Abumandour, 2013). Das et al, (2013) performed a comparative study on the gross morphometry and biometry of the proventriculus of Indian Kadaknath fowl at 0, 7, 28 and 112 days was conducted. The outer surface of proventriculus was glistening and covered with blackish fascia. The weight, volume and thickness was  $0.33 \pm 0.01$  g,  $0.52 \pm 0.01$  g,  $3.93 \pm 0.07$  g and  $9.89 \pm 0.08$  g;  $0.74 \pm 0.01$  cc,  $1.18 \pm 0.10$  cc,  $2.1 \pm 0.07$  cc and  $3.68 \pm 0.10$  cc and  $2.41 \pm 0.07$  mm,  $2.73 \pm 0.09$  mm,  $4.46 \pm 0.04$  mm and  $5.35 \pm 0.12$  mm; the average length was  $12.63 \pm 0.20$  mm,  $14.47 \pm 0.21$  mm,  $23.04 \pm 0.47$  mm, and  $29.35 \pm 0.18$  mm; the average cross sectional area of proventriculus was  $11.5 \pm 0.65$  mm<sup>2</sup>,  $24.3 \pm 0.90$  mm<sup>2</sup>,  $177.1 \pm 2.53$  mm<sup>2</sup> and  $255 \pm 3.53$  mm<sup>2</sup>; while the average diameter was  $6.69 \pm 0.26$  mm,  $8.08 \pm 0.052$  mm,  $10.93 \pm 0.22$  mm and  $14.11 \pm 0.18$  mm on 0, 7, 28, and 112 days of age respectively. The proventriculus sloped from the left to right of median plane. Both openings were narrow and continued interiorly with esophagus and posteriorly with gizzard.

## **2.5 Histology of proventriculus, isthmus and gizzard**

Study on the mural layers of digestive tract in commercial broiler were studied under microscope. The constituent layers included tunica mucosa which is further divided into lamina epithelia, lamina propria, and lamina muscularis; the tunica submucosa, tunica muscularis and serosa excepting the esophagus where outer adventitia was also seen. The proventricular mucosa showed macroscopic folds and numerous microscopic papillae, the lamina propria appeared to have simple glands which converged into a common cavity near the surface. In gizzard, the cuticle was disposed into wavy lines that run parallel to the surface. Several villi were observed in the small intestine (Nasrin et al., 2012). Also, in another study, the proventricular glands in broiler were located in tunica submucosa and with oval to round in shapes. In the gizzard, the straight tubular glands of were limited to the lamina propria. These glands were lined by the simple cuboidal epithelium. Three regions of the of gizzard were identified, viz. the neck, body, and an expanded fundus. Three cell types were found in the glandular epithelium, viz. the chief cells, basal cells and surface epithelial cells (Lambate & Mamde, 2008).

## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.1 Experimental birds**

Gross anatomical and histological observations were performed on proventriculus, gastric isthmus and gizzard from healthy, adult male and female Aseel chickens ( $n=20$ , 10 birds of each sex). Gross morphological parameters were studied immediately after the slaughter at dissection hall of Department of Anatomy and Histology, Sindh Agriculture University Tandojam. The histological studies were conducted in the Tissue Technique laboratory. The birds were slaughtered by cervical dislocation and abdominal cavity was incised. The stomach was exposed after cutting the peritoneum and removing the liver. Samples of proventriculus, gastric isthmus and gizzard were collected immediately after slaughter.

#### **3.2 Gross morphological parameters**

The empty weights of proventriculus, isthmus and gizzard, their weight was measured using electrical balance (Appendix 1). The length, circumference and wall thickness of organ were measured with digital Vernier caliper as shown in Table 3.1. The anatomical location and peripheral relations of these organs were also noted.

