AGE-RELATED GROSS AND HISTOMORPHOLOGICAL CHANGES IN SMALL INTESTINE OF *SAKINI* CHICKEN

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ABSTRACT: The study was carried out to assess the histo-morphological changes in small intestine of *Sakini* chicken from October 2021 to March 2022. Ninety two day-old chicks of *Sakini* breed of both sexes were considered for the study. These birds were reared under the standard husbandry practices and fed with layers starter pellet feed containing 20% crude protein, 3000 Kcal/kg metabolizable energy and *ad libitum* water for 56 days. Forty birds of five different age groups from day 1, day 14, day 28, day 42 and day 56 (eight birds per age group) of trial period were sacrificed. The findings indicated that live body weight gained significantly from 25.22 gm to 533.63 gm as age progressed from day one to day 56. Amongst sex, males had significantly higher body weight (582.25 gm) than females (485.00 gm) at day 56. Length and weight of duodenum, jejunum and ileum increased significantly up to day 28 and day 42 and became steady thereafter. A significant increase in the relative weight of small intestine was observed up to day 28 showing the growth of the small intestine was higher than the rest of the body at an earlier age. Histologically, villus length of duodenum, jejunum and ileum increased from 375.65 µm, 259.25 µm and 209.25 µm at day one to 834.18 µm, 610.57 µm and 447.01 µm at day 56 while apparent villus area increased from 23266.55 $µm^2$, 14053.17 $µm^2$ and 12430.71 $µm^2$ at day one to 109351.55 $µm^2$, 69702.34 $µm^2$ and 44702.80 $µm^2$ at day 56 respectively. The result showed that delayed stabilization of relative weight and length resulted in slower growth of *Sakini* chicken which indicates digestion and absorption capacity increased with advancement of age after stabilization of relative weight and length. An increase in villus height and villus surface area of *Sakini* chicken were the compensatory mechanism to fulfill the increase in nutrient demand of the body. Villus length, villus width, apparent villus area and ratio of villus height and crypt depth were highest for duodenum followed by jejunum and ileum at all ages. These parameters are positively associated with the live body weight so at the initial stage of life duodenum plays the vital role for maintaining the body weight as it is principal site for digestion and absorption. This present research provides novel findings on the baseline information on histo-morphological changes in small intestine of Sakini chicken which can be used as reference for further digestive studies.

Keywords: Sakini; normalized weight; relative length; villus; crypt depth

1. INTRODUCTION

Chicken is an important livestock species in the country, accounting for 9 % of Agricultural Gross Domestic Product and 17.4 % of total meat production with the annual meat production of 62899 metric tons from the population of 75709330 (excluding ducks) in the fiscal year 2018/19 (MoALD 2020; Sapkota et al. 2020). In Nepal, indigenous chicken has about similar market share as commercial chicken and has played an important role in income generation as well as a source of animal protein (SAC 2020).

Sakini (normal feathered), *Ghanti khuile* (naked neck), and *Pwakh ulte* (frizzled feathered) are Nepali indigenous chicken breeds that have been documented (Centre 2020). *Sakini* is the most abundant indigenous breed of Nepal. *Sakini* is a dual-purpose breed and is well known for its hardy nature that leads to resistance to diseases and parasites along with better survivability among the exotic breeds, moreover, indigenous chickens have cultural as well as social significance in several ethnic groups and religions (Sapkota et al. 2020). Organic and pasture-raised poultry are becoming increasingly popular, and demand for these products is gaining popularity worldwide because these backyard poultry producers barely use antibiotics and vaccines (Hanning et al. 2012).

Feed constitutes the major portion of poultry production cost, thus focus has been made so far on nutrition, however, the study on the organ where these nutrient gets absorbed has been overlooked (Gorkhali et al. 2021; Levi et al. 2013). Growth performance and nutrient digestibility have long been used to assess the nutritional value of chicken diets. In addition to these studies, research on the intestinal structure is critical because the small intestine, particularly the absorptive epithelium's crypts, and villi, play an important role in the last stages of nutrient digestion and assimilation (Wang and Peng 2008; Yamauchi 2002). Therefore, the morphometric evaluation of the intestinal length, intestinal weight, crypt depth, villus height, and villus area helps to assess as well as to describe health, digestibility, absorption, and intestinal development in poultry (Laudadio et al. 2012; Miles et al. 2006; Wijtten et al. 2012). Absorptive epithelium more precisely villi and crypts may be different among different breeds of birds (Wang and Peng 2008). Digestive efficiency is directly related to digestive organs (De Verdal et al. 2010). The key measure of gut health is intestinal morphology (Laudadio et al. 2012). Furthermore, the muscular layer that controls gut motility and bolus progression may influence absorption processes (De Verdal et al. 2010).

Although *Sakini* contributes a major portion to the indigenous poultry population, household income and country's economy (Gorkhali et al. 2021), basic research to understand the anatomy of digestive tract of poultry is seldom carried out. In particular, gross, as well as a microscopic study of small intestines of *Sakini* breed of Nepal has not been properly documented which is considered as a novel study in poultry science of Nepal. Such study will also provide the baseline data for further studies that are related to the anatomy of the small intestine of *Sakini* that has relationship with nutrient digestibility and growth. The outcome of this study will also provide a platform to help in describing digestibility, productivity, and efficiency of small intestine for different age groups of the *Sakini* breed. Thus, the present study was designed to assess the differences in the gross and histological parameters in small intestine for different age group of *Sakini* chicken.

