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Functional, Anatomical and Histological Development of Caecum in Rabbits

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ABSTRACT

Rabbit caecum is the largest digestive compartment of Gastrointestinal Tract (GIT) representing a distinct organ for fermentation. This study aimed to investigate development of rabbit caecum with age progress from 3 up to 16 weeks. New Zealand White rabbits were slaughtered at 3, 4, 6, 8, 12 and 16 weeks of age (3 rabbits at each age) to determine fermentation parameters and anatomical and histological characteristics of caecum in relation with growth performance. With age progress, Live Body Weight (LBW), feed intake and weight gain increased ($p<0.05$). At 3 weeks, Volatile Fatty Acids (VFA) was lowest (25.33 mmol L^{-1}) while ammonia-N and pH were highest (13.0 mmol L^{-1} and 7.33, $p<0.05$). VFA doubled at 4 weeks, peaked at 6-8 weeks and decreased at 12-16 weeks ($p<0.05$). Ammonia-N and pH was lowest at 6 and 8 weeks (8.0 mmol L^{-1} and 5.47, $p<0.05$). Caecal Tissue Weight (CTW) was lightest (2.3 g) at 3 weeks and heaviest (33.2 g) at 16 weeks. CTW relative to GIT reduced from 22% at 3 weeks to 16.1% at 16 weeks and increased relative to LBW from 1.5% at 3 weeks to be highest (1.9%) at 8 weeks ($p<0.05$). There was increase in caecal length from 14.2 cm at 3 weeks to 42.1 cm at 12 weeks and in its width from 0.8 cm at 3 weeks to 3.9 cm at 6 weeks ($p<0.05$). Thickness of tunica musculosa, submucosa and mucosa as well as lamina epithelialis mucosa increased at 3-4 weeks and again at 12-16 weeks ($p<0.05$). The current study indicated dramatic changes of anatomical, histological and functional characteristics of rabbit caecum between 3 and 4 weeks. The complete physiological development of rabbit caecum was evidence at 12 weeks of age.

Key words: Rabbit, caecum, development, volatile fatty acids, ammonia-N, anatomy, histology

INTRODUCTION

The pre-weaning period of rabbits is a very critical phase because of the change from milk to an exclusively solid feed intake. Also the digestive system changes from an exclusively hydrolytic system by animal enzymes in the small intestine, to an endogenous digestion followed by a bacterial fermentation in the caecum. Knowledge is essential to determine the nutritional requirement of young rabbits around weaning when they are more sensitive to digestive disorders (Gallois *et al*., 2004). There is an increase of the knowledge in gut morphology changes in young rabbits (Sabatakou *et al*., 1999; Gutierrez *et al*., 2002). However, some difficulties were subsist to evaluate the respective roles of intrinsic factors such as age and extrinsic factors such as feed. So, the understanding of the gut maturation is essential to determine the nutritional requirement of young rabbits around and post-weaning.

In the rabbit, the caecum is colonized by an abundant bacterial flora, mainly strictly anaerobic cellulolytic bacteria that develop strongly around 3 weeks of age and stabilize a few days after weaning (Padilha *et al*., 1995). Increasing feed intake will increase hindgut fermentation and activate microbial metabolism which may stimulate the development of the caecal and colonic weights. The development in the hindgut follows the small intestine which reaches maturity at 8 weeks (Yu and Chiou, 1997). Caecal Volatile Fatty Acids (VFA) level and pH value are largely dependent of the dietary composition and the speed of the intestinal transit (Bellier, 1994). Data concerning the evolution of caecal contents composition and the morphological and histological development of the rabbit caecum are rare.

Changing in characteristics of the caecal contents in rabbits was studied at young ages. The biological significance may be related to the change in digestive weight gain with age progress (Bellier, 1994).

Therefore, the objective of the current study was to investigate developmental age differences in fermentation parameters, anatomical characteristics and histological structure of rabbit caecum at 3, 4, 6, 8, 12 and 16 weeks of age.

