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A Mathematical Model for Calculating Intestinal Villus Surface Area in Broiler Chickens

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ABSTRACT

Villus surface area (V_{SA}) is measured to reflect the active absorption surface of the intestine under different experimental conditions. In context, it has been recommended to use a suitable model for calculating V_{SA} of different shapes. However, no more attention has been paid to this point. Thus, a mathematical model, to calculate the V_{SA} of ridge-like shape villi, was proposed for increasing the accuracy of V_{SA} measuring. Then, to test the proposed model, a comparison between the common (tube-like) and proposed (ridge-like shape) models was done using real 416 villi morphometrics (height and width) of one-day-old specific pathogen-free broiler chicks. Also, Python program® was used to do a microcomputer simulation for comparing between models. Statistically, the apex surface area of the tube-like shape was greater than that of the ridge-like shape ($P<0.0001$). While the total V_{SA} of tube-like shape villus was numerically greater than this of ridge-like shape ones. These results were confirmed by the simulation study. In conclusion, since the villus shape affects the V_{SA}, the proposed model can be recommended for calculating the surface area of the ridge-like shape villi instead of the common ones. Besides villus morphometrics, the villus shape-surface area relationship can be used to explain the changes in absorption capacity and related economic parameters such as growth rate and body weight under different experimental conditions.

Key words: Intestinal morphology, Absorption capacity, Feed conversion, Simulation, Mucosal surface.

INTRODUCTION

The status of the gastrointestinal tract (GIT) affects the health and live performance of animals (Laudadio et al. 2012; Kogut and Arsenault 2016). The small intestine is a crucial part of the GIT, where food digestion and nutrients absorption occur (Svihus 2014). Structurally, the chicken's intestine is the longest organ of the GIT and comprised of three sections. Villi are considered the functional unit in the intestine. They are vascular projections that make up the lining of the small intestine to increases the capacity of the absorption area (Balbi and Ciarletta 2013). The intestinal morphology has been found to be greatly related to the absorptive function (Sittiya and Yamauchi 2014).

It has been reported that the villus does not function as a uniform unit and water is mainly absorbed through its apical part (Lee 1969; McElligott et al. 1975). Abbas et al. (1989) mentioned that the high activity of the apical part can be due to its unique structure with higher vascular intensity. Also, they referred to the importance of villus architecture and its effect on the mucosal surface area. On the other hand, Kapadia and Baker (1976) mentioned that the change from finger-shaped to ridge-shaped villi did not affect the villus mucosal surface area. Although this contentious point of research is of importance, it has not received enough attention in the previous studies.

The normal shape of villi is the finger or tube-like shape, however other shapes such as ridge-like shape is also observed in the chicken's intestine. The alteration in the villus morphology and morphometry are affected by many factors such as genetic endowment, age, health status, environmental factors, and their interactions (Gabella 1985; van Leeuwen et al. 2004; Yamauchi et al. 2010; de Verdal et al. 2010; El Sabry et al. 2013, 2015; Collins et al. 2020). For example, Uni et al. (2003) found that the length and shape of chicks' villi changed during the last trimester of the hatching period and at day of hatch. van Leeuwen et al. (2004) and Laudadio et al. (2012) reported that the crude protein and amino acids

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deficiencies, as well as high fiber levels in the diets provided to broiler chicks, affected the villi morphology and morphometry. Besides, addition of some supplements to chicken's ration has a great influence on intestinal integrity (Rajani et al. 2016; Teng and Kim 2018; Prakatur et al. 2019; Santos et al. 2019). Recent studies revealed that low-molecular-weight nucleotides have a serious impact on the intestinal villi's height and the nutrients' absorption in broilers (Brudnicki et al. 2017; Wu et al. 2018; Khedr et al. 2020).

Several poultry diseases including *Clostridia* (Xu et al. 2020), *Salmonella* (Mohamed et al. 2019), coccidia spp. (Hayakawa et al. 2014; Abebe and Gugsa 2018), reovirus (Rebel et al. 2006; Read-Snyder et al. 2009), and rotavirus (Villarreal et al. 2006) can affect the intestinal morphology and villus integrity. Also, some mycotoxins such as trichothecenes (T-2) and deoxynivalenol induce injury and necrosis of the tips of the villi due to radiomimetic action (Hoerr et al. 1981; Santos et al. 2021).