2. MATERIALS AND METHODS

2.1 Study area and time frame

The birds were reared in the poultry farm of National Animal Breeding and Genetics Research Centre (NABGRC), Nepal Agriculture Research Council (NARC), Khumaltar Lalitpur, Nepal from October to December 2021. All the gross examinations were performed at NABGRC and histological slides were prepared in the facility of laboratory of Centre for Molecular Dynamics Nepal (CMDN), Kathmandu. The histological slides were studied and analyzed at the microbiology laboratory of Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. All these studies were completed within the time frame of six months (from October 2021 to March 2022).

2.2 Animals and diet

Ninety-two, day-old chicks of *Sakini* breed having both sexes were considered for the study. These birds were reared under the standard husbandry practices recommended by National Avian Research Program, Parwanipur, Bara district. Feeds and water were given *ad libitum* throughout the study period. Layers starter (L1) pellet feed having crude protein 20% and metabolizable energy 3000 Kcal/kg prepared by Nimbus Company was given for entire trial period of 56 days (8 weeks).

2.3 Sample collection and assessment

For gross and histological study, the experimental animals were assigned to five different age groups such as day 1, day 14, day 28, day 42, and day 56 having equal number of males and females.

For each age group, eight apparently healthy birds (four males and four females) were sampled and sacrificed after overnight fasting for gross examination while four out of eight sacrificed birds were taken for histological study. In total, forty (20 males and 20 females) apparently healthy *Sakini* breed of chickens were randomly selected from the flock of ninety-two mixed sexes chicks for the study.

2.4 Gross examination

Following measurements were taken for the gross examination:

- Live body weight (gram)
- Weight of each segment of the small intestine i.e., duodenum, jejunum, and ileum (gram)
- Length of duodenum, jejunum, and ileum (centimeter)
- Normalized weight of segments as [weight (g)/ live body weight (g)]×100 (Alshamy et al. 2018; Incharoen et al. 2010)
- The relative length of segments: [intestinal segments length (cm)/ live body weight (g)] ×100 (Alshamy et al. 2018; Incharoen et al. 2010)
- Intestinal density as: weight of segment (g)/ length of segment (cm) (Alshamy et al. 2018; Taylor and Jones 2004)

2.5 Histological slide preparation

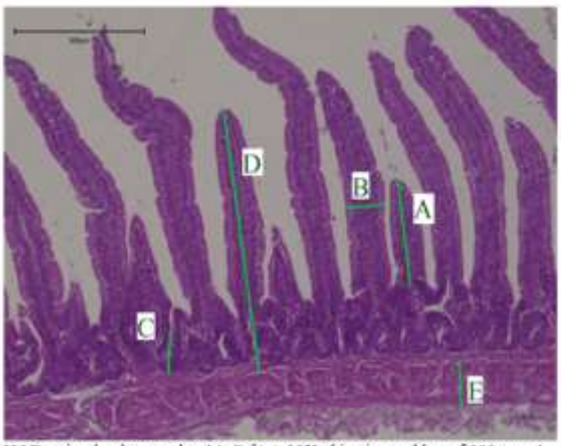
Samples of size 1cm×1cm from each segment of the small intestine were taken from the middle of the duodenum, jejunum, and ileum and were fixed in neutral buffered formalin to preserve the architecture of tissue (Alshamy et al. 2018 and De Verdal et al. 2010). Histological slide preparation was done as per the protocol of Centre for Molecular Dynamics Nepal (CMDN) (Gurina and Simms 2020). Fixed samples were processed, embedded in paraffin, and finally cut into 5 µm thin slices using a microtome (Leica RM2125 RTS). These sections were fixed in glass slides and stained with Hematoxylin and Eosin stain (H&E stain) and finally mounted with DPX for future use.

2.6 Histological examination

Histomorphology and histomorphometric examination of sections were performed by using the Invitrogen™ EVOS™ M5000 cell imaging system. Following variables were measured from each section of each segment of the small intestine based on the protocol described by the previous studies (Alshamy et al. 2018; Incharoen et al. 2010 and Uni et al. 1999) (Figure 1).

An average of five measurements were taken per intestinal segment for histo-morphometric studies.

- Villus height: Villi from their base at the crypt's entrance to their distal points were measured. Only villi that are full finger-shaped and well-oriented were taken
- Villus width: Taken at the middle of the villus
- Apparent villus surface area: Villus height × villus width (De Verdal et al. 2010)
- Crypt depth: From the crypt base to the closest villus base
- Villus height/crypt depth ratio
- Tunica mucosa thickness: Distance from tunica muscularis mucosae to villus tip
- Tunica muscularis thickness: The distance between the lamina muscularis mucosae internally and the tunica serosa externally were considered for this variable.



H&E stain; duodenum; day 14; *Sakini*; 10X objective and bar of 300 µm; A= villus length, B= villus width, C= crypt depth, D= tunica mucosa thickness, and E= tunica muscularis thickness.

Figure 1: Histomorphometric measurements guidance

2.7 Statistical analysis

The mean and standard error were calculated from the data for each variable. Test of significance between the age groups for each variable was obtained by one-way ANOVA through IBM™ SPSS statistics version 23 after testing the equality variances by Levene's test. After obtaining significant results, post hoc analyses were carried out for multiple pair comparison by Least Significant Difference (LSD). An independent sample t-test was performed to compare the means of the variable between males and females of a single age group. The level of significance was taken as 5 % for one way ANOVA.

