DYNAMICS OF THE OVIDUCT MICROMORPHOMETRY OF JAPANESE QUAIL, IN RELATION WITH LAYING CURVE PHASE

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Abstract

In laying hens hybrids, the morpho-physiological bases of table eggs production are well known. Within this context, the dynamics of the morphological and histometrical traits of the oviduct were investigated in another avian species of interest for poultry farming – Japanese quail, in relation with laying curve stages. A population of 400 females was studied, throughout the 8-35 weeks age span. During the 4 key moments of the laying curve - beginning (9 weeks), peak (20 weeks), plateau (26 weeks), ending (35 weeks) – 7 quails were slaughtered in order to sample the oviducts and to investigate them morphologically and histometrically on cross sections cut through magnum and uterus functional the processed through subsequent paraffin inclusion technique and trichrome staining (HEMB). The microscopic investigations run on 20 readings per trait and anatomical region, via MoticImages+3.0 software connected to a Motic M230 microscope, revealed that both mucosa thickness, folds, and size of its epithelial cells in magnum and uterus followed a developmental trend in accordance with the oviduct functional rhythm, overlapped with the apparent laying curve (initial slow increase, then strong hypertrophy during peak of production and a gradual decreasing throughout plateau, followed by atrophy and discontinuities of the epithelium towards the end of laying). Mucosa thickness varied between 1379 µm (beginning of lay) and 1462 µm (peak) in the albuminogenic tract and between 2003-2154 µm in the shell gland (beginning – peak), while the laying rate varied from 45% (beginning) to 85.4% (peak), to 71.3% (plateau) and to 52.75%, towards the end of laying.

Key words: Japanese quail, oviduct, laying curve, magnum, uterus, histometry

INTRODUCTION

The oviduct is a muscular-membranous, sinuous cavity organ that connects the ovary to the cloaca and is intended for fertilization of the egg, transport and deposit of egg layers over the yolk (4 layers of albumen, 2 shell membranes, the mineral shell itself and the cuticula) [14, 19]. In fowl, there is a single functional oviduct (the left one) that forms from the left Muller canal during embryonic development [2], while the right side of the reproductive system gradually atrophiasis till hatching. The oviduct is formed, in the cranio-caudal direction, by five segments: infundibulum, magnum (albuminogenic chamber), isthmus (isthmus), uterus (shell chamber) and vagina [18, 21]. Magnum is the longest part of the oviduct with a role in the secretion and deposition of egg white layers [7]. The development of this segment is based on the increase in the thickness of the mucosa, as laying becomes more intense, it consists of primary folds, on which are arranged the secondary and tertiary folds that have the role of increasing the secretory surface of tubular glands cells deep within the mucosa [15]. The surface epithelium is prismatic, containing ciliated cells and cup-shaped secretory cells [13]. The musculosa of this segment is highlighted by the organization of bands of

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muscle fibers parallel to the spiral direction, favoring the advance of the egg in formation in the albuminogenic chamber and causing the secretion from the outside of the epithelium of albumen layers and of the retinacules (challazes).

Then, the same muscle fibers push in a peristaltic-rotational manner the egg into the isthmus [20]. The uterus (shell chamber) is a dilated segment of the oviduct, the site of mineral shell formation. The mucosa has rare leaf-like folds, which give the mucous tunics a discontinuous appearance. The secretory glands are developed deeper in mucosa and secrete uterine fluid, rich in matrix proteins of the shell, but also in a concentrated solution of calcium and magnesium carbonate, which will deposit in the form of crystals, around the protein matrix, on the foundation represented by the outer shell membrane [16, 17].

There are several studies realized on laying hens [3, 4] that report on the dynamics of the oviduct layers development in relation with laying intensity.

The higher size of such elements (mucosa, submucosa, musculosa, serosa) was observed in laying peak [6].

Within this context, the aim of this study was to assess the morpho-histometric peculiarities of Japanese quail oviduct, especially of its most active segments (magnum and uterus), in relation with the laying intensity, that could vary between 79-91 % during the laying peak, under the influence of several factors (genetics, feeding, thermal stress) [1, 10, 14, 22].

MATERIAL AND METHODS

Hypothesys: the morphology and histology of the oviduct segments magnum and uterus vary in accordance with laying intensity.