Over time, the alteration in villus architecture became a side result, as well as villus shape, has not taken into account in the villus surface area (Vs_A) calculations. For instance, van Leeuwen et al. (2004); Laudadio et al. (2012); Nain et al. (2012) and Xue et al. (2018) mentioned the changes in villus architecture in their studies, however, the V_{SA} for all groups with either tube or ridge-like shapes villi was calculated using Sakamoto model [founded by Sakamoto and Yamauchi (2000)]. This model is easy and applicable for calculating Vs_A of normal villi: $Vs_A = (2\pi) \times (V_W/2) \times (V_h)$, where V_W = villus width and V_h = villus height (Fig. 1).

Having a better interpretation for villus data may need a new mathematical model to accurately measure the mucosal area of abnormal villus architectures. Therefore, a new model for measuring the V_{SA} of the ridge-like shape is proposed and compared to the tube like-shape as a commonly used model.

MATERIALS AND METHODS

Proposed Model

Morphologically, villus comprises of two parts: 1) villus apex that can be: hemisphere in normal tube-like shape villus or cone in abnormal ridge-like shape ones and 2) villus body: is the rest of villus with a cylinder shape (Fig. 1 and 2). The following equations were derived for calculating Vs_A according to villus shape (Table 1).

Intestinal Histomorphology

This study was approved by the Institutional Animal Care and Use Committee of Cairo University Protocol No. (CU-II-F-28-19). Here, the villi data were obtained as follow: Thirty-two, day-old specific pathogen-free broiler chicks were sacrificed, and 1-cm segments were collected from their jejunum. Then, the samples were prepared, fixed, and embedded in paraffin. Three cross sections (5-µm in thickness) were mounted onto glass slides and then stained with hematoxylin and eosin (Bancroft and Gamble 2008). The sections were captured as images using a light microscopy (4X magnification). After screening the images, only the height and width of clearly intact villi (n=416) were measured using Sigma Scan Pro5 program. Height and width of villi were applied in the equations for comparing the commonly used and the proposed models.

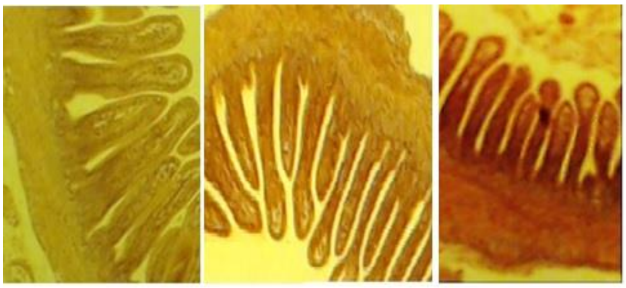

Fig. 1: Tube and ridge-like shape villi in jejunum of specific pathogen free broiler chick

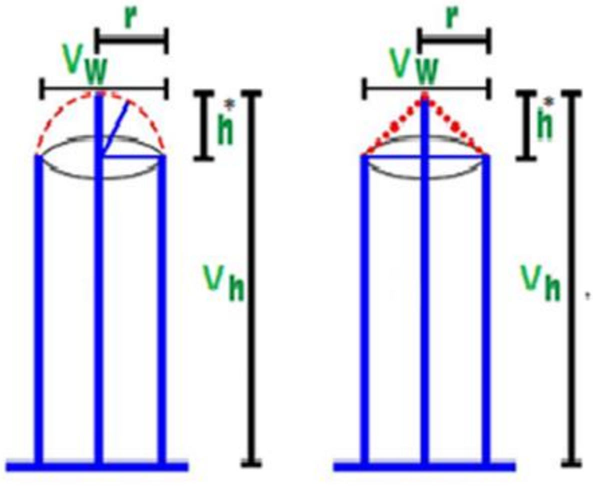

Fig. 2: Outlines of villus tube and ridge-like shapes. Villus Height: V_h, Villus Width: V_W, radius: r = h* =0.5Vw.