3. RESULTS AND DISCUSSION

3.1 Gross morphology

The gross morphology in small intestine from the current study indicated that there was an ill demarcation between duodenum, jejunum, and ileum for all the age groups of *Sakini* chicken. Meckel's diverticulum which was present in all studied birds was found well marked up to 14 days, thereafter the size was decreased, although some authors had reported that it was absent in 40% of chickens (Maierl et al. 2016). This diverticulum was considered as the demarcation between jejunum and ileum in this study which is similar to previous authors (Alshamy et al. 2018; De Verdal et al. 2010 and Nasrin et al. 2012) while Jain (2009) considered the ileo-caecal ligament to demarcate ileum from the jejunum. The present study provides the normal gross and microscopic feature of the small intestine that might correlates with gut health of the chicken (Shazali et al. 2019; Li et al. 2019).

3.2 Gross morphometry

3.2.1 Live body weight

Live body weight was significantly ($p<0.05$) increased from day one to day 56 of age as in previous studies (Jain 2009 and Mabelebele et al. 2017). Average live body weight of *Sakini* in the present study had higher body weight in comparison to the study by Sah et al. (2007) on native chickens of Nepal of respective age except at day one where body weight was lighter in *Sakini* in the current finding. Live body weight of *Sakini* at day old and day 56 was lighter than the Ross 308 broiler and even to Venda chicken which is indigenous to South Africa (Mabelebele et al. 2014; 2017). *Sakini* chicken of the present study also had lighter weight than hybrids: CARI Shyama and Vanaraja breeds (Jain 2009). Comparable body weight was seen with Nigerian indigenous breeds i.e. Normal feathered, Naked neck, and Frizzled feather as their weights were taken from over one-year-old birds and weights were 879.33 gm, 847.33 gm, and 849.67 gm respectively (Mahmud et al. 2015) while *Sakini* breed in the present study weighted 533.63 gm in just 56 days.

Male had significantly ($p<0.05$) higher body weight at day 56 of age having an average body weight of 582.25 gm while the female had 485.00 gm (Figure 2) which was in agreement to the findings of Miles et al. (2006). The authors explained that males grew faster when age-progressed than females. In contrast to this finding, Mahmud et al. (2015) found no difference in body weight between sexes and breeds of three Nigerian indigenous breeds of chicken.

3.2.2 Length of small intestine segment

There was a significant increase ($p<0.05$) in the length of all segments of small intestine from day one to day 28 (Figure 3). After day 28, increase in length was constant ($p>0.05$) for all segments and small intestine as a whole (Table 1). Length of ileum was higher followed by jejunum and duodenum at all ages similar to earlier similar studies (Mabelebele et al. 2014; 2017 and Mahmud et al. 2015). Length of the segment together with ratio of villus height and crypt depth associates with gut health and its digestive and absorptive capacity which in turn associated with better live weight (Gilani et al. 2021; Biasato et al. 2018; Shazali et al. 2019; Li et al. 2019).

3.2.3 Weight of small intestine segment

There was a significant ($p<0.05$) increase in weight of all segments from day one to day 42. Significant increase in weight of segments of small intestine might be correlated with better gut health which in turn better performance of bird (Mabelebele et al. 2017). Between day 42 and day 56, weight increased non-significantly ($p>0.05$) for all segments (Figure 3, Table 1). Jejunum was heaviest followed by duodenum and lightest in ileum at all ages which is in agreement to Mahmud et al. (2015) but in contrast to Mabelebele et al. (2014; 2017) who found that duodenum being heaviest and ileum being the lightest at earlier age. This scenario was reversed at 90 days for both Ross 308 and Venda chicken (Mabelebele et al. 2017). Values of overall length and weight of small intestine in the present study were less than Ross 308 broiler

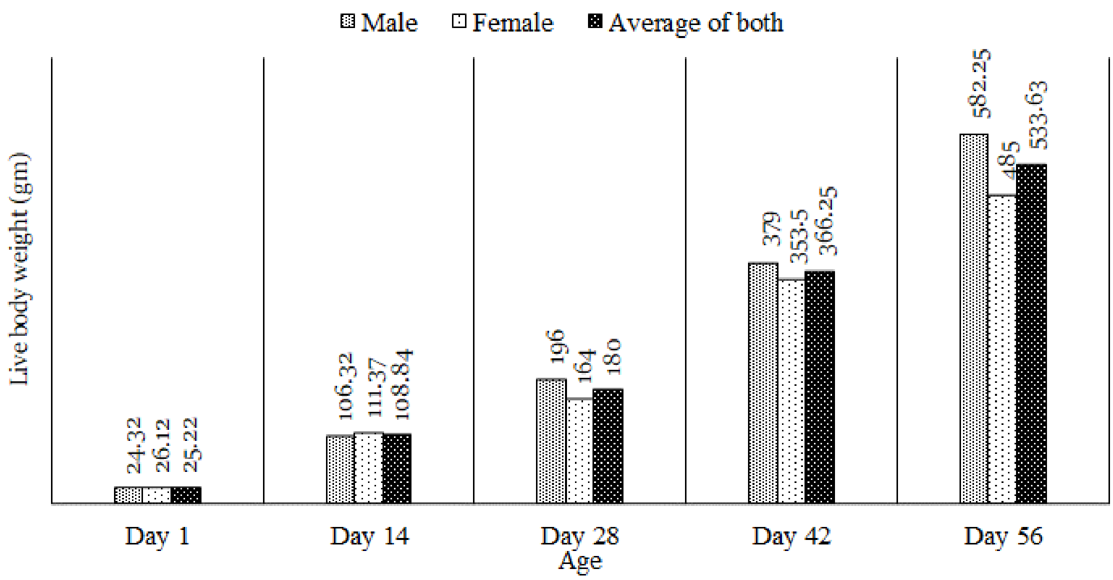


Figure 2: Live body weights of male, female and average of both at different ages.