Biological material: 400 Japanese quails (*Coturnix coturnix japonica*) raised in cages for laying, aged 8-36 weeks, fed on standard laying diet.

Twenty-eight individuals were slaughtered (7 quail x 4 laying moments) to sample oviducts: laying onset (aged 9 weeks); peak (aged 20 weeks); plateau (26 weeks); laying end (35 weeks).

Sample processing and analysis:

* Histological sampling from magnum and uterus from each 7 oviducts/laying curve moments;

* Preparation of 56 histological smears through paraffin inclusion technique and HEMB staining [5];

* Microphotography reading on Motic M230 microscope;

* Cyto-histometry measurements (n=20 readings/studied element/laying moment): mucosa thickness; length of mucosa villi; height of epithelial cells; musculosa thickness; serosa thickness [12].

Data processing (GraphPad Prism 9.3.1 software & Ms.Excel 2016): Descriptive statistics [8]; Charts of the values of oviduct morphological elements in relation with laying curve.

RESULTS AND DISCUSSIONS

Laying dynamics. In the studied quail population, sexual maturity was reached at the age of 9 weeks, and egg yields of 185-192 pieces was obtained daily, which resulted in an intensity of laying, at the beginning of laying, from 46.52% to 48.00%, with a weekly average of 44.96% (table 1, fig. 1). Throughout the peak of laying (quails aged 20 weeks), the daily yield ranged between 339 and 345 eggs, hence a percentage of laying between 84.75 and 86.25, respectively an average of 85.43%.

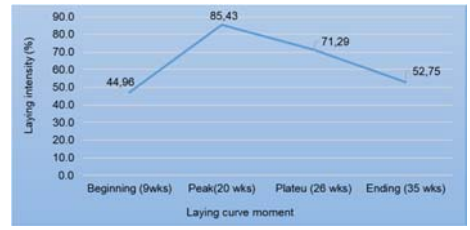


Fig. 1 Dynamics of laying
in the studied quails population

Throughout the plateau period (quails aged 26 weeks), the daily yields ranged between 288 and 283 eggs, resulting a laying intensity between 70.75-72.00, with an average of 71.29%. At the end of laying (quails age 35 weeks), daily yield was

comprised within the 209-313 eggs interval, resulting in a percentage of laying between 53.25 and 52.25, with an average of 52.75%.

Table 1 Dynamics of laying curve, in relation with its key moments

Day of data collection	Beginning (age 9 weeks)		Peak (age 20 weeks)		Plateau (age 26 weeks)		Ending (age 35 weeks)	
	Laid eggs	% laying	Laid eggs	% laying	Laid eggs	% laying	Laid eggs	% laying
d1	185	46.25	339	84.75	288	72.00	213	53.25
d2	187	46.75	340	85.00	287	71.75	212	53.00
d3	188	47.00	341	85.25	286	71.50	212	53.00
d4	189	47.25	341	85.25	285	71.25	211	52.75
d5	190	47.50	342	85.50	284	71.00	210	52.50
d6	191	47.75	344	86.00	283	70.75	210	52.50
d7	192	48.00	345	86.25	283	70.75	209	52.25
Week average		**44.96**		**85.43**		**71.29**		**52.75**

Morphohytometry of magnum.

Whole mucosa. At the beginning of laying (9th week of life), the magnum mucosa had a thickness between 1343.4 and 1392.6µm (average of 1379.8±2.55µm) (table 2, fig. 2). As the laying intensified, the mucosa became much more biologically active, reaching a thickness between 1423.3 and 1487.2µm (average at the peak of laying of 1462.6±3.49µm; 6.00% more developed than in the beginning). In the plateau stage laying, the size of the magnum mucosa thickness was measured between 1402.2 and 1458.8 µm, obtaining an average of 1422.8±2.71 µm, respectively 3.11% more developed than the beginning of laying. In age week 35 (end of laying), the magnum mucosa had a thickness between 1361.8 and 1464.8µm, (average of 1394.6±3.42µm, +1.07% thicker than on the laying onset).

