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Morphometric and histological characteristics of the stomach and reproductive organs in Japanese quail

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ABSTRACT

Knowledge of the anatomical features of the digestive and reproductive systems in Japanese quails makes it possible to purposefully influence their growth and development, using breeding and selection in the right direction to preserve the health of animal and increase its productivity. Japanese quail at certain stages of postembryonic ontogenesis—neonatal (daily), juvenile (30 days), puberty (60 days), and morpho-functional maturity (90 and 240 days) were used in this study. A morphometric assessment of the stomach, ovary, and testes of Japanese quails and the development of its linear index were analysed based on the obtained data. Also, a histological study at different ages was conducted. The materials included the glandular and muscular sections of the stomach, ovaries, and testes obtained from clinically healthy Japanese quails. The material was taken in the middle of a certain stage of postembryonic ontogenesis and consisted of 10 specimens for each age group. The dissected organs of Japanese quails was weighed on an electronic balance to determine the absolute weight in grams. Next, the relative weight of the organ was calculated. Additionally, venire calipers were used to measure the studied organs length and width. In addition to studying the histological structure of the stomach, ovary, and testes. The Japanese quail's stomach was characterized by an age-related staging of formation, with the glandular part growing most intensively until 30 days and the muscular part forming only at 90 days. In the case of histological study, in the glandular stomach, the submucosal layer occupied an average of 63.03% of the total surface area on the 90th day, while the muscle layer increased by 6.32%, equivalent to an increase of 1.98 times. As for the muscular stomach. The rate of development of the inner layer of the muscular stomach varied from day 1 to day 720, increasing its surface area by 10.01 ± 0. A direct correlation between avian reproductive health, ovarian and testicular mass was recorded. Quail mature sexually between four and five weeks of age. At around 6 weeks of age, females begun to lay. In the time between 60 and 150 days of age, the ovary developed most rapidly.

Keywords: Allometry, Morphometric characteristics, Stomach, Reproductive organs, Japanese Quail.
Article type: Research Article.

INTRODUCTION

Quail farming is one of the most productive industries that can outpace the pace of agricultural development and chicken farming which is used very intensively (Angel Daniel *et al.* 2022). High levels of science and biological standards enable us to achieve better results at the level of animal productivity as well as protect it from diseases (Alabdallah *et al.* 2021). Although Japanese quail is a promising species for the production of eggs rich in nutrients and minerals, and tasty meat, which is appreciated by consumers, the morpho-functional characteristics of this species are not yet well understood (Angel Daniel *et al.* 2022). Therefore, further research is needed to elucidate

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the relationships that may exist between various systems and organs of the quail, especially the digestive and reproductive organs, which are of great scientific and practical interest in quail production and reproduction. To solve this problem, it is necessary to pay special attention to the morphology and physiology of the digestive and reproductive systems in Japanese quail which have a significant impact on the ontogenetic process of animal development. Moreover, it is important to identify and study the most important critical periods in the formation of body systems, to improve the productive and reproductive qualities of Japanese quails and prevent their various diseases (Alabdallah *et al.* 2021). The gastrointestinal tract of vertebrates is a dynamic and energy-intensive organ system. Various anatomical and physiological parameters of the gastrointestinal tract are regularly used in clinical studies to assess growth dynamics and related physiological functions during the normal and abnormal development of birds. This knowledge is not only added to the literature on these types of birds, but it will also help to understand their biology and domestication. Moreover, it will be useful for pathologists and clinicians, especially when conducting post-mortem examinations (Hena *et al.* 2012). An important factor in poultry productivity is the development of the digestive system. The organs that make up this system develop significantly in the final phase of the growth of the embryo. On the 18th day of incubation, they make up about 1% of the mass of the embryo, and in the brood, they already account for 3.5% of the total mass. This increase in intestinal weight is associated with the development of intestinal villi (Uni *et al.* 2003). At the time of hatching, the bird's digestive system is anatomically complete, but its functionality is imperfect. Serious morphophysiological changes take place in the gastrointestinal tract, which will ensure greater efficiency of digestion and absorption processes (Nitsan *et al.* 1995; Uni & Ferket 2004).

In the quail and pigeon, the muscular gizzard or gizzard has been considered as a spheroidal organ located immediately caudal to the proventriculus and located partly between the lobes and partly behind the left lobe of the liver. It has a flattened, round shape, somewhat reminiscent of a convex lens, one side of which is slightly larger than the other. Each surface is covered with a shiny layer of tendinous tissue, thicker in the center and thinner towards the edges (Pesek 1999). Stomachs differ in birds depending on the diet. Vegetarian birds have a developed masticatory function, while granivorous and insectivorous birds have a significant masticatory function (Duke 1997; Gartrell 2000). The morphology of the stomach in birds is different depending on the species, the stomachs of chickens are spindle-shaped and have gastric papillae, on the upper surface of which processes are projected. The papillae secrete gastric juice mixed with mucin, hydrochloric acid, and gastric enzymes **(**Ahmed Abd-Galil *et al.* 2011**;** Silva *et al.* 2012**)**. The weight and length of the stomach in quail were 4.06 ± 0.22 g and 2.38 ± 0.20 cm with a relative weight of 31.6% and a relative length of 2.54% **(**Ahmed Abd-Galil *et al.* 2011**)**, while the weight and length of the stomach of the pigeon were 6.86 ± 0.69 g and 2.75 ± 0.25 cm, and their relative weight percentage and stomach length were 45.3% and 3.2%, respectively **(**Hena *et al.* 2012). Poultry is bred for meat and sold as individual animals. As a result, producers want to obtain as many chicks as possible in order to maximize profits. Certain critical reproductive parameters, such as fertility, hatchability, and embryonic mortality, influence a breeding flock's ability to produce a particular number of chicks (Narinc *et al.* 2013). Poultry reproduction differs greatly from that of other farm animal species. Just the left gonad in quail develops into an ovary, as it does in the majority of bird species.

