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RESEARCH ARTICLE

Pre-Hatch Growth and Development of Selected Internal Organs of Domestic Duck (*Anas platyrhynchos*)

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ABSTRACT

Normal growth and development pattern of selected internal organs of domestic duck (*Anas platyrhynchos*) during pre-hatch period was studied in this project. Tongue, esophagus, proventiculus, gizzard, small and large intestines, liver, kidneys, trachea, lungs, brain, eye balls and heart were studied for morphometric measurement. A total of 120 healthy fertile domestic duck eggs were equally divided into 24 groups (n=5). Eggs were weighed, labeled and incubated. All groups were examined during incubation; collection of embryos was started from day five on daily basis but recordable observations were obtained from day 10 of incubation. Means and standard errors of mass and length of selected internal organs were calculated for each age group till hatching. Growth rate of duck was measured by applying Janoscheck growth curve to age group means. Results revealed a sigmoid to exponential growth curves for organs' maturity. Growth pattern grouped organs into: eyes, brain and trachea with early rapid growth; liver and some digestive organs having intermediate growth; while heart, lungs, kidneys and esophagus attained maximum maturity towards the end of incubation. It is conceivable from the data that most of the parameters under study show complex allometry, i.e., the ratio of relative growth rates between organ and whole body changes several times however, these transitions are gradual.

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INTRODUCTION

With increasing demand for duck meat products and by products, the duck meat industry is expanding rapidly at a growth rate of 10-15% annually; a similar trend is emerging in Pakistan. There are more than 40 breeds of domestic duck. The white Pekin duck (*Anas platyrhynchos)* is the most common variety raised for eggs, meat and feather. Ducks are grown to about 7 weeks of age and make average 95 g/day with a feed conversion of under 2.15 to 1. In other words, 2.15 kg of feed is required to produce 1kg of duck meat (Stein, 2012). Size of the duck egg is 10-15 gram larger than chicken egg. Broiler /green ducks are very fast growing than chicken, with better growth rate and feed efficiency (Rajput *et al*., 2014). For a good health and profitable production of avian species, it is very important to understand embryonic development during incubation and peri-hatch time (Barri, 2008) as the ecological factors including temperature humidity and rainfall may be important factors for alteration in establishment and maturation of embryos (Guillemain *et al*., 2013).

Besides, the avian embryo provides a multifaceted model to study developmental mechanisms because of its accessibility to microsurgery, fluorescence cell labeling, in vivo imaging, and molecular manipulation (Kulesa *et al.,* 2013). Analysis of organs in terms of size, weight and shape during incubation could be important to define complex procedures of organogenesis and teratogenesis, comparison of growth among different age groups, physio-pathological responses to drugs, disease conditions and the evaluation of therapeutic drugs and environmental stresses (Arora, 2011; Johnson and Kochhar, 2012; Scanes, 2014).

There is a shortage of well characterized allometric information in the literature for assessing the pre-hatch growth in the domestic duck. Hence, this study was aimed to describe allometric growth of embryo as well as to establish growth curves using the Janoscheck growth model.

MATERIALS AND METHODS

Collection of eggs: A total of 150 fresh eggs of uniform size and weight were purchased from University Poultry Club Faisalabad, from a randomly breeding colony of domestic duck which was provided commercial duck feed *ad libitum*. During the collection of fertile eggs it was considered that age, feed, management conditions and genotype of laying ducks were uniform; however it was not possible to determine gender of egg at that time because it is rarely possible to differentiate between the sexes of duckbills till 3rd week of life (Krause, 1992).

Incubation: The selected 120 eggs were cleaned with dry muslin cloth, labeled and divided into 24 groups at random such that each group contained five eggs. All eggs were weighed to nearest 0.01 g with an electric balance before setting in incubator. Eggs were randomly distributed in a single tray of incubator according to treatment replication and incubated (37°C with 65-70% of relative humidity). Position of eggs was sharp end down and they were automatically turned after every half an hour along the entire course of incubation.

Collection of embryos: Starting from day 5th of incubation, one group of eggs per day was randomly removed from incubator up to day 28th. These eggs were weighed and broken out into a petri-dish containing normal saline solution according to Peebles *et al*., 1998. Embryos which looked abnormal or retorted were disposed off.