strain at 42 days studied by Mabelebele et al. 2017. Heavier and longer small intestine of Ross 308 when compared to *Sakini* breed was due to the heavier body weight (Mabelebele et al. 2017) of broiler than indigenous *Sakini*. These evidences suggest that higher body weight tends to have a heavier and longer gastrointestinal tract. In the current study, jejunum and ileum had similar lengths at all ages but the weight of jejunum was noted higher than ileum which is in consonance to Mahmud et al. 2015. The authors further substantiated that this could be due to higher digestibility of jejunum than the ileum which is also supported by higher value of villus length, villus width and apparent villus surface area of jejunum than ileum at all ages. Similar lengths of jejunum and ileum also suggested that Meckel's diverticulum almost equally divided the latter two segments of the small intestine. In contrast to this finding, Jain (2009) and John et al. (2012) found that jejunum as longest and ileum being the least. This was due to difference in the measurement procedure as they didn't consider Meckel's diverticulum as demarcation between jejunum and ileum.

3.2.4 Normalized weight and relative length of small intestine segment

There was a significant ($p<0.05$) increase in normalized weight from day one up to day 28, however, from day 28 up to day 56 there was a significant ($p<0.05$) decrease in value for all the small intestinal segments and for

Table 1: Gross morphometric measurements (Mean±SE) of small intestine of Sakini at different age

Variables	Age of chicks	Duodenum	Jejunum	Ileum	Total of small intestine
Length (cm)	Day one	7.09±0.26^{a}	13.31±0.73^{a}	12.91±0.32^{a}	33.31±1.23^{a}
	Day 14	13.14±0.54^{b}	21.95±0.92^{b}	23.81±1.02^{b}	58.90±2.28^{b}
	Day 28	19.35±0.60^{c}	36.79±1.82^{c}	38.68±1.20^{c}	94.81±3.31^{c}
	Day 42	22.64±1.07^{d}	37.69±2.08^{cd}	36.85±2.40^{c}	97.18±5.25^{c}
	Day 56	23.91±0.68^{d}	41.30±1.83^{d}	41.50±2.41^{c}	106.71±4.47^{c}
Weight (gm)	Day one	0.24±0.02^{a}	0.23±0.02^{a}	0.18±0.01^{a}	0.64±0.04^{a}
	Day 14	2.01±0.13^{b}	2.45±0.16^{b}	1.60±0.12^{b}	6.05±0.39^{b}
	Day 28	4.63±0.24^{c}	7.05±0.38^{c}	4.20±0.17^{c}	15.88±0.76^{c}
	Day 42	7.51±0.95^{d}	11.32±1.56^{d}	6.31±0.78^{d}	25.14±3.27^{d}
	Day 56	8.85±0.55^{d}	12.26±0.63^{d}	6.96±0.46^{d}	28.06±1.51^{d}
Normalized weight (gm per 100gm BW)	Day one	0.95±0.06^{a}	0.89±0.09^{a}	0.70±0.06^{a}	2.55±0.15^{a}
	Day 14	1.86±0.08^{b}	2.26±0.09^{b}	1.47±0.06^{bd}	5.59±0.22^{b}
	Day 28	2.58±0.10^{c}	3.93±0.17^{c}	2.35±0.10^{c}	8.87±0.33^{c}
	Day 42	2.03±0.19^{b}	3.05±0.32^{d}	1.71±0.16^{b}	6.79±0.65^{d}
	Day 56	1.68±0.13^{b}	2.32±0.15^{b}	1.31±0.07^{d}	5.31±0.32^{b}
Relative length (cm per 100gm BW)	Day one	28.30±1.24^{a}	53.13±3.04^{a}	51.64±2.18^{a}	133.06±6.03^{a}
	Day 14	12.28±0.61^{b}	20.48±0.97^{b}	22.17±0.86^{b}	54.94±2.28^{b}
	Day 28	10.90±0.59^{b}	20.65±1.20^{b}	21.67±0.75^{b}	53.22±2.42^{b}
	Day 42	6.20±0.22^{c}	10.32±0.47^{c}	10.09±0.54^{c}	26.61±1.12^{c}
	Day 56	4.55±0.25^{c}	7.86±0.54^{c}	7.84±0.50^{c}	20.24±1.21^{c}
Intestinal Density (gm/cm)	Day one	0.034±0.001^{a}	0.017±0.001^{a}	0.014±0.001^{a}	0.019±0.001^{a}
	Day 14	0.153±0.007^{b}	0.111±0.004^{b}	0.067±0.003^{b}	0.102±0.004^{b}
	Day 28	0.241±0.015^{c}	0.193±0.009^{c}	0.109±0.003^{c}	0.168±0.006^{c}
	Day 42	0.325±0.026^{d}	0.293±0.024^{d}	0.169±0.012^{d}	0.253±0.018^{d}
	Day 56	0.369±0.017^{d}	0.299±0.016^{d}	0.168±0.006^{d}	0.263±0.009^{d}

Note: Values with different superscripts within single column of the single variable were significantly different from one another ($p<0.05$)

whole small intestine (Table 1).Normalized weight, relative length, and intestinal density of each segment mimicked the small intestine as a whole which is similar to the finding of Alshamy et al. 2018.

In the current study, relative length decreased significantly up to day 14 and stabilized at day 42 onwards until the study period (Figure 4) which is supported by Alshamy et al. (2018) who found that that relative length of slow-growing Lohman Dual (LD) stabilized after 42 days and Ross broiler stabilized from 25 days.