The ovary is a polymorphic, granular body with an irregular form that is situated close to the left kidney's cranial lobe in the lumbar area of the body. The size of the ovary is determined by its maturity, or more particularly, the size of the eggs produced there. There are cortical and cerebral zones in the ovary, but their interactions are not always well characterized (Krotova *et al.* 2015). In males, there are two testicles, one on each side of the body's midline. The testis is covered by the capsule (three layers are made of dense, irregular connective tissue that primarily contains collagen fibres and a small number of elastic fibres). Testicular septa divide the testis into around 250 lobes. Each testicular lobule is occupied by 1-4 U-shaped, double-ended seminiferous tubules (which contain the spermatogenic cells that produce the sperm; (Kühnel 2003). In this work, an allometric equation is proposed that takes the age factor into account. Allometric equations make it possible to non-standardly evaluate and calculate anatomical constants for a particular animal species. Thus, knowledge of the anatomical features of the digestive and reproductive systems of Japanese quails makes it possible to purposefully influence their growth and development, using breeding and selection in the right direction to maintain the health of the animal and increase its productivity. Adequate development of the stomach directly affects the growth and productivity of any living organism, in particular the Japanese quail. Also, a histological study of the stomach, ovary, and testes of quails at different ages was conducted.

MATERIALS AND METHODS

The study was carried out in the experimental research laboratory and vivarium of the Department of Veterinary Medicine of the Agrarian and Technological Institute of the Peoples' Friendship University in Russia in the period from 2019 to 2022. The object of research was Japanese quail at certain stages of postembryonic ontogenesis: neonatal (daily), juvenile (30 days), puberty (60 days), and morpho-functional maturity (90 and 240 days). Each of these stages is characterized by certain features and has a different duration in Japanese quails, therefore, to increase the objectivity of the study, the material was taken in the middle of a certain stage of postembryonic ontogenesis in the amount of 10 specimens of each age group. The conditions for keeping and feeding Japanese quails corresponded to the zootechnical standards for this type of bird in conditions of industrial breeding. Water and the commercial pelleted feed were provided without restriction throughout the experiment. A lighting plan of 12 hours per day was used. The temperature and relative humidity were roughly 24 °C and 70%, respectively. Table 1 displays the composition of the feed.

Table 1. Composition of the diets provided to Japanese quails.

Nutrient	Unit	Value
Metabolic energy	kcal/100 g	285
Crude protein	%	20
Fat	%	3.5
Fibers	%	5.1
Linoleic acid	%	1.8
Lysine	%	1.05
Methionine	%	0.50
Methionine + cystine	%	0.72
Tryptophan	%	0.2
Threonine	%	0.55
Calcium	%	3.3
Sodium	%	0.16
P	%	0.8
Available P	%	0.75
NaCl	%	0.33

The glandular and muscular sections of the stomach, ovary, and testes were examined using macro- and micro-preparations, and the relative weight of the organ was calculated as a percentage of the live weight.

$$\mathbf{Mf.rel = Mf. \bullet 100/Mt}$$

where Mf is the absolute mass of the stomach (g); Mt is body weight (g).

Allometric equations are used to detect and describe the structure and functioning of an organism, and to assess the quantitative and qualitative deviations of individual animal species from the general model. The constancy of relative growth is described by the allometric equation: allometric ratios.

$$\mathbf{Y = a*X^{b}}$$

where Y- is the value of one variable; X- is the value of another variable; a- is the value of Y at the value of X equal to 1; b- is the ratio of the growth rates of variables Y and X.

Histopreparations are made using samples of the stomach (glandular and muscular), ovary, and testes that have been treated with a 7–10% solution of neutral formalin. Using a sledge microtome, each sample was divided into 10–15 cross-sectional slices with a thickness of 5–10 microns. These cross-sections were then stained using Ehrlich hematoxylin and an aqueous solution of eosin (5). A compound digital microscope (Olympus CX23, Tokyo, Japan) was used to quantify the histomorphometry of these various histological components, and ImageJ software was used to measure the pictures' thickness or diameter in micrometers (Java-based, version 1.52v, LOCI University of Wisconsin-Madison; Schindelin *et al.* 2012).

Biometric analysis of the digital material was performed using the updated methods of the data analysis package Microsoft Excel 2010.

RESULTS AND DISCUSSION

Morphological study of the digestive and reproductive organs in quail birds

The stomach of Japanese quail consists of two parts: the glandular (pars glandularis), where digestive enzymes are produced, and the muscular (pars muscularis), where the mechanical processing of food takes place. It is located in the anterior part of the left half of the abdominal cavity of the bird's body. The glandular section has a pear-shaped gray-pink colour, and the muscular section of the stomach is shaped like a disk with thick walls of dark red colour (Sherri 2003; Ahmed Abd-Galil *et al.* 2011; Lei 2015; Wilkinson *et al.* 2018; Alabdallach *et al.* 2020; Hristov 2021). We generally noticed a gradual change in the body weight of Japanese quails, body length, and mass index upwards. The body weight of Japanese quail increased 21.3 times from the first day of life to the 30th day from 8.03 ± 0.06 g to 171.41 ± 1.18 g. From the 30th to the 60th day, the increase in body weight was 15.3 times. The body weight of Japanese quail increased only 1.1 times over the period from the 60th to 90th days. From the 90th to 240th days, its increase was minimal from 211.25 ± 1.49 g to 215.22 ± 3.56 g. Ultimately, the total weight gain from day 1 to day 240 was 207.2 g, which averaged 0.86 g per day. However, the body weight increased most intensively in the first month of Japanese quail, which should be taken into account when drawing up a feeding ration (Ahmed Abd-Galil *et al.* 2011; Silva *et al.* 2012; Grieser *et al.* 2015; Cruz *et al.* 2019). Table 2 depicts the dynamics of body weight growth and mass index in Japanese quails.