MATERIALS AND METHODS

Rabbits used in this study were taken from rabbit herd of Private Rabbit Production Farm in Zian Region, Dakahlyia Governorate, Egypt. The experimental work was carried out at the Laboratory of Animal Physiology, Faculty of Agriculture, Mansoura University, Egypt, during the period from November 2010 to March 2011.

Animals: A total of 83 NZW rabbits was used in this study. Three non-sexed rabbits were slaughtered at 3 weeks of age to determined caecum function and caecal characteristics at this age. However, 80 male rabbits weaned at 4 weeks were housed in pairs in flat-deck cages made of galvanized wire (50×60×40 cm) and supplied with automatic drinking system to determined growth performance parameters at 4 (n = 80), 6 (n = 77), 8 (n = 74), 12 (n = 71) and 16 (n = 68) weeks of age, whereas 3 rabbits were randomly chosen to slaughtered at each age from 4 up to 16 weeks.

Rabbits were fed diet of their dams during the interval from 3 to 4 weeks of age (weaning), then they were fed a basal diet in pelleted form which was formulated to meet or exceed all the essential nutrient requirements of growing rabbits according to De Blas and Mateos (1998). Rabbits were fed *ad libitum* and feed was offered at 8 a.m. and 4 p.m. The basal diet composed of 40% berseem hay, 25% barley, 8% soybean, 23.5% wheat bran, 3% molasses and 0.5% premix. It contained 17.8% CP, 15.5% CF on dry matter basis.

Experimental procedures: During an experimental period from 4 up to 16 weeks of age, Live Body Weight (LBW) and feed intake was recorded at 4, 6, 8, 12 and 16 weeks of age, except during the interval from 3-4 weeks. it was difficult to record feed intake of bunnies during the suckling period. Then, average daily gain and feed conversion ratio were calculated at different age intervals (4-6, 6-8, 8-12 and 12-16 weeks).

Three rabbits were randomly taken and weighed before slaughter at 3, 4, 6, 8, 12 and 16 weeks of age to study the anatomical and histological development and 3 weeks of age was considered as 0 time of the developmental processes. After complete bleeding, the Gastrointestinal Tract (GIT) of each animal was removed and full caecum was separated and weighed with and without contents. The pH value in the contents of the caecum was measured using digital pH-meter and samples from these contents were taken to determine caecal activity and Dry Matter (DM). Total Volatile Fatty Acids (VFA) concentration was determined on the wet samples of caecal contents using the method described by Warner (1964). However, ammonia-N concentration was determined in the caecal contents using standard solution of magnesium oxide distillation according to the method of AOAC (1990).

After empting all caecal contents, the caecal tissues were gently washed by saline solution (0.9% NaCl), dried by clynex paper and weighed. The caecal dimensions (length and width) were measured and then specimens were taken from the caecum (middle region) to fix in 10% neutral formalin for 24-48 h.

The histological study took place at the Physiology Laboratory of Animal Production Department, Faculty of Agriculture, Mansoura University. The fixed specimens were washed by running tap water, dehydrated in ascending grades of alcohol, cleared, impregnated, embedded in paraffin wax-blocks, cut into thin sections (7-10 μm) and stained by heamatoxylin and eosin (Bancroft *et al.*, 1990). The stained sections were examined by microscope to measure thickness of tunica mucosa, tunica submucosa, tunica musculosa and lamina epithelialis mucosa using eye-piece.

Statistical analysis: The obtained data were statistically analyzed according to Snedecor and Cochran (1982) using computer program of SAS (2004). The significant differences among means at different ages during the experiment period were tested by multiple Range Test (Duncan, 1955).

For all data, the completely randomized design was used to establish the effect of age (6 ages) and the statistical model was:

$$Y_{ij} = U + A_i + e_{ij}$$

where, Y_{ij} is observed values of growth parameters of rabbits and anatomical and histological characteristics of the caecum; U is Overall mean; A_i is age and e_{ij} is Random error.