Statistical Analysis

The t-test was applied to compare the results of apex $_{SA}$ means of the tube and ridge-like shape villi models. Also, the same test was done to compare between total V_{SA} of tube and ridge-like shapes villi using JMP program®. In addition, Python program®, version 3.8 was used to create 50,000 cases and compare between the common and proposed models. It was assumed that the villus height was 1 mm, while the villus width ranged between 0.2-0.4 mm (Fig. 3a).

RESULTS

The results showed that the apex surface area of the tube-like shape villus was greater than the apex of the ridge-like shape one- ($P<0.0001$). The difference in apex surface affected the total V_{SA}, being the V_{SA} of tube-like shape was numerically ($P=0.155$) greater than the ridge-like shape (Table 2). These results mathematically confirm the difference in V_{SA} between normal villi and abnormal ones.

The results of the simulation test confirmed that a clear difference between apex $_{SA}$ that calculated the common and proposed models, being the apex of tube-like shape was larger than this of ridge-like shape ones (Fig. 3b). Moreover, using simulation test showed that the total V_{SA} of tube-like shape villi was (800 mm^2 / 50.000 villi) greater than this ridge-like shape villi (Fig. 3c). In addition, a strong positive relation between the villus width and villus surface area was observed (Fig. 3d).

Table 1: Two models for calculating normal (tube-like shape) and abnormal (ridge-like shape) villi surface area

Normal villus, tube-like shape surface area	Abnormal villus, ridge-like shape surface area
Hemisphere apex $_{SA}$	Cone apex $_{SA}$
$2*\pi*r^2$	$=\pi * r * L$ where $L^2=r^2+r^2$ and $h^*=r$
$=2*\pi*(0.5*V_W)^2$ $=2*\pi*0.25*V_W^2$	$L^2=2r^2$ L= sqrt($2r^2$) L= sqrt (2)*r $L=2^{0.5}*r$ $=\pi*r*2^{0.5}*r$ $=2^{0.5}*\pi$
$=0.5*\pi*V_W^2$	$*(0.5V_W)^2$
Total cylinder surface area = $2*\pi*r*h$	Adjusted Cylinder $_{SA}$ $=2*\pi*r*(V_h-r)$
$=2*\pi*0.5*V_W*(V_h-0.5*V_W)$	$=\pi*V_W*(V_h-0.5 V_W)$, which was used in both methods
Total V_{SA} of tube-like shape villus:	Total V_{SA} of ridge-like shape villus:
$V_{SA}=\pi*V_W*(V_h-0.5 V_W)+0.5*\pi*V_W^2$.	$V_{SA}=\pi*V_W*(V_h-0.5 V_W)+2^{0.5}*\pi*(0.5V_W)^2$
$=\pi[V_W*(V_h-0.5 V_W)+0.5*V_W^2]$	$=\pi[V_W*(V_h-0.5 V_W)+2^{0.5}*(0.5V_W)^2]$

Villus surface area: V_{SA}, Villus Height: V_h, Villus Width: V_W, π: pi and r: radius= $h^*=(0.5 V_W)$.

Table 2: Comparing apex and total surface area of villus that calculated using two different methods (n= 416)

Parameter Area (mm^2)	Hemisphere Normal	Ridge SE (±) Proposed	P-Value
Apex surface	0.0016±0.0000^a	0.0011±0.0000^b	<0.0001
Total villus surface	0.01500146±0.0003	0.0146±0.0003	<0.1556

Means±SE with different superscripts, within a trait, differ significantly.