Table 2. Dynamics of body weight growth and massiveness index in Japanese quails.

Age (days)	N	Body weight (g)	Body length (cm)	Mass index
		M ± m	M ± m	
1	10	8.03 ± 0.06*	3.33 ± 0.15*	2.41*
30	10	171.41 ± 1.18	6.45 ± 0.14	26.57
60	10	186.68 ± 1.81	7.77 ± 0.08	24.02
90	10	211.25 ± 1.49	8.58 ± 0.05	24.62
240	10	215.22 ± 3.56	8.80 ± 0.08	24.00

The length of the body exhibited similar dynamics. It maximally increased by 1.9 times in the period from 1 to 30 days of age (6.45 ± 0.14 cm). Thus, the total torso length increased by 5.47 cm during the study period, which is a daily increase of 0.02 cm. To solve the set tasks, we studied the absolute weight of the stomach of Japanese quails, which at the day old was 0.51 ± 0.17 g, i.e., increased by almost 5 times. By the end of the juvenile period, that is, by the age of 60 days, the growth rate slowed down significantly, and the absolute mass of the quail stomach was only 4.11 ± 0.19 g, i.e., it increased only 1.7 times. Nasrin *et al.* (2012) revealed that the weight of the gizzard was 5.32 g in broiler chickens. Table 3 displays the morphometric characteristics of the stomach of Japanese quails in the studied periods of post-incubation ontogenesis.

Table 3. Morphometric characteristics of the stomach of Japanese quails in the studied periods of post-incubation ontogenesis.

Age (days)	Absolute mass (g)	Relative mass (%)	Length, mm (Mean ± SD)		Width, mm (Mean ± SD)	
			Glandular stomach	Muscular stomach	Glandular stomach	Muscular stomach
1	0.51 ± 0.17	6.89 ± 1.95	06.02 ± 0.02*	12.4 ± 0.01*	3.08 ± 0.22*	8.20 ± 0.10*
30	2.42 ± 0.19	5.21 ± 0.34	13.63 ± 0.05	21.63 ± 0.04	6.08 ± 0.23	18.34 ± 0.22
60	4.11 ± 0.19	2.37 ± 0.06*	14.21 ± 0.03	25.4 ± 0.02	6.41 ± 0.38	20.17 ± 0.14
90	8.42 ± 0.36*	4.00 ± 0.17	16.63 ± 0.02	29.07 ± 0.03	8.41 ± 0.07	21.32 ± 0.31
240	6.58 ± 1.11	3.23 ± 0.20	16.08 ± 0.04	26.05 ± 0.06	8.49 ± 0.17	22.40 ± 0.17

During the period from the 60th to the 90th days, the absolute mass of the quail stomach doubled from 4.11 ± 0.19 g to 8.42 ± 0.36 g, i.e., 2.2 times. This is followed by a significant decrease in the mass of the quail stomach between the 90th and 240th days of the bird's life by 1.3 times. At the last stage of our experiment, there was a slight increase in the absolute mass of the quail stomach from 6.58 ± 1.11 g to 7.47 ± 0.32 g, i.e., 1.1 times (Kadhim *et al.* 2010). Thus, from the 1st to the 90th day of bird life, a gradual increase in the absolute weight of the stomach was observed in Japanese quails, and at the age of 90 days, its maximum value was noted (Table 2). We also studied the relative weight of the stomach in Japanese quails, which at the age of one day was 6.89 ± 1.95%. Her changes were not uniform during the study. Indeed, we noted a decrease in the relative weight of the stomach in Japanese quails by the age of 30 days to 5.21 ± 0.34%, i.e., it decreased by 1.3 times. This trend continued and the relative weight of the stomach in Japanese quails decreased by the age of 60 days to 2.37 ± 0.06%, i.e. 2.2 times. However, by the age of 90 days, there was a significant increase in this indicator to 4.00 ± 0.17%, i.e. 1.7 times. By the age of 240 days, the relative weight of the stomach in Japanese quails slightly decreased to 3.23 ± 0.20% and, finally, by the age of 720 days it reached 4.36 ± 0.79% (Santos *et al.* 2005; Ahmed Abd-Galil *et al.* 2011). The ostrich gizzard shape looked like a hen's gizzard, but the ostrich gizzard weight (1001–1150 g) was 12 times heavier than the weight in the hen's gizzard (52.0–81.0 g; Cooper & Mahroze 2004**;** Sales 2006**;** Table 3). Thus, the weight indices of the stomach in Japanese quails changed unevenly and reached stable values only by the age of 240 days. To solve the problem, using the data of body weight and the absolute weight of the stomach of Japanese quails from the moment of hatching to 720 days of age, we, using a computer and a set of applied programs, empirically calculated the original allometric equation, which, unlike those proposed earlier, takes into account the age factor (B, months) and has the following form:

$$\mathbf{Mf = (2.18\text{-}0.62^{B})\ X_{Mt}^{0.63}}$$

In this case, the variable Y corresponds to the absolute mass of the stomach (Mf) and is calculated in grams. The variable X corresponds to absolute weight (Mt) and is calculated in kilograms. For b, which shows the ratio of the growth rates of variables Y and X, different growth rates are shown in this case (0.63 for quail). The a value is more complex but takes into account the age factor (B), which is calculated in months for a particular animal species. When calculating the value of a, the concept of a constant is introduced that characterizes some types of quail animals (2.18). This parameter, like the value of b, is directly related to the size of the animal and tends to decrease. As shown by biostatistical analysis, the proposed new allometric equation has an average level of deviation from real data of 10–15%, which makes it possible to recommend this allometric equation for research projects. The total length of the stomach in all studied quails increased 2.3 times from the first day to the 30th day of life (35.26 ± 0.05 mm). By the 60th day, this indicator increased by 4.35 mm to 39.61 ± 0.02 mm. This dynamics was observed up to 240 days of age and amounted to 42.13 ± 0.05 mm. Thus, the total length of the stomach during the study period increased by 23.7 mm, which is a daily increase of 0.1 mm (Ahmed Abd-Galil *et al.* 2001). Hassan & Moussa (2012) reported the length of the proventriculus in pigeons to be 26 ± 2.16 mm and was 60 ± 5.16 mm in ducks. The length of the proventriculus (PV) in the Muscovy duck was 56 ± 6.43 mm, as reported by Madkour (2015). The study (Zaima *et al.* 2021) indicated that the PV length and weight were increased among all the young groups and also the mean values of the adult group were higher than the young group's readings. Wu *et al.* (2010) reported that the PV length and weight was 60 mm and 35.9 g, respectively, in pheasants (Table 3). The length of the glandular stomach gradually increased from the first to the 90th day from 06.02 mm to 16.63 mm, which was 10.61 mm equivalent to a length of 2.8 times by the 1st day. The daily increase over this period was 0.11 mm. This increase was very large from day 1 to day 30 (7.61 mm), which is 2.36 times the length on day 1. The glandular stomach decreased in length by 0.55 mm from the 90th to the 240th day. Every day of this period there was a decrease of 0.003 mm, although very slight, it should be noted. At the level of the muscular stomach, the length developed in the same trend as at the level of the glandular stomach, however in different proportions .Thus, from the first to the 30th day, the progress was 9.23 mm, which is 1.7 times more. From day 30 to day 60 the increase was very small at 0.14 mm and then increased to 7.29 mm from day 60 to day 90. Thus, the muscular section of the stomach increased in length by 2.3 times from the first to the 90th day by 16.40 mm. The results obtained are consistent with the data of Kretov (2018) and indicate that the stomach grows most intensively in the first month of quail life, especially its muscular section. The total width of the stomach in all studied quails increased 2.2 times from the first day to the 30th day of life (24.48 ± 0.23 mm). By the 60th day, this indicator increased by 2.1 mm to 26.58 ± 0.26 mm. This dynamics was observed up to 240 days of age and amounted to 31.3 ± 0.17. Thus, the total width of the stomach during the study period increased by 20.02 mm, which is a daily increase of 0.08 mm. The width of the glandular stomach gradually increased from the first to the 90th day from 3.08 mm to 8.41 mm, which is 5.33 mm equivalent to a length of 2.7 times by the 1st day. The daily increase over this period was 0.06 mm. This increase was very large from day 1 to day 30 (3 mm), which is 0.9 times the length on day 1. At the level of the muscular stomach, the width developed in the same trend as at the level of the glandular stomach, however in different proportions. Thus, from the first to the 30th day, the progress was 10.44 mm, which is 2.2 times more. From day 30 to day 60 the increase was very small at 1.83 mm and then increased to 21.32 mm from day 60 to day 90. Thus, the muscular section of the stomach increased in width by 2.4 times from the first to the 90th day by 13.1 mm (Subedi *et al.* 2008**;** Table 3). To characterize the age-related processes of stomach formation in Japanese quails, we propose to calculate the linear index of the stomach (Is, %), taking into account the total length (L; mm) and total height (Vs; mm) of the stomach:

Is = (L – Vs)/ (L + Vs) ×100

As our studies have shown, the maximum linear index of the stomach of Japanese quails was observed at a daily age (24%). It changed most significantly in the first month of a quail's life and by the age of 30 days it was only 17.4%, i.e., it decreased by almost 1.4 times (Fig. 4). By the end of the juvenile period (the 60th day), the growth rate slowed down and the linear index of the stomach of Japanese quails was only 15.3%, i.e., it decreased by almost 1.1 times. Further, this indicator of linear growth of the ventricle increased again and by the beginning of oviposition of the quail was 21.2% in 90-day-old quails. Subsequently, the linear index of the ventricle of Japanese quails decreased and by the age of 240 days was only 14.7%. As the present study have shown, by the time of hatching, daily Japanese quails exhibited the most intensively developed stomach, as evidenced by its maximum linear index (24%), which significantly decreased to 15.3% by the time of puberty. However, by the time of oviposition increased again to 21 .2% (HASSAN & MOUSSA 2012). In our study of the morphological changes of the testicles, we found an increase in the weight, length, and width of the right and left testicles of male quails by age, with an average of 1.37, 1.21, and 1.23, respectively (Table 4). As for the ovary of female quail birds, we noticed an elevation in the weight of the ovary by age, as it reached 50% at the age of 90 days when compared to the age of 30 days. We also found an upraise in the length and width of the ovary by age, at a rate of 2.61 and 2.29, respectively (Table 5). Early in the quail embryonic development, the sexual organs are differentiated. Both sexes have bilateral, bi-potential gonadal anlagen, which are made up of a medulla and a cortex, and their structures are indistinguishable before differentiation.

In the medulla of males, the primary sex cords mature into seminiferous tubules that contain spermatocytes and sertoli cells. In birds, there are two testicles, one on each side of the body's midline. An exocrine and an endocrine function are shared by the testis, a complex tubular gland.