Collection of organs: The embryos were incised and internal organs were collected. The tongue was measured from base to the apex while width from 3 different sites like base, body and apex then average value was taken. Whole digestive tract from esophagus to anus was cut apart. Esophagus was cut out near to its point of entry into gizzard. Trachea was cut at the point of bifurcation into bronchi and its length was recorded from syrinx to the point of bifurcation. Liver was kept for some time before weighing to remove the blood if any. Heart was removed by cutting major blood vessels from their base. Pericardium and fat were also excised. The fibrous and fatty tissue was cut aside from both of the kidneys and they were taken out from the sockets. Weights of all selected internal organs were measured carefully with an electric balance up to nearest milligrams. Length of esophagus and trachea, and length and width of tongue was measured by Vernier caliper (mm). Janoscheck growth curve following Gille and Salomon, 1999 was fitted to age group means by non-linear regression procedure of Mondragon and Borchers (2005).

$$W = A - (A - W_0). \text{Exp} (- k . t^p)$$

In the above function, W is the corresponding weight, which is measured in grams, at time t, measured in days. A is the asymptotic value (in g), W_0 the weight at birth (t=0) and k and p parameters determining the ascent and shape of the growth curve. The values of A, k and p can be judged by previous study. The co-ordinates of point of inflection for the Janoscheck growth curve are

$$t = ((p - 1) / (p \times k))^{1/p} \quad \text{and}$$
$$W_i = A-(A-w_0)*\text{Exp} (-(p-1)/p)$$

Statistical analysis: Statistical analysis of the data was carried out according to statistical procedures of Snecdecor and Cochran (1991). The level of significance was ≤ 0.05. The growth curve of domestic duck was measured by applying Janoscheck growth curve.

RESULTS

In this study, domestic duck's embryos and its internal organs were studied for changes in their masses and dimensions (length and width) as the course of time. The Janoscheck growth curve was applied to weight, length and width of different parts of digestive system and some selected internal organs including brain, eye, heart, trachea, lungs, kidneys and whole embryo weight of the domestic duck (*Anas platyrhynchos*).

The curve based on measured and predicated means and result growth curves for each organ studied are graphically illustrated in Fig. 1. Results of current study showed that Janoscheck growth curve provided best fit model and body growth data in domestic duck. In addition, characteristics parameters of growth curve of all organs were studied. Selected growth curve characteristics are presented in Table 1 and 2. A close scrutiny of the data revealed following growth patterns:

1. Body mass as well as most of the organs showed a sigmoid growth pattern.
2. From both age–related and allometric patterns, four basic growth patterns can be deduced. Central nervous system, exponential type, body mass associated type, and wing type.
3. Some of the digestive organs, however, showed exponential growth. They are characterized by positive allometry.
4. Parameters with similar percentage growth compared to body mass exhibit isometry. This is present in organs like kidneys and heart.

Some of the organs showed complex trend and the ratio of relative growth between organ and whole body changed several times.

DISCUSSION

The Janoschek growth curve provides an excellent fit to the most diverse growth data (Gille and Salomon, 1995). Its flexibility is similar to that of the Richards model and allows application to most sigmoid and exponential growth courses. Contrary to three-parameter models as the Gompertz, logistic, or Bertalanffy growth curves, the ratio of inflection ordinate and asymptote are flexible for both the Janoschek and the Richards growth curve. Because initial parameter estimates are easily obtainable and procedural problems rarely occur, we therefore preferred the Janoschek growth curve.

Weights of most of the selected internal organs like brain, eye, heart, kidneys as well as lengths of tongue, esophagus and trachea showed a consistent growth with the growth of the embryo weight and exhibited a sigmoid curve pattern. Width of tongue, weights of proventriculus, gizzard, intestines, liver and lungs however, presented

exponential type of growth curve. Similar growth curves were obtained by Arora (2011) for the internal organs of *Coturnix japonica*.

On the basis of point of inflection (POI), we can classify these organs into three distinct types; organs with early POI (day 12-17) including eye, tongue, brain and trachea; organs with late POI (day 24-28) including heart, lungs, kidneys and esophagus; organs like proventiculus, gizzard, intestines and liver were found in between two categories i.e., POI (day 20-23).