#### **3.3 Histological parameters**

For general histology, the organs were opened longitudinally, and the contents washed out with saline solution. The organs were opened longitudinally and specimens of  $2 \times 2 \times 0.5 \text{ cm}^3$  size were collected. The sample were processed by paraffin embedding technique. The samples were fixed in 10% neutral buffered Formalin (NBF) in phosphate buffered saline (PBS; pH 7.2) solution for 48 h. The specimens were washed (2x5 min each) in distilled water to remove the salts. The samples were dehydrated in ascending grades of Ethanol (EtOH); starting from 75% EtOH for 2h, 85% EtOH 1h, two changes of 95% EtOH for 1h each and, finally; two changes of 100% EtOH for 1h each. The specimens were then cleared in 2 changes of Xylene for 30 min each. The samples were transferred to hot air oven at 60 °C for infiltration in 2 changes of melted paraffin for 1h each, and paraffin embedded tissue blocks were obtained. The slides were washed in a solution of 0.5% HCl in distilled water overnight. Slides were then washed in running tap water to remove any traces of the acid, rinsed in distilled water and dried in incubator at 39 °C overnight. Sectioning was accomplished using manual rotary microtome Leica RM2235 (Leica Biosystems, USA). Sections of 5-7  $\mu\text{m}$  thickness were obtained and flattened in warm water bath at 42 °C. The sections were then mounted over the pre-cleaned

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glass slides and dried overnight in incubator at 39 °C. The next day, sections were stained with Hematoxylin and Eosin (H&E) stain. The parameters for histological studies were identification of the different layers of the wall; viz. tunica mucosa, tunica sub-mucosa, tunica muscularis and serosa (Table 3.2) at low and high magnifications. Photographs were taken with Optikan—pro (OPTKA, Italy) microscope.

3.4 Statistical analysis

Data was expressed as Mean ± SD. The data was analyzed by ANOVA using SPSS version 17.0 statistical software. The means were compared by Student’s t-test.

**Table 3.1 Gross anatomical measurements on proventriculus, gastric isthmus and gizzard of adult male and female of Aseel chicken**

Parameter		Weight (g)	Length (cm)	Circumference (cm)	Wall Thickness (cm)	Location / Peripheral Relations
Proventriculus	M					
	F					
Isthmus	M					
	F					
Gizzard	M					
	F					

Gross morphological parameters were studied immediately after at dissection hall of Department of Anatomy and Histology, Sindh Agriculture University Tandojam. The histological studies were conducted in the Tissue Technique Laboratory, Department of Anatomy and Histology.

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**Table 3.2 Histological observations on proventriculus, gastric isthmus and gizzard of adult male and female of Aseel chicken**

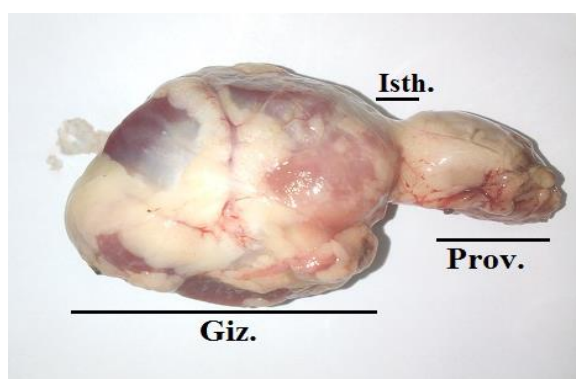
Parameter	Tunica mucosa (Glands, lamina propria, muscularis mucosa)	Mucosal papillae	Tunica sub-mucosa	Tunica muscularis and serosa	Remarks
Proventriculus					
Isthmus					
Gizzard					

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## CHAPTER 4

### RESULTS

Gross morphological and histomorphological observations were performed on the proventriculus, gastric isthmus and gizzard of healthy, adult, male and female Aseel chicken (Figure 4.1).



**Figure 4.1** Gross morphology of proventriculus, isthmus and gizzard of adult Aseel chicken.

Prov., proventriculus; Isth., isthmus; and Giz., gizzard.

#### **4.1 Gross morphology of proventriculus of adult male and female Aseel chicken**

##### **4.1.1 Weight of proventriculus**

The mean values for the weight of proventriculus of adult male and female Aseel chicken were  $4.30 \pm 0.26$  g, and  $3.63 \pm 0.25$  g respectively (Figure 4.2). Statistically, the difference of weight between the male and female birds was non-significant ( $P < 0.078$ ).