RESULTS AND DISCUSSION

Growth performance of growing rabbits

Live body weight (LBW): As illustrated in Fig. 1, LBW of rabbits showed linear pattern by increasing ($p<0.05$) with age progress from 3 up to 16 weeks of age. De Blas *et al.* (1986) found that growth rate was optimal in the range of 10-15% dietary crud fiber (corresponding to 13-25% ADF) while a lower growth rate was observed with a crude fiber level below 10% (dietary starch level of 30%). This is in agreement with the present results, whereas CF content in the basal diet was 15.58%.

Results of growth performance parameters of rabbits in terms of Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) during different age intervals from 4 up to 16 weeks shown in Table 1 revealed significant age effect on all parameters. Average daily gain showed significant ($p<0.05$) increase from 4-6 weeks (21.1 g day^{-1}), reaching the maximal values up to 8-12 weeks (30.6 g day^{-1}), then significantly ($p<0.05$) decreased to 23.6 g day^{-1} during the interval from 12 to 16 weeks of age. In this respect, it was reported that ADG of NZW rabbits fed different diets at various age intervals under the local conditions ranged between 17.32 and 27.39 g/h/day (Abd El-Moneim, 1990) or between 17.7 and 21.0 g/h/day (Attyia, 1996).

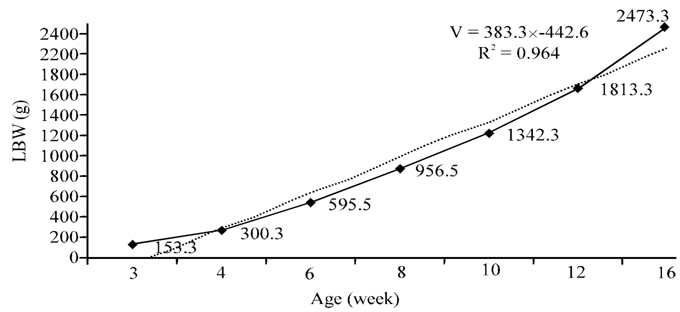

Fig. 1: Average live body weight of growing NZW rabbits at consecutive ages from 3 up to 16 weeks

Table 1: Growth performance parameters of growing NZW rabbits at different age intervals

	Growth performance		
Age (week)	ADG (g)	DFI (g)	FCR (DFI/ADG)
4-6	21.1±0.9^{b}	49.1±1.04^{d}	2.33±0.12^{d}
6-8	25.7±1.5^{ab}	93.0±1.95^{c}	3.62±0.36^{c}
8-12	30.6±1.7^{a}	149.2±3.25^{b}	4.88±0.24^{b}
12-16	23.6±1.5^{b}	173.6±6.36^{a}	7.36±0.20^{a}

Means denoted within the same column with different superscripts are significantly different at $p<0.05$

Table 2: Development of caecal parameters in NZW rabbits with age progress

	Caecal parameter		
Age (week)	pH value	VFA (mmol L^{-1})	Ammonia-N (mmol L^{-1})
3	7.33±0.15^{a}	25.33±1.4^{d}	13.00±0.58^{a}
4	6.10±0.12^{b}	52.67±0.8^{c}	11.33±0.67^{a}
6	5.97±0.23^{bc}	68.00±1.1^{a}	8.00±0.58^{b}
8	5.47±0.09^{c}	71.33±2.4^{a}	7.33±0.33^{b}
12	6.17±0.13^{b}	64.33±0.8^{b}	8.33±0.33^{b}
16	6.07±0.09^{b}	62.33±0.8^{b}	7.24±0.33^{b}

Means denoted within the same column with different superscripts are significantly different at $p<0.05$

In accordance with the highest trend of increase in ADG during the interval from 8-12 weeks of age in this study, Debray *et al.* (2002) found that ADG was 35.9-39.9, 38.0-42.1 and 43.6-44.9 g day^{-1} during pre-weaning interval (18-32 days), after weaning (32-45 day) and 45-70 day of age, respectively. It was observed that rabbits increased ($p<0.05$) ADFI with age progress. The ADFI increased from 49.1 g at 4-6 weeks to 93.0, 149.2 and 173.6 g at the intervals of 6-8, 8-12 and 12-16 weeks of age, respectively (Table 1).