The brain showed a rapid growth next to eyes. The curve showed a sigmoid or divergent trend and the point of inflection was near to hatch. Brain weight showed slightly negative allometry during entire course of incubation. The brain size increased rapidly as compared to embryo weight in the end stages of incubation. Gille *et al*. (2000) supported that high growth rate of brain along with prolonged incubation period was the predisposing factors for high cerebralization of *Anserifom* species. In fact the anseriform birds (ducks and geese) are precocial (e.g., hatchlings feed on their own) in which major neurogenesis completes before hatching and unlike parrots and song birds evolve a disproportional telencephalon without delaying telencephalonic neurogenesis (Iwaniuk and Hurd, 2005).

The eyes developed and grew in weight quite fast during the early periods of incubation and exhibited very early point of inflection i.e., day 16. Up till day 10, they were the most prominent and the heaviest organs of the embryo. After day 17, they gained weight comparatively at a slower rate. The higher growth rate of brain when compared to eye weight in late incubation combined with prolonged incubation time probably enables the high cerebralization in Anseriform species where the nucleus basalis and the complexus paleostriatus are well developed (Starck, 1993). Gille *et al.* (2000) also reported a similar pattern in embryonic geese.

Like eye and brain, heart was the most prominent tissues in the domestic duck embryo. Heart presented quite high rate of development from day 8 as presented by Chan and Burggren (2005) and Yang and Siegel (1998) in chick embryos. This higher percentage might be due to its role as supply organ in the body. The storage of glycogen by the heart may also be contributing in its weight as reported by Boerjan (2005). It kept on gaining its mass with the increase in the mass of embryo and time of incubation in sigmoid fashion. This trend of weight gain was in agreement with the findings of Yang and Siegel (1998). Just before hatching, heart weight did not increase as the embryo weight (Yang and Siegel, 1998).

The lungs were the paired organs. They did not demonstrate any early fast pace of development. The statistical result predicted that it got mature towards the end of incubation period. Its curve reached to its maximum towards day 25 of incubation. On the other hand trachea developed on sigmoid growth pattern. Another paired organ was kidneys. They are one of the organs that reached maturity at the end time (day 26) of hatch. They acquired a declined growth rate post 18 day.

The digestive organs were one of the fastest growing organs in the embryo. Gizzard, liver and proventriculus exhibited accelerated rate. The growth curves of all these three organs followed almost a straight line. Proventriculus, though it was a rapidly growing organ, its growth rate was not appreciable between day 10 and day 15. Almost similar trend was expressed by liver and gizzard. As liver and heart are supply organs, their growth was recorded quite high. Similar findings were reported by Christensen *et al*. (2002) in broiler embryos. Liver showed stability in weight gain towards the time of hatching and point of inflection as recorded as late as 20 days. Similarly, poultry at day 19 and turkey at day 25 showed a consistent growth rate (Sell *et al*., 1991). Yang and Siegel (1998) had also documented that liver weight did not increase appreciably as the embryo weight increased just before hatch.

Uni *et al.* (2003) reported regarding the weight of intestines that it weighs approximately 1% of the total body weight at day 17 in chicken and this percentage increased up to 3.5% toward the end of incubation. Regardless of the rapid late growth rate of the intestines, many other studies indicated that intestines get full maturity after hatch. The immaturity of intestines was also reported by Iji (2008) at the time of hatch in embryonic ostrich. Sell *et al*. (1991) reported that the small intestine in turkeys undergoes considerable development during incubation but it is still immature at hatch. The immaturity of all digestive organs was also reported by Ravindran (2003) even at the time of hatch in broiler chicken. Uni *et al*. (1995) stated that the late rapid developmental rate was found in all parts of the intestine including liver and pancreas. Later, Tong *et al.* (2013) reported a linear pattern of increasing embryonic weight followed by aggressive growth during last 48 hours before hatching when duodenum weight doubled in chickens. This change indicates that a great portion of embryonic investment in intestinal tissue is concentrated during the last 48 hours before hatching, probably in preparation for post hatch digestion of nutrients. In order for independently forage and digest food immediately after hatch, birds must develop before hatch the necessary complement of digestive enzymes and mucosal maturity with the absorptive capacity to use ingested feed. This prenatal development of digestive capacity increases as the avian embryos orally consume their amniotic fluid before hatch (Uni and Ferket, 2004; Moran, 2007). The nutrients within the amnion comprise the first meal of the embryo and along with yolk infusion into the intestine (Esteban *et al.*, 1991; Noy *et al.*, 1996); they facilitate enteric development toward hatch (Uni *et al.*, 2003).