If we only observe absolute value of weight and length it may mislead because there is no any correlation with body weight so normalized weight and length is must to access the digestive and absorptive capacity. In initial phase, birds are not efficient in terms of body weight performance which can be depicted by increased normalized weight and decreased relative length. In early stage of life most of the energy is utilized to develop visceral organs including small intestine which is later on shifted to whole body development so in earlier life digestibility and efficiency of digestion and absorption is low as compared to later stage of life (Uni et al. 1999; Alshamy et al. 2018). In the present study, normalized weight of the small intestine peaked at day 28 (Figure 4) while previous researchers found it be

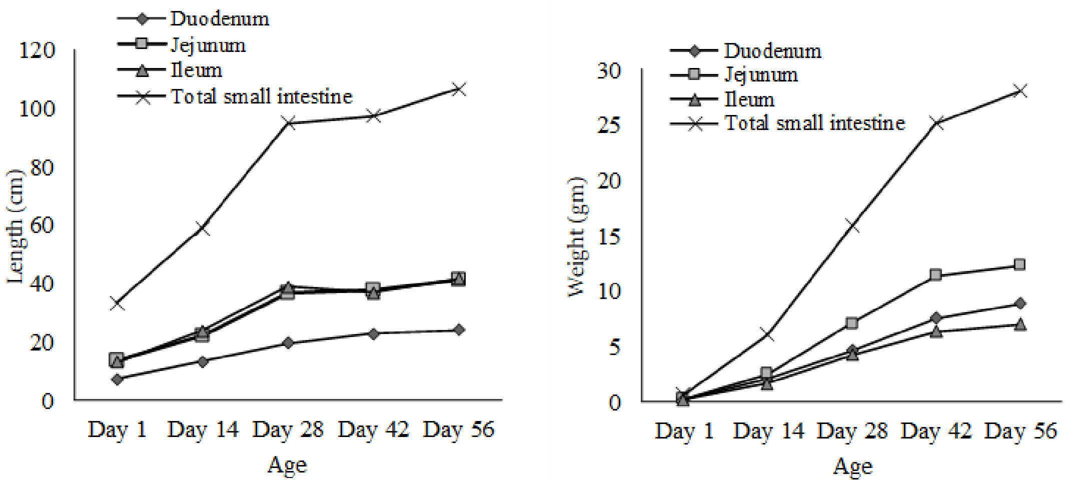


Figure 3: Length and weight of small intestine segments at different ages

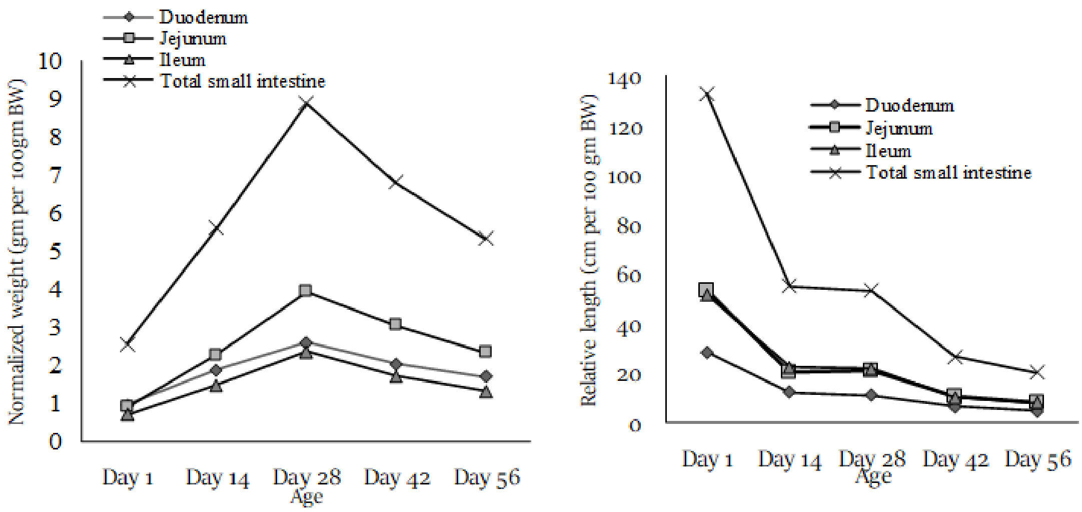


Figure 4: Normalized weight and relative length small intestine segments at different ages

from 7 to 12 days (Alshamy et al. 2018; Uni et al. 1999 and Wijtten et al. 2012). It shows there was delayed stabilization of normalized weight and relative length which ultimately leads to slow growth of *Sakini* chickens compared to Lohman Dual studied by Alshamy et al. (2018). *Sakini* chickens of present study also had a higher normalized weight and relative length as compared to fast-growing birds suggesting *Sakini*'s small intestine was not efficient as the broilers in earlier stage (Al-Marzooq et al. 2019 and Alshamy et al. 2018).

3.2.5 Intestinal density of small intestine segment

Intestinal density was found to be increased from day one with the values of 0.034, 0.017, 0.014 and 0.019 to day 56 with values 0.369, 0.299, 0.168 and 0.263 for duodenum, jejunum, ileum and the small intestine in total respectively. Intestinal density was found significant ($p<0.05$) from day one up to day 42, however, between day 42 and day 56 the increase was non-significant for all segments and the small intestine as a whole ($p>0.05$) (Figure 5, Table 1). Intestinal density was higher in duodenum followed by jejunum and least in ileum at all the ages studied. Higher value of intestinal density of duodenum than other segments at all ages suggested that duodenum had higher absorptive and digestive capacity than jejunum and ileum (Alshamy et al. 2018). Since the absolute length of intestinal segments remained steady after day 28 absolute weight of intestinal segments remained increased for the compensatory mechanism to fulfill the higher demand of birds after day 28 in our study suggesting that more digestive and absorptive capacity of intestine at later ages (Alshamy et al. 2018). This is also supported by the histological study that the apparent villus area, tunica mucosa thickness increased with increasing age in our study (Table 2).