Mucosa folds. At the beginning of laying (9th week of life), the length of the magnum mucosa folds varied between 1301.2 and 1322.2µm, resulting an average of 1306.1±1.01µm. As the laying intensified, the folds of the mucosa became more biologically active, reaching a thickness between 1405.2 and 1425.4µm, resulting an average during egg-laying peak of 1416.2±1.34 µm. Thus, compared to the beginning of laying, the length of the mucosa folds was 8.43% higher. In the plateau phase of laying, the size of the folds was between 1351.2 and 1395.4µm, obtaining an average of 1373.6±2.77 µm (+5.17% longer than in the beginning of laying). In week 35, towards the end of laying, the length of the magnum mucosa folds varied between 1321.4 and 1362.4µm, obtaining an average of 1343.0 ± 2.91µm (+2.83% higher vs. laying beginning).

Mucosa epithelial cells. The cells are prismatic cilliated, aspect observed as well in other studies [9]. During laying onset, the height of the epithelial cells of the magnum had a size between 15.2 and 27.4µm, resulting an average of 22.4±0.78 µm. As egg production increased, the epithelial cells of the magnum became more biologically active, reaching a height of 16.2-29.6µm, with a week average, at the peak of laying (week 20), of 25.3± 0.96µm (12.94% more developed than at the beginning). During the plateau period (week 26), the height of the epithelial cells of the magnum was measured between 11.2 and 29.4µm, reaching an average of 25.1±1.03µm, respectively +12.05% more developed than on laying onset. During week 35, towards the end of laying, the height of the mucosa epithelial cells varied between 12.4 and 28.6µm, obtaining an average of 24.9±1.12µm (+11,2% vs. laying onset).

Musculoasa. At the beginning of laying (age 9 weeks), musculosa had a thickness between 15.4 and 26.2µm, resulting in an average value of 21.1 ± 0.67µm (table 2, fig. 2). As the laying intensified, the musculature

became thicker and varied between 15.7µm and 28.4 µm, with a weekly average during the laying peak of 22.8 ± 0.76 µm. Compared to the laying onset, the magnum musculosa was 8.05% more developed. In the plateau phase (week 26), the size of the magnum musculosa was between 16.3-28.9µm, obtaining an average of 21.7±0.78µm, resulting in a degree of development 2.84% higher compared to the laying onset. In week 35 (end of laying), the magnum musculosa varied between 16.1-27.9µm, obtaining an average of 21.4 ± 0.78µm (+1.24 thicker than in the beginning).

Table 2 Dynamics of magnum structural elements, in relation laying key moments

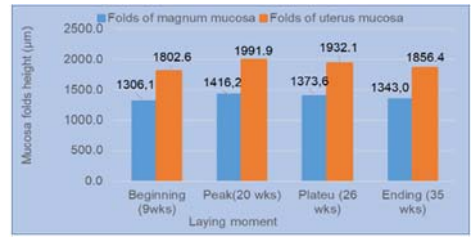
Assessed trait	Statistical descriptors	Beginning (9 weeks)	Peak (20 weeks)	Plateau (26 weeks)	Ending (35 weeks)
Whole mucosa thickness	Mean (µm)	1379.80	1462.60	1422.80	1394.60
	±StdDev (µm)	11.40	15.60	12.10	15.30
	±MeanStdError (µm)	2.55	3.49	2.71	3.42
	Variation (%)	0.83	1.07	0.85	1.10
Mucosa folds height	Mean (µm)	1306.10	1416.20	1373.60	1343.00
	±StdDev (µm)	4.50	6.00	12.40	13.00
	±MeanStdError (µm)	1.01	1.34	2.77	2.91
	Variation (%)	0.34	0.42	0.90	0.97
Mucosa epithelial cells height	Mean (µm)	22.40	25.30	25.10	24.90
	±StdDev (µm)	3.50	4.30	4.60	5.00
	±MeanStdError (µm)	0.78	0.96	1.03	1.12
	Variation (%)	15.63	17.00	18.33	20.08
Musculosa thickness	Mean (µm)	21.10	22.80	21.70	21.40
	±StdDev (µm)	3.00	3.40	3.50	3.50
	±MeanStdError (µm)	0.67	0.76	0.78	0.78
	Variation (%)	14.22	14.91	16.13	16.36
Serosa thickness	Mean (µm)	12.90	13.70	13.50	13.50
	±StdDev (µm)	1.90	2.80	2.50	2.50
	±MeanStdError (µm)	0.42	0.63	0.56	0.56
	Variation (%)	14.73	20.44	18.52	18.52