The seminiferous tubules' lining epithelium produces spermatozoa as part of their exocrine function. A particular interstitial (Leydig) cell in the intertubular connective tissue performs the endocrine function by producing the male sex hormone testosterone (Aughey & Frye 2001; Jamieson 2007). According to our findings, the development of testes increased significantly by the age, and the left testis was wider and shorter than the right one. The start of sexual maturity is influenced by both social and photoperiodic influences. Depending on the lighting pattern, domestic quail mature sexually between four and five weeks of age. At around 6 weeks of age, females begin to lay (Cheng *et al.* 2010).

Table 4. The morphometric characteristics of testes in Japanese quail.

Parameter	Day 30	Day 60	Day 90
Weight of testes (g)	0.03 ± 0.005*	1.12 ± 0.055	3.1 ± 0.41
Testes index (%)	0.07 ± 0.006*	0.72 ± 0.09	1.71 ± 0.2
Weight of the right testis (g)	0.011 ± 0.007*	0.64 ± 0.05	1.4 ± 0.23
Weight of the left testis (g)	0.018 ± 0.003*	0.48 ± 0.005	1.6 ± 0.2
Right testis length (mm)	9.1 ± 1.5*	16.82 ± 0.5	21.3 ± 2
Length of left testis (mm)	8 ± 1*	12.71 ± 0.5	18.1 ± 2.2
Right Testis Width (mm)	5.2 ± 1.1	7.90 ± 0.085	11.2 ± 2.4
Left testis width (mm)	6.7 ± 0.9	8.9 ± 0.09	14.3 ± 2.3*

Table 5. The morphometric characteristics of the ovary in Japanese quail.

Parameter	Day 30	Day 60	Day 90
Ovary weight (g)	0.019 ± 0.004*	0.87 ± 0.09	0.90 ± 0.04
Relative Ovary Mass	0.04 ± 0.009*	0.43 ± 0.06	0.36 ± 0.02
Ovary length (mm)	9 ± 0*	17.2 ± 1.3	23.34 ± 4
Ovary Width (mm)	4.33 ± 0.33*	11.7 ± 1.5	13.54 ± 0.47

In the time between 60 and 150 days of age, the ovary develops most rapidly (Krotova *et al.* 2015). In the cortical zone, ovocyte development occurs. The first order ovocyte enters the oviduct during ovulation, where it undergoes additional maturation, fertilization, the production of egg shells, and the embryo's early stages of development (Subedi *et al.* 2008).

Histological study of the digestive and reproductive organs in quail birds

Birds' stomachs are divided into three sections: the glandular, the muscular, and the pyloric. The species' particular reproductive practices and nutrition exhibit an impact on the anatomical topography and microstructure of the individual components (Abdul Ridha *et al.* 2019; Daibes *et al.* 2020). According to the type of diet, the size of the various digestive system parts in birds varies (Langlois 2003).

The black-tailed crake's compound tubular glands, like those in other omnivorous birds such as the red jungle fowl (Gartrell 2000), duck and pigeon (HASSAN & MOUSSA 2012), and *Coturnix coturnix* (Zaher *et al.* 2012), occupied the thickest part of the wall of PV, while in the PV of a carnivorous falcon, the muscle layer took up the thickest portion of the wall (Abumandour 2013; Lei 2015), which perhaps large foods require more force to be forced into the gizzard. So, the size of the food may also display an impact on the stomach's histological structure. The PV’s four layers and other histologically distinct components are depicted in Figs. 1-2. Similar strata were also observed in several species of birds (Ogunkoya, & Cook 2009; Batah *et al.* 2012; Hassan & Moussa 2012; Abumandour 2013; Al Saffar & Al Samawy 2014; Al Saffar & Al Samawy 2014) as well as in mammals. Although many authors concurred that the PV and gizzard of Japanese quail, (Liman *et al.* 2010), (Rocha & Lima 1998) and also the PV and gizzard of other birds (Zhu *et al.* 2013), including ostrich (*Struthio camelus*) (King *et al.* 2000; Cooper 2004), Guinea fowl (*Numida meleagris*) (Selvan *et al.* 2008), quail (Attia 2008), Japanese quail (King *et al.* 2000) and Coot bird (Batah *et al.* 2012) all have three layers. Although Marshall & Folley (1965) discovered only three layers in the wall of PV in Asiatic swiftest (*Collocalia* spp.), in which only mucosa, muscularis, and serosa were detected.

Proventriculus (PV): The four primary layers of the quail PV microscopic components, including their dimensions, are shown in Table 6. Simple columnar cells lined the folds in the tunica mucosa, which had folds of varied heights. These cells responded positively to the PAS, which then reacted with the mucin granules, a finding similar to that reported in domestic chicken by Hodge (1974). The core of the mucosa is filled with a layer called the lamina propria, which is made up of white fibres, fibroblast cells, and a lot of surface gastric glands that are tubular in shape. This finding was in line with Jassem *et al.* (2016).