3.3 Histomorphology

All segments had four basic layers of hollow organs with ill-defined tela submucosa and tunica serosa. Crypts of Liberkuhn or intestinal glands were extended up to the tunica muscularis mucosae. Fibers of muscularis mucosae were extended up to the villus tip (Figure 6). Contraction of these muscle fibers present in villus were responsible for lateral movement and shortening of villus which helps in absorption as well as the passage of food (Eurell & Frappier 2006). Submucosal duodenal glands were absent at all ages.

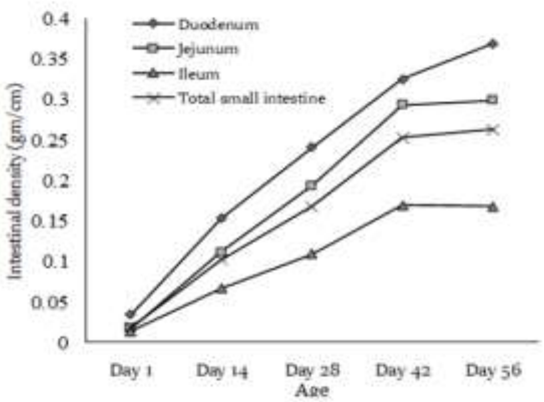


Figure 5: Intestinal density of small intestine segments at different age

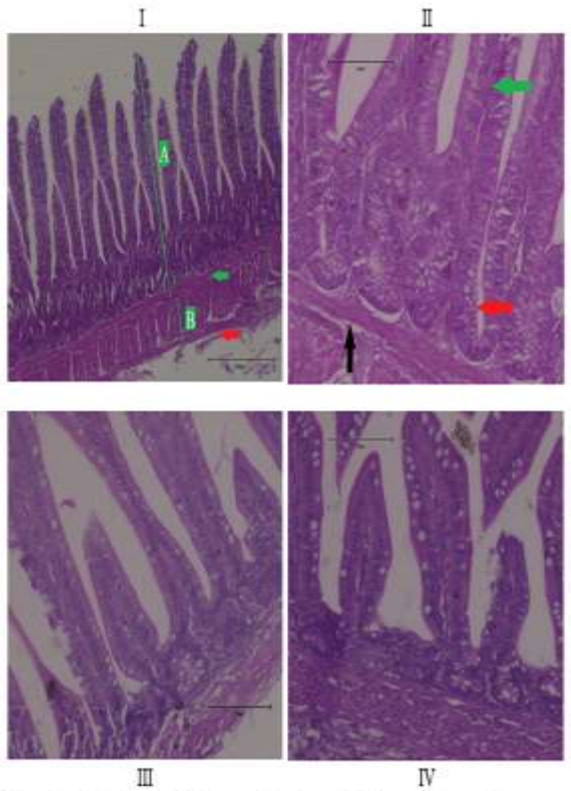
Tunica muscularis has a thick inner circular and thin outer longitudinal muscle layer in all segments while this thickness varies with age and location.

Duodenum was found to be tallest and finger-shaped while jejunum is relatively smaller and finger or tongue-shaped and lowest height was seen in ileum which is spatula-shaped. The density of goblet cells increased from duodenum to ileum as reported by Eurell & Frappier (2006). The number of goblet cells were also found in Crypts of Liberkuhn or intestinal glands. In a single villus, the density of goblet cells decreased from base to tip as mentioned by Bacha & Bacha (2012) (Figure 6). These findings depicts taller villi and less goblet cells in duodenum which might results in better digestion and absorption capacity than ileum (Jain 2009).

3.4 Histo-morphometry

3.4.1 Villus length, villus width and apparent villus area

In the present study, longer villus length of duodenum than jejunum and ileum were similar to the findings of De Verdal et al. 2010; Mabelebele et al. 2017; Rana et al. 2015 and Yamauchi 2002 who mentioned that duodenum had highest digestive and absorptive capacity and least in the ileum (Svihus and Itani 2019). Moreover, apparent villus area and villus width were also highest for duodenum in all ages of the *Sakini* chicken (Table 2). It is observed that there is no formation of additional villi which means the villi present at hatching only change its shape and size (Moon and Skartvedt 1975). Besides, the relative weight after day 28 and relative length from day one of small intestine decreased continuously over time in the current study which is similar to Alshamy et al. 2018. Increasing villus and microvillus length is the only way to cope with the increasing demand of body (Uni et al. 1999 and Wijtten et al. 2012). As birds get older, the absorption capacity of the small intestine is less dependent on an increase in length and weight rather it depends more on increasing overall villus surface area keeping the light weight of birds to fly (Wijtten et al. 2012) which is why the apparent villus area increased over time in this study. This also depicts absorptive capacity is increased with increasing age.