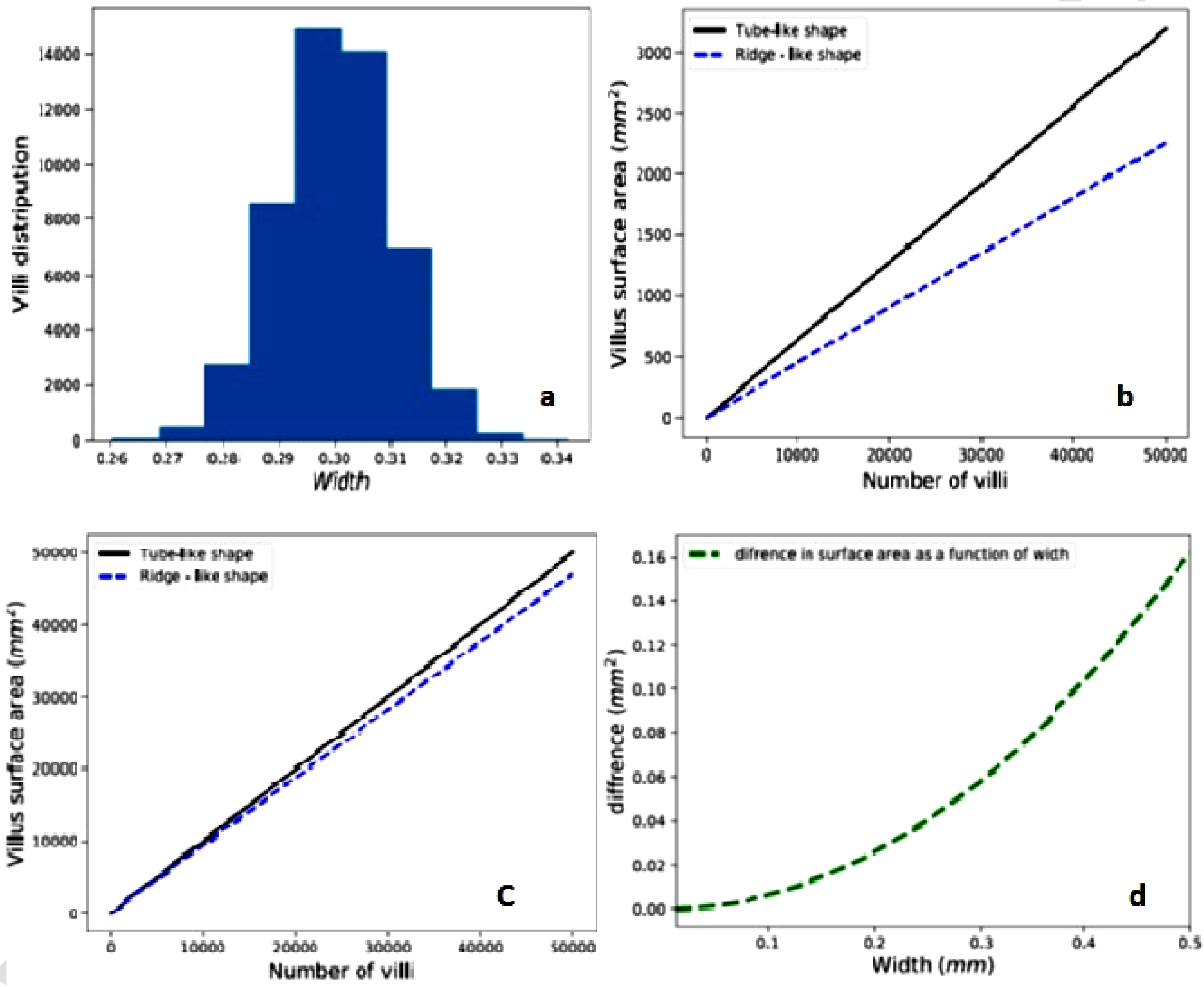

Fig. 3a: The distribution of the villus width data that used in the simulation study using Python program. **b:** Demonstrating the difference between apex surface areas of the tube- and ridge-like shapes villi. **c:** Comparing surface areas of the tube and ridge-like shapes villi using Python program. **d:** Relation between villus width and villus surface using Python program.