The proventricular lobes and glands, which made up the thickest part of the proventricular wall, were located in the tunica submucosa, which made up the majority of PV (Matias *et al.* 2021). Inner circular and outer longitudinal smooth muscle fibres were found in the tunica muscularis' two layers, in accordance with findings from other studies (Nitsan *et al.* 1995; Kadhim *et al.* 2010; Batah *et al.* 2012; Dahekar *et al.* 2014; Al Saffar & Al Samawy 2014; Madkour & Mohamed 2018). According to these findings, the outer longitudinal muscle fibres were thin and were defined by flat basic cells. The inner circular smooth muscle fibres were thicker distinguishing by round basic cells dispersed throughout the muscle bundles. The back-and-forth movement of feeds in the PV and gizzard is caused by the tunica muscularis (Langlois 2003). The PV outermost layer, the tunica serosa, is made up of smooth muscle fibres. This layer also contains dense connective tissue, in which, blood vessels and nerves are located (Rossi *et al.* 2005; Al Saffar & Al Samawy 2014; Dahekar *et al.* 2014; Jassem *et al.* 2016; Table 6). We observed the uneven development of the surface of the various layers of the glandular stomach from the examination. The mucous layer and axillary layer took up the majority of space (Matias *et al.* 2021). The tunica mucosa and the tunica submucosa, are the thickest due to the numerous proventricular glands found in this layer, while the tunica muscularis, and the tunica serosa are the thinnest. So, on the first day, the muscle layer occupied 16.67 ± 3.30 of the total surface; on the 30th day, it decreased and occupied 10.01 ± 0.02 or 6.66 ± 0.28. On the 60th day, there was a significant increase of 2.67 times in comparison with the 30th day, passing to 26.70 ± 3.27. On the 90th day, the muscle layer occupied 16.60 ± 3.23 of the total surface, i.e., a decrease of 10.10% compared to the previous 60 days.

It occupied the maximum surface on the 720th day (33.35 ± 3.23). As a result, the area occupied by the muscle layer increased by two times during the study period. Similar findings in ducks were reported by Qureshi *et al.* (2017).

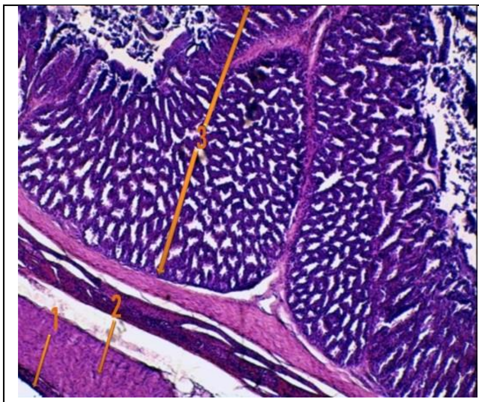	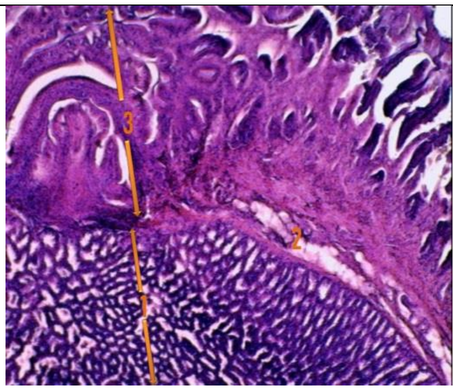
Fig. 1. Glandular stomach of Japanese quail at the age of 1 day; 1. serosa, 2. Muscular layer; 3. tubular glands (H & E 40 X).	**Fig. 2.** Glandular stomach of Japanese quail at the age of 30 days; 1. Muscular layer; 2. Submucosa; 3. Mucous membrane (H & E; 40 X).

Table 6. Histological results of the stomach.

Stomach type	Layers	Age (days)					
		1	30	60	90	240	720
Glandular stomach	Mucosa	16.67 ± 3.30	10.01 ± 0.02*	26.70 ± 3.27	16.60 ± 3.23	23.28 ± 3.32	33.35 ± 3.23
	Submucousa	63.33 ± 8.78	60.03 ± 5.78	63.31 ± 3.29	70.06 ± 5.67	66.69 ± 3.30	50.01 ± 5.70
	Muscular layer	16.69 ± 3.29	23.29 ± 3.29	3.32 ± 3.26*	6.65 ± 3.30	6.68 ± 3.28	3.29 ± 3.30*
	Serosa	3.31 ± 3.27*	6.70 ± 3.28	6.67 ± 3.28	6.69 ± 3.28	3.35 ± 3.30*	13.35 ± 3.32
Muscular stomach	Cuticle	10.03 ± 0.01	3.31 ± 3.05*	10.05 ± 0.02	6.70 ± 3.30	6.66 ± 3.28	33.28 ± 3.27
	Submucosa	10.02 ± 0.02	3.33 ± 3.10*	6.64 ± 3.31	20.02 ± 5.81	6.70 ± 3.30	10.02 ± 0.58
	Muscle	76.70 ± 3.34	90.01 ± 0.02	76.61 ± 3.07	66.68 ± 3.34	53.29 ± 3.26	26.69 ± 3.30*
	Fatty tissue	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	26.68 ± 3.31*	16.70 ± 3.27*
	Serosa	3.30 ± 3.32*	3.35 ± 3.06*	6.70 ± 3.35	6.72 ± 3.37	6.69 ± 3.30	13.31 ± 3.31

The thickness of each layer of the PV and gizzard was positively associated by aging. The submucosal layer was wider on days 1 and 60 and occupied an average of 63.32 ± 6.03 of the total surface area. However, it was on the 90th day that it reached its maximum area (70.06 ± 5.70), which was 6.73% higher, which corresponds to an increase of 1.10 times compared to the first day. This trend continued up to the 720th day (50.01 ± 5.67), which is 20.05 ± 0.57 less than on the 90th day, or 1.4 times lower. The muscle layer increased by 6.60 ± 0.03 from the 1st to the 30th day, from 16.69 ± 3.29 to 23.29 ± 3.29, which is 1.40 times higher. Then there was a significant decrease in its area for 60 and 720 days from 23.29 ± 3.32 to 3.29 ± 3.30, a decrease of 19.97 ± 0.03, i.e., 8.02 times lower. On the other hand, the serosa exhibited positive growth from the days 1 to 720. Indeed, it increased from 3.31 ± 3.27% to 6.70 ± 3.28%, i.e., it doubled its surface area from the first day. The same trend continued from day 60 to day 720, increasing from 6.67 ± 3.28% to 13.35 ± 3.32%, which is equivalent to an elevation of 1.98 times (Ahmed Abd-Galil *et al.* 2011). In the study on the striated scops owl (Al Saffar & Al Samawy 2014), the mean thickness of the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa were about five, six, seventeen, and five times, respectively, thicker than in quail.