Rabbit's feed intake depends basically on nutrient contents and/or palatability of diets. The daily dry matter intake is determined by the actual energy need of the animal and/or protein and fiber level of its ration (Fekete and Bokori, 1985). These findings may explain dietary requirements of rabbits to grow with age progress, being lower during early ages (4-12 weeks) and higher at later ages (12-16 weeks, Table 2). Similar trend was obtained by Debray *et al.* (2002).

It is of interest to note that FCR showed a reversible situation to ADFI at different age intervals, being the best (2.33, $p<0.05$) during 4-6 weeks, then reduced ($p<0.05$) to be the poorest (7.36) during 12-16 weeks of age (Table 1). Similarly, De Blas *et al.* (1986) observed that feed conversion efficiency declines with age progress, being the most efficient at 3 weeks of age and the least expensive gains are made with 4-6 weeks old interval, so it is important to make maximum use of their full growth potential during that period.

Development in caecal function

Concentration of volatile fatty acids (VFA): The evolution of VFA concentration in caecal contents in rabbits (Table 2), it reflected the lowest ($p<0.05$) values (25.33 mmol L^{-1}) at 3 weeks, doubled ($p<0.05$) at 4 weeks, reached the maximal ($p<0.05$) values between 6 and 8 weeks and then markedly decreased ($p<0.05$) at 12 and 16 weeks of age.

In accordance with the present trend, Kovacs *et al.* (2004) found that VFA concentration in caecal content of rabbits was 12.8, 13.3, 45.7, 93.3, 91.8 and 104.2 mmol kg^{-1} at 7, 14, 21, 28, 35 and 42 days of age. Also, Piattoni *et al.* (1995) observed significant ($p<0.01$) changes in concentration of VFA in caecal contents of rabbits with age progress, being the highest on day 42 and 56 of age. In adult rabbits, Marounek *et al.* (2004) found that concentration of VFA was 104.8 μmol g^{-1}.

The present values of VFA concentration on day at 4-12 weeks of age are in agreement with the range from 31.8 to 88.5 mmol L^{-1} in rabbits (Gracia *et al.*, 1996; Motta-Frreira *et al.*, 1996). The observed lowest VFA at 3 weeks of age was mainly associated with type of feeding during the suckling period. Till 21 days of age, young rabbits were fed a single meal (milk) which is digested only in stomach and small intestine via enzymic digestion. So, concentration of VFA was very small and the microbial fermentation is nil. In the very young rabbit (1st weeks of life) the stomach wall secretes a pepsin and another peptidase, rennin or chymosin (Angelo and Srivastava, 1979).

It is of interest to note that the change in VFA level at early ages was mainly related to type of feeding. In this respect, some investigators found that the removal of milk from 18 days of age (early weaning) is followed 4 days later by a high concentration of VFA and a lower caecal pH compared with control rabbits of the same age (Maertens and Paittoni, 2001).

The detected highest increase in VFA production between 42 and 56 days of age was mainly attributed to quick change in feeding behavior as the young rabbit moves from a single meal of milk per day (from birth to 21 days old) to a large number of solids and liquid meals. During the 4th weeks of age, the ingestion of solids food and water begins to exceed that of milk (Gidenne and Fortun-Lamothe, 2002). Bellier (1994) reported a plateau of VFA level on 56 days of age. Piattoni *et al.* (1995) reported the gradual increase in VFA level was associated with increasing solid feed intake, doubling significantly ($p<0.01$) on 56 days of age. Padilha *et al.* (1995) found that as a consequence of the age-related changes in the microbial population, production of VFA increases in caecal contents of rabbits with age progress.