Serosa. At the beginning of laying, the serosa had a thickness between 10.2-18.2µm, resulting an average value of 12.90±0.42µm. As the egg-laying intensified, the serosa became thicker, reaching 11.1-18.6µm, with a weekly average during the laying peak period of 13.7±0.63µm (+6.2 % from the laying onset). In the plateau phase (week 26), the thickness of the serosa varied between 11.8 and 17.6µm, obtaining an average of 13.5±0.56µm (degree of development 4.65% higher than the moment the beginning of laying). In week 35 (end of laying), the serum of the magnum had a size between 11.6 and 17.4µm, obtaining an average of 13.5±0.63µm.

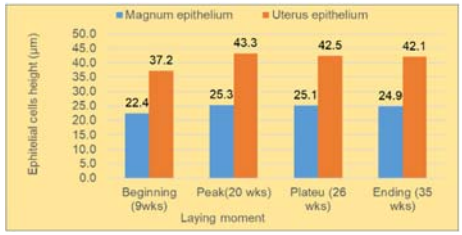
Morphohystometry of uterus

Whole mucosa. At the beginning of laying (age 9 weeks), the lining of the uterus had a thickness between 1995.4 and 2009.2µm, resulting in an average value of 2003.4±3.2µm (table 3, fig. 2). As the laying intensified, the mucosa became more biologically active, reaching a thickness between 2122.2 and 2182.2µm and a weekly average during the laying peak period of 2154.2±3.85µm. Thus, compared to the time of laying, the lining of the uterus was 7.52% more developed.

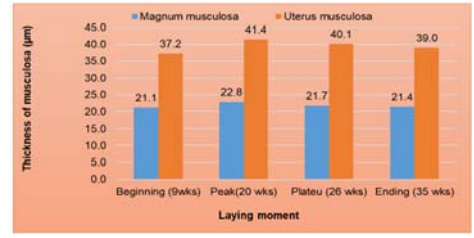
a) Whole mucosa thickness



b) Mucosa folds height



c) Epithelial cells height



d) Musculosa thickness

e) Serosa thickness

Fig. 2 – Dynamics of oviduct wall elements size, in relation with laying curve

Table 3 Dynamics of uterus structural elements, in relation laying key moments

Assessed trait	Statistical descriptors	Beginning (9 weeks)	Peak (20 weeks)	Plateau (26 weeks)	Ending (35 weeks)
Whole mucosa thickness	Mean (µm)	2003.40	2154.20	2100.40	2005.60
	±StdDev (µm)	14.30	17.21	13.84	15.65
	±MeanStdError (µm)	3.20	3.85	3.09	3.50
	Variation (%)	0.73	0.81	0.69	0.72
Mucosa folds height	Mean (µm)	1802.60	1991.90	1932.10	1856.40
	±StdDev (µm)	6.11	8.09	13.66	14.38
	±MeanStdError (µm)	1.37	1.81	3.05	3.22
	Variation (%)	0.34	0.41	0.71	0.77
Mucosa epithelial cells height	Mean (µm)	37.20	43.30	42.50	42.10
	±StdDev (µm)	6.18	8.25	7.38	6.91
	±MeanStdError (µm)	1.38	1.84	1.65	1.55
	Variation (%)	16.61	19.05	17.36	16.41
Musculosa thickness	Mean (µm)	37.20	41.40	40.10	39.00
	±StdDev (µm)	4.61	5.29	4.82	5.08
	±MeanStdError (µm)	1.03	1.18	1.08	1.14
	Variation (%)	12.39	12.78	12.02	13.03
Serosa thickness	Mean (µm)	17.80	18.20	18.10	18.00
	±StdDev (µm)	2.55	2.93	2.71	3.08
	±MeanStdError (µm)	0.57	0.66	0.61	0.69
	Variation (%)	14.33	16.10	14.97	17.11

Throughout the plateau phase (week 26), the size of the lining of the uterus was between 2079.8 and 2121.7µm, obtaining an average of 2100.9±3.09µm, resulting in a degree of development 4.82% higher compared to the onset of laying. In week 35 (end of laying), the lining of the uterus had a thickness between 1997.4 and 2017.5µm, with an average of 2005.6±3.5µm.