The Gizzard: The gizzard mucous membrane has branched folds that are lined by simple columnar epithelial cells. Above the mucous membrane is a thick layer of cuticle that reacts positively to the PAS marker when acidic mucin is present, which is consistent with findings from other authors (Zaher 2012). The fact that the cuticle is well developed, showed the connection between it and the type of bird diet. This finding is consistent with Al-Saffar & Al-Samawy's (2015) who reported that the lamina propria of mallards was composed of dense connective tissue and numerous deep simple tubular glands that dilated in the fold's foundation. These glands are lined by simple columnar epithelial cells. This discovery is consistent with the description of the *Coturnix coturnix* by Zaher *et al.* (2012), who found crypts in the folds' foundation. This finding, which was also made in the Red-Capped Cardinal by Catroxo *et al.* (1997), shows that the muscularis mucosa is lacking. The smooth muscle tissue is organized into an internal longitudinal layer and a circular outer layer in the muscularis layer, which is well developed. This outcome is consistent with data from coot root collected by Batah *et al.* (2012). Blood vessels and nerve endings were abundant in the serosa, which is made up of connective tissue and covered with simple squamous epithelium. This outcome is comparable to what was seen in chickens by (Caceci 2006; Fig. 3). The most important note is the presence of a layer of adipose tissue on the 240th and 720th days of the study (46). The rate of development of the inner layer of the muscular part of the stomach, which is the cuticle, changed unevenly at different stages of development. This percentage decreased from the first to the 30th day by another 3.03 times and increased by another 3.03 times by the 60th day. Similarly, from the 60th to the 240th day, the indicator decreased by 1.51 times, which is the cuticle, and changed unevenly at different stages of development. This percentage decreased from the first to the 30th day by another 3.03 times and increased by another 3.03 times by the 60th day. Similarly, from the 60th to the 240th day, the indicator decreased by 1.51 times. However, the increase in cuticle area was very significant between days 240 and 720 (5 times): from 6.66 ± 3.28 to 33.28 ± 3.27 (Qureshi *et al.* 2017). In ducks, the thickness of each layer of the gizzard increased by age in a positive correlation. The layer under the mucous membrane increased from the first to the 90th day by another 2 times, and from the 90th to the 720th day it decreased by 2 times lower. The second observation is that the muscle layer was the most extensive, with the highest recorded on day 30 being 90.01 ± 0.02. Then this indicator decreased from the 30th to the 720th day from 90.01 ± 0.02 to 26.69 ± 3.30, which is 3.37 times lower (Table 6). Similar findings for the tunica muscularis were reported by Qureshi *et al.* (2017) and Starck & Rahman (2003) for immature, adult, and elderly quail groups. Adipose tissue was present only on the 240th day (26.68 ± 3.31), and on the 720th day, it was 16.70 ± 3.27, which corresponds to the aging of quails in our study. The serous layer, on the other hand, increased very uniformly from day 1 to day 720, from 3.30 ± 3.32 to 13.31 ± 3.31, increasing surface area by 10.01 ± 0.01%, which is 4.03 times higher. According to Qureshi *et al.* (2017) and Starck & Rahman (2003), the tunica serosa's outermost layer had considerably ($p < 0.05$) less development in adults than in younger groups. Similar information on the tunica serosa in ostriches was reported by Wang *et al.* (2017; Table 6).

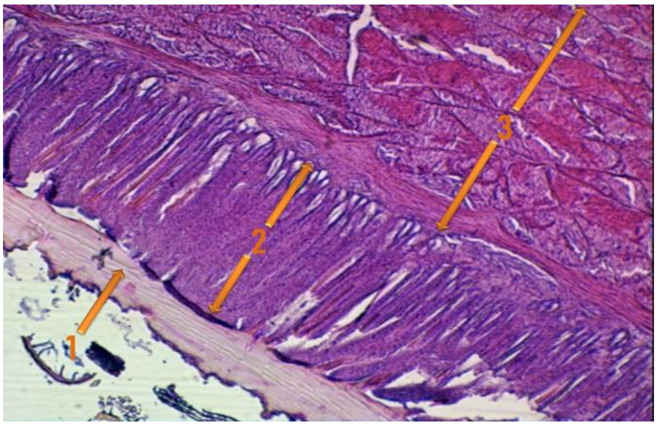

Fig. 3. Muscular stomach of Japanese quail at the age of 30 days; 1. Cuticle; 2. Mucosa with submucosa; 3. Muscular layer (H & E; 40 X).