Concentration of ammonia-N: Concentration of ammonia-N in caecal contents of rabbits significantly ($p<0.05$) reduced by age progress, being the highest at 3 weeks and the lowest at 16 weeks of age (Table 3). The lowest rate of ammonia-N reduction was observed between 3-6 weeks of age (Table 2). In disagreement with the present results, Piattoni *et al.* (1995) observed that concentration of ammonia-N in the caecal contents did not differ significantly between 22 and 56 days of age and ranged between 12.6 mmol kg^{-1} on day 56 and 17.4 mmol kg^{-1} on day 36 of age. However, in caecal contents of adult rabbits (2.87±0.36 kg), concentration of ammonia-N was 23.3 μmol g^{-1} (Marounek *et al.*, 2004). The noted confliction in the obtained results regard to ammonia-N concentration and those reported by the previous authors may be related to breed of rabbit, method of determination, content of dietary crude protein and/or protein

Table 3: Anatomical development of caecal tissue in NZW rabbits with age progress

Age (week)	Caecal fresh tissue weight			Caecal measurement	
	Absolute (g)	As a percentage of total tissues of GIT	As a percentage of LBW	Length (cm)	Width (cm)
3	2.3±0.10[f]	22.0±0.96[a]	1.5±0.05[bc]	14.2±0.1[e]	0.8±0.05[d]
4	4.3±0.31[e]	20.7±0.46[ab]	1.4±0.07[cd]	22.3±0.9[d]	1.6±0.11[c]
6	12.9±0.58[d]	18.4±0.72[b]	1.7±0.05[ab]	32.8±1.2[c]	3.9±0.05[a]
8	18.5±1.14[c]	19.7±1.01[ab]	1.9±0.12[a]	37.9±0.9[b]	3.0±0.05[b]
12	23.1±0.91[b]	19.2±0.49[b]	1.4±0.07[cd]	42.1±1.1[a]	3.2±0.15[b]
16	33.2±1.17[a]	16.1±0.67[c]	1.3±0.03[d]	44.2±1.3[a]	3.1±0.10[b]

Means denoted within the same column with different superscripts are significantly different at $p<0.05$. GIT: Gastrointestinal tract. LBW: Live body weight

utilization. In this way, an increment in caecal ammonia concentration was related by different authors (Gracia *et al.*, 1996; Caraban *et al.*, 1997) to an increase in the dietary digestible crude protein content when the protein intake exceeds the nutritional requirement. The urea recycling from blood to the caecum might be increase, leading to an elevation in the caecal ammonia concentration. Belenguar *et al.* (2000) found that ammonia concentration was 3.6 and 7.4 mmol L^{-1} for low and high barley diets (14.9 and 44%) and 3.65 and 3.2 mmol L^{-1} for low and high corn diets (13.4 and 40.5%).

Caecal pH values: Table 2 shows that pH values in caecal contents markedly decreased ($p<0.05$) between 3 and 4 weeks of age (pre-weaning interval) and showed insignificant changes up to 16 weeks of age. It is worthy noting that the obtained caecal pH of rabbits varied inversely to the increase in VFA concentration at successive ages (De Blas *et al.*, 1986). The fermentative activity, characterized mainly by the VFA concentration, is almost nil at 2 weeks of age and then increases rapidly with the start of ingestion of solid food, hence the fall in pH (Chiou *et al.*, 1994).

The present pH values at more than 3 weeks of age are nearly similar to that reported by Marounek *et al.* (2004), who found pH value of 5.81 in caecal content of adult rabbits. Also, Belenguar *et al.* (2000) showed that caecal pH in rabbits fed different diets based on barley or corn ranged between 6.01 and 6.17. In general, fiber level could affect caecal pH value depending on the source of dietary fiber (Caraban *et al.*, 1997).