I: Jejunum; Day 14; A: Tunica mucosa, B: Tunica muscularis, Green arrow: Tela submucosa, Red arrow: Tunica serosa
II: Duodenum; Day 14; Black arrow: tunica muscularis mucosae, green arrow: fibers of tunica muscularis mucosae in villus and red arrow: goblet cells in the intestinal gland.
III: Duodenum; Day one; smaller number of goblet cells
IV: Ileum; Day one; greater number of goblet cells
10X objective with bar: 300 µm for I and 40X objective with bar: 75 µm for II, III and IV

Figure 6: Histomorphology of segments of small intestine; H & E stain

Sakini in the present study had relatively shorter villi length for all small intestine segments than Uttara fowl of the same age studied by Rana et al. (2015) suggesting low absorptive power of *Sakini* over Uttara fowl. At 42 days, Ross 308 broiler studied by Mabelebele et al. (2017) had higher villi length for all three segments than *Sakini* in the present study suggesting fast-growing broiler has more absorptive capacity than slow-growing *Sakini*. This is because the body weight was found to be correlated with villus length (Alshamy et al. 2018) and Ross 308 had higher body weight at the same age. The present study also suggestive that the duodenum had the highest villi and least in ileum for grower (8 weeks) but pullet (16 weeks) had the highest villi length for jejunum and ileum (Jain 2009) which suggests that in earlier age anterior portion has better digestive and absorptive property which shifts to the posterior portion of the intestine as birds become older.

3.4.2 Crypt depth, Villus length/crypt depth ratio & Tunica mucosa thickness

Similar to current study, Levi et al. (2013) found that the crypt depth increased with age (Table 2) while Alshamy et al. (2018) stated that crypt depth decreased with increasing age. Crypts are regarded as villus factories and deeper crypts indicate rapid epithelial turnover (Biasato et al. 2018 and Choct 2009) that permits renewal of villus as needed and helps in the absorption and performance of birds. Higher villi and deeper crypts are associated with better performance but with decreasing villus length: crypt depth ratio suggests it needs more energy for renewal of villi that reduce the performance (Miles et al. 2006 and Oliveira et al. 2008). With the similar villus length, digestive and absorptive capacity can vary due to variation in crypt depth (Mabelebele et al. 2017) so villus length: crypt depth ratio is also a good indicator for evaluating absorptive capacity. Increase in villus length, crypt depth, villus length : crypt depth ratio and tunica mucosa are associated with an increase in digestive and absorptive capacity (Adibmoradi et al. 2006; Mirelly et al. 2009 and Montagne et al. 2003). In consonance to these findings, the

Table 2: Changes in histological measurements of small intestinal segments of Sakini chicken from day 1 to day 56 (Mean ± SE)

Variables	Age	Duodenum	Jejunum	Ileum
Villus length (µm)	Day one	375.65±84.08^{a}	259.25±16.26^{a}	209.25±50.76^{a}
	Day 14	498.06±40.72^{ab}	314.35±16.91^{a}	234.23±9.67^{a}
	Day 28	595.31±45.96^{bc}	544.63±11.15^{b}	263.53±10.14^{a}
	Day 42	706.88±67.11^{cd}	672.44±24.36^{c}	377.92±42.62^{b}
	Day 56	834.18±19.50^{d}	610.57±80.09^{bc}	447.01±16.48^{b}
Villus width (µm)	Day one	56.66±8.52^{a}	53.96±1.75^{a}	53.42±8.25^{a}
	Day 14	100.27±2.43^{b}	77.22±3.45^{b}	79.22±3.63^{b}
	Day 28	124.84±6.65^{c}	117.47±7.19^{c}	113.92±3.93^{c}
	Day 42	119.04±5.54^{bc}	118.42±9.41^{c}	105.12±3.05^{c}
	Day 56	131.62±10.83^{c}	113.37±7.74^{c}	100.08±6.98^{c}
Crypt depth (µm)	Day one	33.61±3.89^{a}	32.32±1.11^{a}	29.66±3.20^{a}
	Day 14	93.48±4.31^{a}	138.99±8.44^{b}	129.88±14.79^{b}
	Day 28	283.14±68.95^{ab}	267.6±39.81^{c}	203.7±34.57^{c}
	Day 42	268.54±32.57^{ab}	246.79±24.91^{c}	146.34±25.23^{bc}
	Day 56	472.85±175.82^{b}	262.54±15.93^{c}	203.42±14.48^{c}
Villus length/crypt depth ratio	Day one	10.7±1.75^{a}	8.04±0.49^{a}	6.85±0.96^{a}
	Day 14	5.33±0.32^{b}	2.30±0.24^{b}	1.89±0.25^{b}
	Day 28	2.37±0.41^{c}	2.18±0.32^{b}	1.40±0.21^{b}
	Day 42	2.66±0.09^{c}	2.81±0.31^{b}	2.67±0.29^{b}
	Day 56	2.36±0.56^{c}	2.34±0.28^{b}	2.22±0.14^{b}
Apparent villus area (µm^{2})	Day one	23266.55±7099.81^{a}	14053.17±1255.19^{a}	12430.71±5198.29^{a}
	Day 14	50121.86±4978.41^{b}	24120.45±704.82^{a}	18520.10±901.42^{a}
	Day 28	74023.54±5560.93^{c}	63885.98±3637.60^{b}	29990.95±1337.58^{b}
	Day 42	83137.66±4502.09^{c}	79144.54±5121.50^{b}	39556.36±4170.33^{bc}
	Day 56	109351.55±7368.21^{d}	69702.34±10844.36^{b}	44702.80±3455.61^{c}
Tunica mucosa thickness (µm)	Day one	444.59±89.32^{a}	272.2±16.45^{a}	256.37±55.92^{a}
	Day 14	631.92±19.43^{a}	485.21±12.45^{b}	386.35±10.95^{ab}
	Day 28	974.05±125.56^{b}	845.39±33.03^{c}	509.15±45.42^{bc}
	Day 42	1112.07±124.29^{b}	963.1±20.23^{cd}	567.02±64.85^{cd}
	Day 56	1241.11±22.85^{b}	999.38±89.74^{d}	694.89±35.81^{d}
Tunica muscularis thickness (µm)	Day one	46.83±4.61^{a}	38.74±7.71^{a}	43.5±7.63^{a}
	Day 14	155.87±36.34^{b}	155.13±15.78^{b}	151.39±11.57^{b}
	Day 28	215.13±16.95^{bc}	213.42±16.74^{bc}	207.22±32.00^{c}
	Day 42	315.84±52.91^{d}	264.35±35.54^{c}	236.44±7.57^{c}
	Day 56	300.43±22.99^{cd}	279.42±23.67^{c}	316.88±17.28^{d}