When we studied the testis tissue in male quails, we noticed a decrease in the septum and capsule of 20% and 19%, respectively, at the age of 90 days. As for the spermatogenic epithelium, we noticed an elevation by age, reaching 28% at the age of 90 days. In the ovary, we noticed a decrease in the thickness of the capsule by aging, when it was 90 days old, by 18%. As for the follicular epithelium, we found an increase in its thickness by aging, when it reached 26% at the age of 90 days (Table 7). According to McGary *et al.* (2002), there is a direct correlation between avian reproductive health and testicular mass. A direct link between sperm production and testicular mass has been reported in broiler breeders (Leeson & Summers 2010). Testicular size is the primary indicator of spermatogenesis since seminiferous tubules and germinal components account for approximately 98% of the total mass of the testes (Herve *et al.* 2018). There are two basic parts of the testis' interstitial tissue. The first is the boundary tissue, a dense layer of myofibroblasts and connective tissue that tightly encircles the seminiferous tubule. The other is the interstitial tissue, a loose connective tissue that sits between seminiferous tubules. It expresses itself fully as angular regions or wedges between three or more neighbouring seminiferous tubules (Al Tememy 2010). Seminiferous tubules are connected to straight tubules (tubuli recti), which are continuous with a network of anastomosing channels that make up the rate testis, at their terminal segments by a transitional zone that is bordered by sustentacular cells. The rate testis has a simple squamous or cuboidal epithelium. It is encircled by the mediastinum's loose connective tissue and is drained by seven to twenty efferent ducts. The simple columnar or pseudostratified epithelium that lines the efferent ducts contains some ciliated cells. Each duct has a large lumen and a thin layer of connective tissue called the lamina propria (Aughey & Frye 2001; Fig. 4).

Table 7. Stereometric analysis of the testes and ovary of Japanese quail.

Parameter	Testes			Ovary		
	Stroma		Parenchyma	Stroma		Parenchyma
	Septa	Capsule	Spermatogenic epithelium	Septa	Capsule	Follicular epithelium
1 day	23.2 ± 3.1	21.5 ± 2.7*	55.3 ± 1.9	18.9 ± 1.9	17 ± 2.7	64.1 ± 3.4
30 days	25.2 ± 2.7	13.4 ± 3.3	61.4 ± 1.3	16.3 ± 2.1	14.3 ± 1.7	69.4 ± 1.3
60 days	18.4 ± 3.1	15.2 ± 1.3	66.4 ± 2.5	15.7 ± 2.6	12.1 ± 2.1	72.2 ± 2.4
90 days	16.8 ± 3.1*	12 ± 1.8	71.2 ± 2.3*	16.7 ± 2.3	9.7 ± 1.2*	73.6 ± 3.5

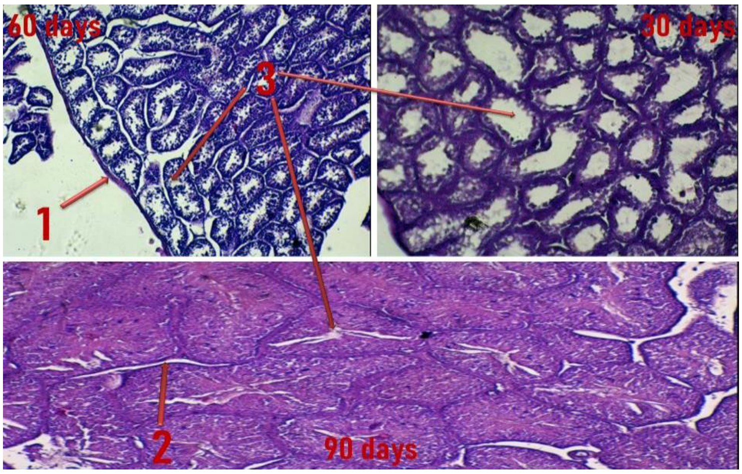

Fig. 4. Histological structure of testes in Japanese quail birds; 1. capsule, 2. Albuginea, 3. Convoluted efferent tubules (H & E; 40 X).

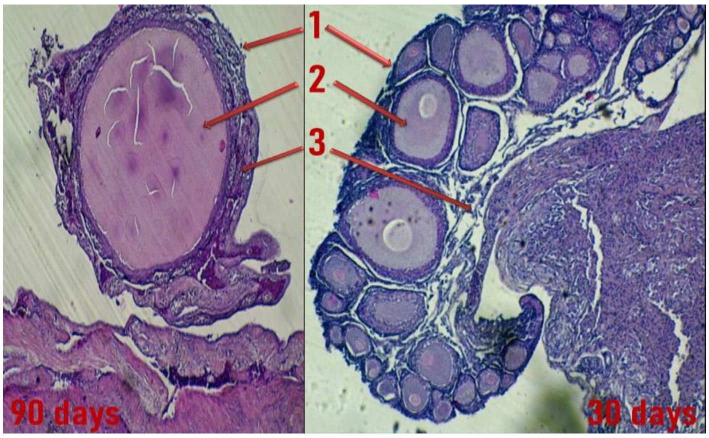

Fig. 5. Histological structure of ovary in Japanese quail birds; 1. cortical zone, 2. oocyte, 3. follicular epithelium (H & E; 40 X).

The number of follicles in ovary reaches over 900 during the oviposition stage, but they shrink to microscopic sizes between the ovipositors. The follicular epithelium stays affixed to the wall of the empty follicle following ovulation. It doesn't produce the yellow body. There are numerous blood arteries in the stroma of the medulla. It contains medullary interstitial cells and lacunae covered with single-layer epithelium (Asem *et al.* 2000; Song & Silversides 2006; Fig. 5). The ovary of birds has two main functions: the development of germ cells and the synthesis of sex hormones (Berg 2000).

CONCLUSION

The proposed new allometric equation has an average deviation of 10-15% from real-life data, which is acceptable for the development of a biomedical profile. Japanese quails have two independent champers (glandular and muscular), with the glandular section growing most intensively until 30 days and the muscular section reaching its final development only at 90 days. A direct correlation between avian reproductive health, ovarian and testicular mass is recorded. Testicular size is the primary indicator of spermatogenesis since seminiferous tubules and germinal components account for approximately 98% of the total mass of the testes. Quail mature sexually between four and five weeks of age. At around 6 weeks of age, females begin to lay. In the time between 60 and 150 days of age, the ovary develops most rapidly.

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