Morphological and anatomical development of rabbit caecum

Fresh tissue weight: Table 3 reveals that rabbit caecum showed markedly continues increase ($p<0.05$) in absolute fresh tissue weight with age progress, being ($p<0.05$) the lightest (2.3 g) at 3 weeks and the heaviest (33.2 g) at 16 weeks of age. When the caecal weight was expressed as a percentage of fresh tissue weight total Gastrointestinal Tract (GIT), it showed gradual reduction ($p<0.05$) from 22% at 3 weeks to 16.1% at 16 weeks of age. This was mainly attributed in marked and continues increase in proximal colon weight relative to GIT with age progress. On the other hand, caecal weight relative to LBW showed insignificant changes with age progress from 3 weeks, reaching the highest ($p<0.05$) values (1.9%) up to 8 weeks of age, then decreased ($p<0.05$) to 1.3% at 16 weeks of age. This finding may indicate higher rate of increase in caecal weight than in LBW with age progress up to 8 weeks of age, followed by lower rate up to 16 weeks of age.

Also, it was observed that, the pronounced increase ($p<0.05$) in absolute caecal fresh tissue weight was associated with gradual increase ($p<0.05$) in length of the caecum only up to 12 weeks

Table 4: Anatomical development of caecal contents in NZW rabbits with age progress

	Caecal content weight			
Age (week)	Absolute (g)	As a percentage of total tissues of GIT	As a percentage of LBW	DM (%) in caecal contents
3	4.4±0.02[e]	39.3±0.95[b]	2.9±0.07[c]	21.64±0.73
4	18.7±1.4[d]	41.3±2.3[b]	6.2±0.40[a]	22.10±0.74
6	45.7±2.1[c]	42.6±1.7[b]	6.2±0.29[a]	20.49±1.49
8	63.7±2.6[b]	48.0±2.1[a]	6.6±0.34[a]	19.56±1.51
12	83.7±5.2[a]	46.7±2.4[a]	5.2±0.34[b]	20.46±1.31
16	91.7±5.5[a]	44.3±1.4[ab]	3.5±0.19[c]	21.23±1.32

Means denoted within the same column with different superscripts are significantly different at $p<0.05$. GIT: Gastrointestinal tract. LBW: Live body weight

and only in width of the caecum up to 6 weeks of age. Such results may suggest marked increase in both caecal measurements up to 6 weeks and only enlargement in the caecum up to 12 weeks of age (Table 4).

In agreement with the present changes in fresh tissue weight, Piattoni *et al.* (1995) found that fresh tissue weight of rabbit caecum increased ($p<0.01$) from 22 up to 56 days of age. Also, Marounek *et al.* (1999) found that weight of rabbit caecum increased ($p<0.05$) from 4 up to 11 weeks of age. Piattoni *et al.* (1995) found that the caecal relative weight to LBW significantly ($p<0.01$) increased between 22 and 36 days of age, thereafter decreased at 42 and 56 days of age. In the current study, relative weight of the caecum significantly increased from 3-8 weeks, thereafter it showed marked decrease. This variation may relate to breed and feeding system.

It is of interest to note that such trend of change in weight and measurements of rabbit caecum with age progress was associated with increasing feed intake and complete development in its function for microbial fermentation up to 12 weeks of age. Increasing feed intake with age progress will increase hindgut fermentation and activates microbial metabolism which may stimulate the development of the caecal weights (Yu and Chiou, 1997). This finding is in association with increasing VFA concentration in caecum with age progress in this study (Table 2). Caecum develops strongly around 3 weeks of age and stabilizes a few days after weaning (Padilha *et al.*, 1995).

Caecal contents: Results shown in Table 4 revealed an increase in caecal contents as absolute weight ($p<0.05$) with age progress, being the highest (83.7 g, $p<0.05$) at 12 weeks. However, the caecal content weight relative to total GIT or LBW showed gradual increase ($p<0.05$) from 39.3 and 2.9% at 3 weeks up to the highest values (48 and 6.6%) at 8 weeks of age, respectively. Such trend was in relation to increasing feed consumption with age progress.

Interestingly to note that the rate of increase in caecal contents weight was nearly in positive relationship with the rate of increase in fresh tissue weight with age advancing (Table 4). This trend is in agreement with the findings on rabbits as reported from 22 up to 56 days of age (Piattoni *et al.*, 1995) or from 4 up to 11 weeks of age (Marounek *et al.*, 1999).