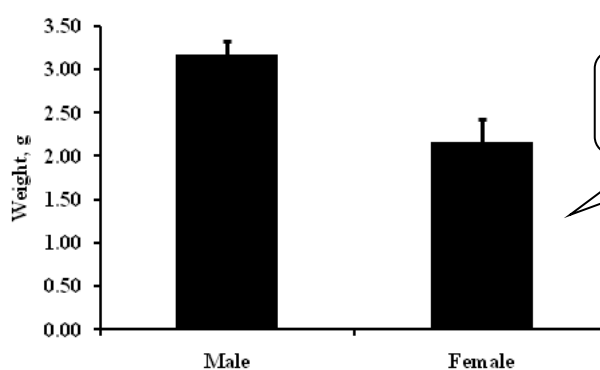


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**Figure 4.2** Graph showing the comparison of weight of proventriculus of adult male and female Aseel chicken

## CHAPTER 5

### DISCUSSION

The digestive system of birds is the main entity focused by the researchers with the goal of improving the feed efficiency and weight gain from commercial poultry. Much of the literature is directed toward the intestines and their importance in the absorption and uptake of the nutrients in the body of chicken. However, the glandular and muscular parts of the avian stomach are main compartments where the base components of the feed are broken down and made available to the intestines for further digestion and absorption. These organs were found very important with the particular reference to digestive tract and mechanism, function of these organs grinding of hard feed, digestive enzymes release and resistance against disease in the animals, birds and in the human beings. Generally, Aseel chicken are held in flocks of a few birds in rural areas as a backyard poultry farming. The feed provided to these birds is not enough or of low quality and mostly left unattended, such that the owners do not care for their daily feed intake (Abedullah & Bukhsh, 2007), compared to the broiler strains which are selectively raised for high feed efficiency and fed with high grade balanced diet.

Several authors have studied the morphology of digestive tract organs in different species of birds including ostrich, Japanese quail, red jungle fowl, broiler and Kadaknath fowl (Abumandour, 2013; Babar et al., 2012; Kostuch & Duke, 1975; Rideau et al., 2013; Sturkie, 2000; Zaher et al., 2012). However, most of the information available is on broiler and Red jungle fowl, but the information about histomorphology of digestive organs of Aseel chicken are unavailable. Therefore, most of the results in the present investigation being discussed are those from different species / breeds of birds.

#### **5.1 Gross morphology of proventriculus, isthmus and gizzard of Aseel chicken**

The gross morphological study on proventriculus in Kadaknath fowls reported that the long axis of the proventriculus extends obliquely from left to the right of the median plane with a narrowing of its lumen at both ends. The luminal surface of the proventriculus in this breed appeared whitish and was lined with visible short, round papillae. Externally, the proventriculus was covered with a glistening thin layer. The organ was continuous with the gizzard via a constricted, short isthmus (Das et al., 2013).



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## **CHAPTER 6**

### **CONCLUSIONS AND RECOMMENDATIONS**

#### **6.1 Conclusions**

Features of gross morphology (weight and size measurements) of the proventriculus, gastric isthmus and gizzard are different among the two sexes of Aseel chicken. The relative histomorphology of the mucosa, submucosa and muscularis of proventriculus, gastric isthmus and gizzard entails their specific functions, in the glandular digestion, gastric motility and food volume regulation and further grinding of the ingesta respectively in Aseel chicken. Aseel breed appears to be promising to fulfill the demands of the more backward rural community where the broiler farming might have drawbacks of harsh weather.

#### **6.2 Recommendations**

Further studies may be performed from day 1 of post-hatching to the adult age on weekly basis, so that a clear trend of changes may be observed at gross morphological and histomorphological level. Histochemistry using various stains, e.g. Periodic Acid-Schiff (PAS) and Alcian blue for mucins, bromophenol blue for staining of acid-base areas of the avian stomach, or a combinations of other immunohistochemical techniques may performed to elucidate the precise chemical composition of the cells appears to be imperative.

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**If appropriate, the 'Recommendations' section may be further divided into three subheadings i.e., 'Recommendations for farmers', 'Recommendations for policy makers' and 'Recommendations for researchers'.**

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## APPENDICES

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### Appendix 1 Comparative live body weight (kg) of male and female adult Aseel chicken

No	Male	Female
1.	2.53	0.908
2.	2.41	0.95
3.	2.742	1.25
4.	2.741	1.405
5.	2.3	1.597
6.	1.416	1.185
7.	2.445	1.191
8.	2.416	1.04
9.	2.53	1.00
10.	2.325	1.20
Mean	2.386	1.172
SD	0.372	0.213
Minimum	1.416	0.908
Maximun	2.742	1.597

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Signature.....

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