Regarding maturity; brain, eye, tongue, esophagus, and trachea were among the most matured organs at hatching while lungs, kidneys and gizzard were the least. The maturity of lungs was predicted toward the end of incubation. At day 10 of incubation heart, gizzard, liver, kidneys and intestines were less mature organs (<5%) of the duck embryo compared to eye (11.682%), brain (12.251%), tongue (15.931%), esophagus (64.128%) and trachea (64.357%).

Throughout the incubation period, weight of the embryo was directly proportional to age of the embryo. Bruzual *et al*. (2000) in broiler and Arora (2011) in Japanese quail (*Coturnix japonica*) embryo had also reported a positive relationship between them.

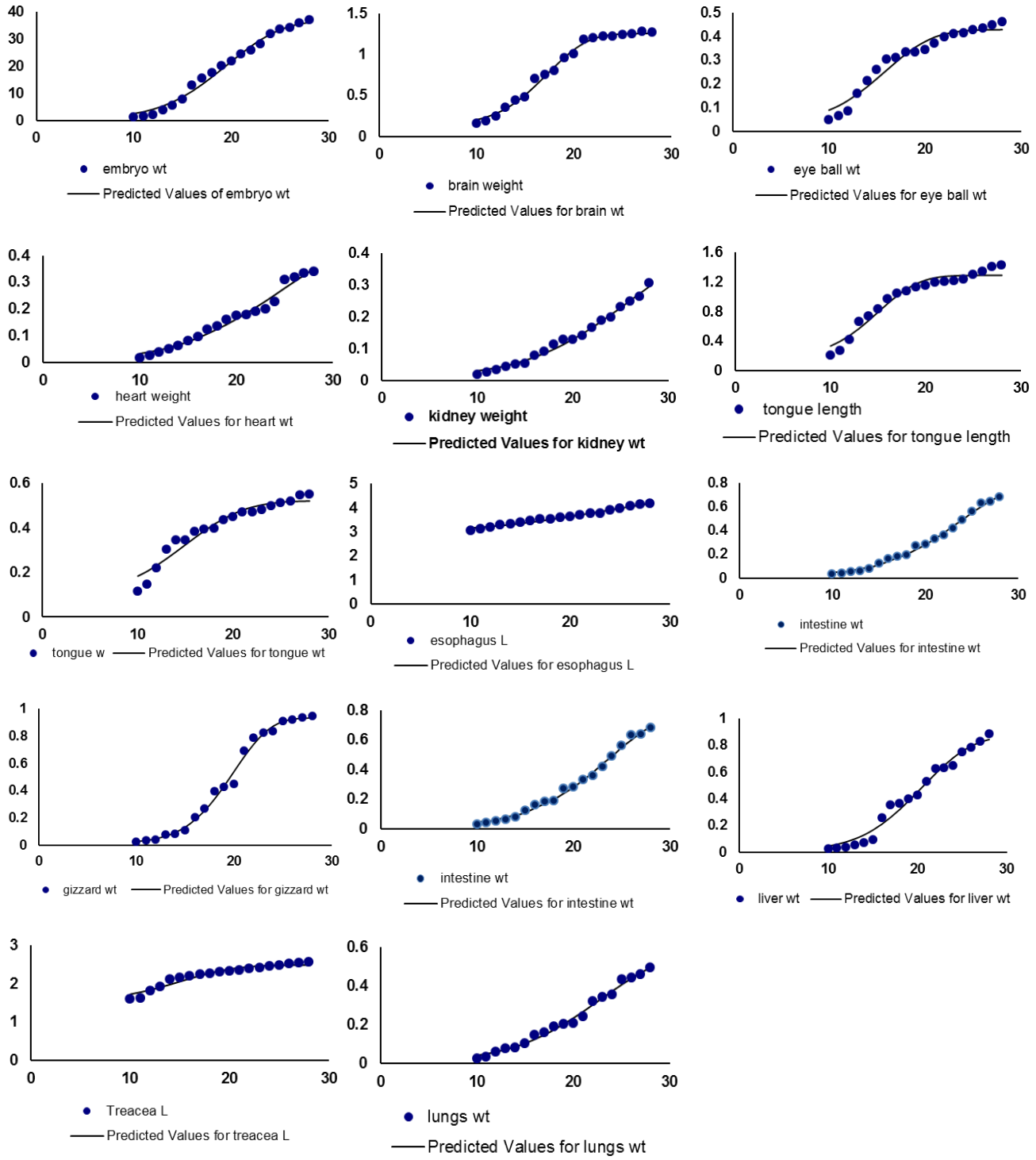

Fig 1: Graphical presentation of measured (·) and predicted mean values (—) and resulting growth curves of selected internal organs of domestic duck (*Anas platyrhynchos*).

Table 1: Characteristics parameters of growth curve of organs weight

Organs	A	$W_{0\,(g)}$	$W_{i\,(g)}$	$\mu_{o(\%)}$	$\mu_{i(\%)}$	$T_{i(days)}$	R^2
Embryo	36.775	1.31	20.483	3.562	55.698	20.347	0.98
Brain	1.257	0.154	0.765	12.251	60.861	17.324	0.99
Eye	0.428	0.05	0.251	11.682	58.740	15.974	0.95
Heart	0.547	0.016	0.283	2.925	51.831	28.177	0.97
Kidney	0.497	0.018	0.263	3.621	53.034	26.776	0.99
Proventriculus	0.286	0.016	0.161	5.594	56.517	22.834	0.99
Gizzard	0.932	0.024	0.541	2.575	58.089	22.834	0.99
Intestine	0.851	0.032	0.473	3.760	55.665	23.688	0.99
Liver	0.873	0.024	0.485	2.749	55.633	20.713	0.97
Lungs	0.606	0.024	0.329	3.960	54.315	24.335	0.99

A=Asymptomatic value in grams; W_0 = weight at 10th day of incubation; Wi=weight at the point of inflection; ti=age at the point of inflection; μ_o=Degree of maturity at day 10; μ_i=Degree of maturity at the point of inflection.