The marked increase in caecal contents with age progress was mainly related to changes in feeding behavior of rabbits which are quick as the young rabbit moves from a single meal of milk from birth to 21 days old to an amount of solid and liquid meals which increase rapidly thereafter. During the 4th weeks of age, the ingestion of solids food and water begins to exceed that of milk (Gidenne and Fortun-Lamothe, 2002).

On the other hand, dry matter (DM) in caecal contents did not differ significantly by advancing age, ranging between 19.56 and 22.10% at all ages studied (Table 5). In comparable

Table 5: Development in thickness (µm) of tunica musculosa, submucosa and mucosa as well as thickness of lamina epithelialis mucosa in caecum of NZW rabbits at successive ages

	Thickness (µm) of caecal wall			
Age (week)	Tunica musculosa	Tunica submucosa	Tunica mucosa	Lamina epithelialis
3	172.5 ± 10.73^{d}	23.8 ± 0.34^{c}	59.0 ± 0.08^{e}	8.3 ± 0.07^{e}
4	395.0 ± 7.41^{c}	30.5 ± 1.23^{b}	90.0 ± 0.62^{de}	9.7 ± 0.10^{d}
6	367.5 ± 11.32^{c}	31.4 ± 1.12^{b}	146.0 ± 2.10^{b}	10.8 ± 0.13^{c}
8	362.5 ± 10.62^{c}	26.1 ± 0.44^{c}	127.0 ± 0.94^{bc}	11.8 ± 0.14^{b}
12	342.5 ± 6.58^{c}	24.1 ± 0.56^{c}	106.0 ± 1.5^{cd}	12.2 ± 0.10^{ab}
16	527.5 ± 6.42^{a}	40.1 ± 1.13^{a}	300.0 ± 4.3^{a}	12.8 ± 0.12^{a}

Means denoted within the same column with different superscripts are significantly different at $p<0.05$

with the present results, Piattoni *et al.* (1995) found that total DM in caecal contents of rabbits on day 22, 25, 28, 32, 36, 42 and 56 was 20.8, 20.8, 20.2, 20.7, 19.0, 20.0 and 25.7%.

Histological characteristics of rabbit caecum: The histological examination revealed that the caecal wall of rabbits at all ages was composed of four tunicae, an outer layer (*T. serosa*), *T. musculosa*, *T. submucosa* and inner layer (*T. mucosa*), respectively and *T. mucosa* included three laminae, outer muscularis, middle propria and inner epithelialis, respectively (Fig. 2).

It is of interest to note that only pronounced differences were recorded in thickness of the examined tunicae and laminae with age progress. Results presented in Table 5 show that thickness of all tunicae (*Musculosa*, *Submucosa* and *Mucosa*) as well as lamina epithelialis markedly increased ($p<0.05$) between 3 and 4 weeks of age. Thereafter, all thicknesses showed inconsistent trend of increase with different levels of significance ($p<0.05$) from 4 up to 12 weeks and again showed dramatic increase ($p<0.05$) from 12 weeks to be the thickest at 16 weeks of age.

It is known that thickness of tunica mucosa in particular lamina epithelialis is very important for increasing absorption of microbial products in the caecum. Johnson *et al.* (1984) reported that the dietary fiber might stimulate multiplication of the mucosal epithelial cells, thus increasing the mucosal secretion. Also, the different sources of fiber influenced different mucosal cells. The observed increase in caecal wall with age progress may be caused by some kind of toxin produced in the distal end of the intestine and caecum by microbes. These toxins thicken the caecal wall and the mucosal membrane (Jacobs and Lupton, 1984). Also, the fermentation products, (VFA) will have a stabilizing effect on caecal and colonic mucosal cells. Caecal fermentation could also produce hydrogen ions that would increase the rate of cell division (Lupton *et al.*, 1988). Concentration of VFA has been suggested as being a simulative factor of mucosal growth (Chiou *et al.*, 1994).