Note: Values with different superscripts within single column of the single variable were significantly different from one another ($p<0.05$)

present study revealed that villus length, villus width, crypt depth, villus length: crypt depth ratio and tunica mucosa thickness were higher for duodenum followed by jejunum and least in ileum (Table 2) suggesting that duodenum had higher digestive and absorptive capacity followed by jejunum and least in ileum at respective ages. In the present study, all the histological parameters were increasing except villus length: crypt depth ratio which was decreasing with age (Figure 6) for all three segments. This finding is in contrast to Alshamy et al. (2018) who observed that this ratio increased with increase in age suggesting the better performance of Ross 308 and Lohmann Dual than *Sakini* chicken in the present study. Decrease in villus height: crypt depth ratio in the present study might be due to the fast turnover of villi tissue consuming more energy resulting in decreased performance than Ross 308 and Lohmann Dual. It is also in agreement with Wijtten et al. 2012 who found out that renewal of epithelium were faster on broiler at early age than older birds where the lifespan of enterocyte increased with age.

3.4.3 Tunica muscularis thickness

The thickness of tunica muscularis affects the rate and power of intestinal motility that affects the absorption process (De Verdal et al. 2010). The thickness of tunica muscularis is correlated with body weight, thus, with the increasing age, the body weight also increases resulting in thicker tunica muscularis over age (Alshamy et al. 2018). The current finding (Table 2) was in agreement with the study by Alshamy et al. 2018. The current study indicated that *Sakini* chicken had thicker muscularis than Uttara fowl (Rana et al. 2015) at respective ages of day one and day 28 suggesting faster passage of ingesta in *Sakini* thereby resulting in less absorption and ultimately less performance (Alshamy et al. 2018) than Uttara fowl. A similar result was found in the study of Ross 308 and Lohmann Dual (Alshamy et al. 2018) where slow-growing Lohmann dual had thicker muscularis than

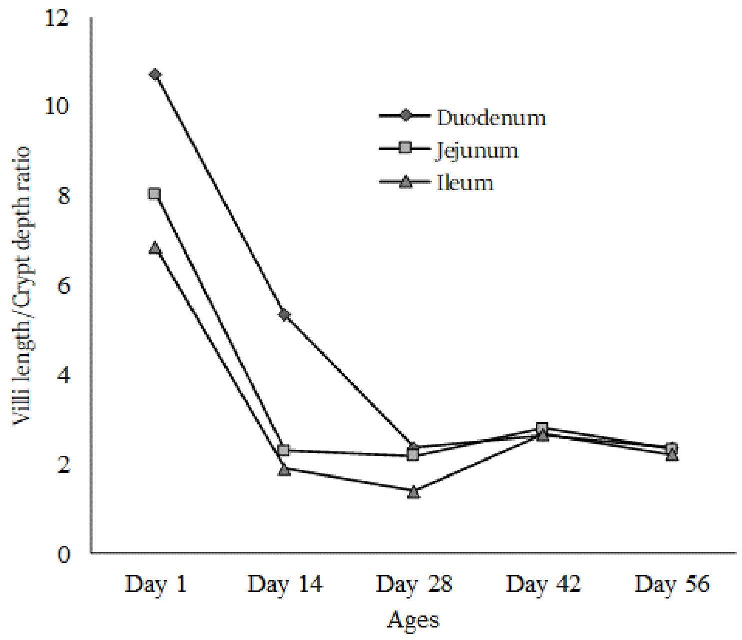


Figure 6: Villus length/crypt depth ratio of segments of small intestine at different ages (µm: micrometer)

Ross broiler resulting in less performance by Lohmann Dual bird. Svihus and Itani 2019 found that retention time of ingesta was only one hour at duodenum and jejunum combined and two hours at ileum. Although duodenum had thicker tunica muscularis it had remarkable and efficient digestive and absorptive capacity than the rest of the small intestine (Svihus and Itani 2019).

4. CONCLUSION & RECOMMENDATION

Body weight gained significantly as age progressed so did the length and weight of small intestine segments up to day 28 and day 42 respectively. In comparison between sexes, males grew faster than females as the age of birds advanced. Growth of the small intestine was higher than the rest of the body at an earlier age. Delayed stabilization of relative weight and length resulted in slower growth of *Sakini*. Digestion and absorption capacity increased with advancement of age after stabilization of relative weight and length. Increased in villus height and villus surface area were the compensatory mechanism to fulfill the increase in nutrient demand of the body since relative weight and length had less emphasis as birds grew so these parameters continuously decreased over time. Villus length, villus width, apparent villus area and ration of villus height and crypt depth were higher for duodenum followed by jejunum and least in ileum at all ages. These parameters are positively associated with the live body weight so at the initial stage of life duodenum plays the vital role for maintaining the body weight as it is principal site for digestion and absorption. Findings from a baseline data of small intestine of Sakini chicken up to 56 days which can be used as reference for further study of digestive tract of chicken.

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