Table 2: Characteristic parameters of growth curve of organs length/width

Organs	$A_{(cm)}$	$W_{0\ (cm)}$	$Wi_{(cm)}$	$u_{o(\%)}$	$u_{i(\%)}$	$T_{i(days)}$	R^2
Tongue length	1.293	0.206	0.792	15.931	61.319	15.240	0.95
Tongue width	0.522	0.116	0.321	22.222	61.633	14.947	0.94
Esophagus length	4.781	3.066	3.880	64.128	81.172	24.442	0.98
Trachea length	2.483	1.598	2.058	64.357	82.903	16.263	0.93

A=Asymptomatic value in grams; W_0 = weight at 10th day of incubation; Wi=weight at the point of inflection; ti=age at the point of inflection; μ_o=Degree of maturity at day 10; μ_i=Degree of maturity at the point of inflection.

Various organs accounted for different percentage from day 10 of incubation till the time of hatching. This was confirmed by the findings of Katanbaf *et al*. (1988) in layers and guinea fowls (Gosomji *et al.,* 2015). The weight percentage of the organs kept on changing at various stages because they had different growth rates. While working on turkey, Fan *et al*. (1998) reported that this depends upon the need and strategy of the growth. They considered this type of growth and development model as a survival strategy.

Conclusions: On the whole, it is inferred that most of the parameters under study showed complex allometry, i.e., the ratio of relative growth rates between organ and whole body changes several times however, these transitions are gradual.

Authors' contribution: ASQ designed the project, supervised lab work and finalized manuscript. Ziaullah, MZA and AM performed laboratory sampling, statistical analysis and prepared preliminary write up.

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