Mucosa folds. At the beginning of laying (9th week), the length of the folds of the lining of the uterus was between a minimum of 1795.2µm and a maximum of 1808.2µm, resulting in an average value of 1802.6±1.37µm. As egg production intensified, the length of the mucosal folds increased, reaching 1982.6-1999.2µm, with a weekly average during the peak laying period of 1991.8±1.81µm. The length of the folds of the lining of the uterus was more prominent by 10.49%, compared to the time of laying. During the plateau period, the length of the folds of the uterine mucosa was between 1922.4 and 1941.2µm, obtaining an average of 1932.10±3.05µm, reaching a degree of development 7.18% higher than at the beginning of laying. In week 35 (end of laying), the length of the folds of the lining of the uterus varied between 1848.6-1883.6µm, obtaining an average of 1856.4±3.22µm.

Mucosa epithelial cells. The epithelium was observed as prismatic ciliated, uni-layered or pseudo-layered, suggesting the great plasticity of the uterus in modifying its volume, in relation with egg passage, aspects observed as well in other studies (11). At the beginning of laying, the height of the epithelial cells of the uterus varied between 32.9 and 43.8µm, resulting in an average of 37.2±1.38µm. As egg production increased, the height of epithelial cells ranged from 39.6 to 46.1µm, reaching a weekly average, at the peak of laying, of 43.1±1.84µm (16.39% more developed than at the beginning of laying). During the plateau period (week 26), epithelial cells size was between 37.4 and 45.8µm, reaching an average of 42.5±1.65µm (14.24% higher degree of development compared to laying onset). Towards the end of laying (week 35), the height of the epithelial cells of the uterus varied between 36.8 and 44.3µm, obtaining an average of 42.10±1.55 µm.

Musculosa. At the beginning of laying, the muscular layer had a thickness between 33.9 and 41.8µm, resulting in an average value of 37.2±1.03µm. As the laying intensified, the muscle of the uterus became stronger, reaching a thickness in the range of 38.6-46.5µm, with a weekly average of 41.40±1.18 µm (Table 3, Fig. 2) (+11.29 % vs. laying onset). In the plateau phase, the thickness of the uterine muscles ranged between 37.4 and 43.8µm, obtaining an average of 40.10±1.08µm, resulting in a degree of development 7.80% higher than at the beginning. In week 35 (end of laying), the musculosa thickness varied between 37.6 and 42.5µm, obtaining an average of 39.00±1.14µm.

Serosa. At the beginning of laying, the uterine serosa had a thickness between 13.4 and 16.2µm, resulting in an average value of 12.39±0.57µm. At the peak of production, the thickness varied between 15.8 and 20.1µm, with a weekly average of 18.20±0.66µm. Thus, compared to the time of onset, the uterus serosa was 46.89% more developed. In the plateau phase, the thickness of the serosa ranged between 15.1 and 22.2µm, obtaining an average of 18.1±0.61µm (+46.1% compared to the beginning of laying). In week 35 (end of laying), the uterine serosa had a size between 14.7 and 21.8µm, obtaining an average of 18.0±0.69µm.

CONCLUSIONS

The dimensional dynamics of magnum and uterus wall elements (mucosa, musculosa, serosa) overlaps on the laying curve shape, therefore the hypothesis of the study was confirmed.

Higher modifiable structural element was mucosa. In magnum, mucosa folds were thicker, more compact and less numerous than in Uterus, where they were longer, thinner, in higher amount and resembled to intestinal villi.

In magnum, the epithelium is one layered, cylindrical ciliated, secretor type, while in uterus, the epithelium is both cylindrical uni-layered or pseudo layered, which indicates a higher plasticity of the mucosa and higher secretory activity (potential for more intense cell lysis and regeneration). Also, this uterus particularity allows this segment to easily modify its shape and volume, in relation with the physiological status (void uterus or uterus filled with egg).

Towards the end of laying, the epithelia degenerated, indicating thus and accentuated erosion of mucosa, especially in uterus. These findings explain the more frequent occurrence of eggs with shell anomalies towards the end of laying.

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