In accordance with the present trend of increase in thickness of tunica mucosa, Van der Hage (1988) found that the intestinal mucous wall developed gradually from birth until adulthood in rabbits. These may be attributed to the increased intake of high dietary fiber causing distension in all segments of the intestine (Yu and Chiou, 1997).

It is worthy noting that thickness of tunica mucosa and thickness of lamina epithelialis mucosa showed dramatic increase between 3 and 4 weeks of age (Table 5). This may mainly attribute to that bunnies start to feed on solid feeds at 3 weeks of age (Cheeke, 1987). In contrast to the pronounced increase in caecal wall thickness between 12 and 16 weeks of age, Yu and Chiou

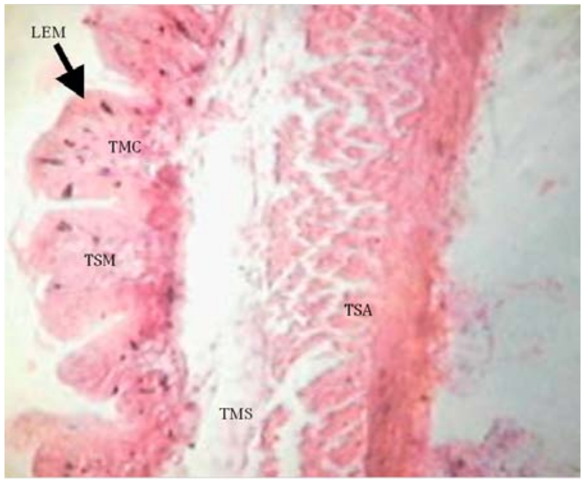

Fig. 2: Cross-section in caecal wall of rabbit at 6 weeks of age showing Tunica Serosa (TSA), Musculosa (TMS), Submucosa (TSM) and Mucosa (TMC) as well as Lamina Epithelialis Mucosa (LEM). (H and E stains, ×200)

(1997) found no significant differences in mucosal depth of the caecal between 8 and 16 weeks of age. It is assumed that the mucosa increases in depth as the rabbits grow and reaches maturity at 8 weeks. Indeed, the mucosa is a major site for digestion and absorption of nutrients but also for interaction with the normal and pathogenic flora (Gallois *et al.*, 2004). In rabbits, the caecal and colon mucosa wall change from 16 days of age with the appearance of ridges (Sabatakou *et al.*, 1999). Therefore, mucosal morphology may be related to digestive physiology (Cheeke and Patton, 1980).

In general, the present results showed that the relationship between thickness of mucosa and submucosa was almost in positive pattern as proved by (Aboul Hamd, 2003). Increasing thickness of tunica mucosa is in need to associate higher thickness in submucosa to increase the absorptive capacity whereas tunica submucosa contained concessive network of blood vessels which are important to absorption (Abdel-Khalek, 2000).

The development of tunica musculosa may be in relation to dietary factors rather than age progress. In this respect, Chiou *et al.* (1994) found that adding pectin or cellulose to the diet of rabbits thickened the caecal muscle layer whereas a supplement of lignin thinned the caecal muscle layer. However, Yu and Chiou (1997) found that after 4 weeks, the muscle layer of rabbits becomes thinner which may be attributed to the increased intake of high dietary fiber causing distension in the caecum. However, the trend of development of the thickness of tunica musculosa may be related to the changes of the diet as found by Khalil (1974) in lambs and it was affected by the nature of diet as reported by Tamate *et al.* (1962) in calves. The present significant ($p<0.05$) increase in thickness of tunica musculosa with advancing age was observed in lambs and kids by Abdel-Khalek (1986).

CONCLUSION

The current study indicated incidence of anatomical, histological and functional development in rabbit caecum with age progress. Dramatic changes had occurred between 3 and 4 weeks of age. The complete physiological development of rabbit caecum was evidence at 12 weeks of age.

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