DISCUSSION

Villus is a unique structure in the intestine of all vertebrates that originating from the epithelial mucosa during embryonic development (Balbi and Ciarletta 2013). The villi number is about 6.000 to 25.000 per square inch of the intestine. These villi are most prevalent at the beginning of the small intestine and diminish in number toward the end of the tract. They range in length from about 0.5 to 1mm. Abbas et al. (1989) reported that the villus could be divided into the apical, middle, and basal thirds, which have different internal structures e.g. the apical part is rich with blood capillaries compared with the other two thirds. In addition, Pappenheimer and Michel (2003) quoted that “Krogh noted that 80 and 90% of blood capillaries are in the upper third of the villi of rabbits and dogs". Hence, it seems that the apex of villi is the most functional part in the absorption process compared with the other parts. It has been demonstrated that the epithelial cells originated from the lower parts of the crypts migrate along the villus surface toward the villi tips within few days for maturation (Yamauchi 2002).

Diseases conditions affecting broiler chickens have severe negative effects on intestinal integrity. For example, reovirus associated with chicken malabsorption syndrome is considered as one of the most important infections causing villus atrophy with low height which adversely affects the intestinal integrity and consequently the final body weight gains (Rebel et al. 2004). The effects of some other viral enteric diseases on the intestinal integrity of quails have been demonstrated (Kim et al. 2020). It has been demonstrated that impairment of villus function is usually happened through increasing the cell losses from the villus apex (Hoerr 1998). Moreover, bacterial infection with *C. perfringens* has been found to decrease the intestinal villus height, as well as villus height to crypt depth ratio (Li et al. 2018), which directly affects the intestinal absorption and growth parameters.

On the other hand, broiler diets supplemented with feed additives such as probiotics or acidifiers showed improved intestinal mucosal layer morphology and structural integrity of the small intestine, and consequently enhance nutrient absorption (Elhassan et al. 2019).

Accordingly, it is very important to find an accurate method for measuring the villus surface area. Measuring V_{SA} can only depend on intestinal cross section, which can provide enough data about the villus morphology and morphometry and minimize the variation of sampling (Mayhew 1988). Sakamoto and Yamauchi (2000) developed an easy method to measure intestinal V_{SA} using only the villus height and villus width. Although this method can be accurately used to measure the normal villus shape, it does not consider ridge villus. Attributed to the distinguished structure and the functional importance of the villus apex, the architecture of the villus apex was suggested to be considered in the V_{SA} measurement. Therefore, the ridge-like shape method was suggested in this study for increasing the accuracy of V_{SA} measuring.

The statistical analysis showed that apex $_{SA}$ of the tube-like shape villi was greater than those of the ridge-like shape ones, and it was confirmed by the results of the simulation test. Moreover, it enabled us to use a large number of cases (50.000) for applying a comparison between the tube and ridge-like shape models that manifests the V_{SA} difference. These results confirmed the assumptions of Lee (1969) and Pappenheimer and Michel (2003), who expected that the change in villus apex area can significantly change the total V_{SA} (difference in V_{SA} * Villus /cm^2* intestine surface area), especially the apex surface as the most active section of villi. Our results are confirmed by the simulation test that demonstrated the difference between tube and ridge-like shape models' results.

It is important to mention the positive relation between the villus $_{SA}$ and the villus width. In most of the previous studies, the villus height is the main factor that can affect V_{SA} (Laudadio et al. 2012; Biloni et al. 2013; Rekiel et al. 2014; Adibmoradi et al. 2016), while this logical relationship was not taken considerable attention. Therefore, we confirm that considering the architecture in the V_{SA} is a must to have accurate results.

In humans, Creamer (1964) found that the change in the villi morphology is followed by changes in the number and livability of epithelial cells on the villus surface. He also expected a change in the mucosal surface due to these changes. In context, accurate V_{SA} values could be important for 1) assessing the intestine development V_{SA} / crypt volume ratio and 2) comparing the efficacy of the normal and abnormal villi (Abbas et al. 1989; Biloni et al. 2013; Adibmoradi et al. 2016).

From another perspective, it has been shown that villi have a strong relationship with the economic parameters (Biloni et al. 2013; Rekiel et al. 2014; Adibmoradi et al. 2016). For instance, insoluble fiber-rich diets increased the sluffing of the tips of the villi and microvilli, which may increase the percentage of the ridge apex (Adibmoradi et al. 2016). Thus, with the ridge-like shape method for calculating the $V_{SA,}$ it can confirm the negative effect of this change from normal villi shape to ridge-like shape on both nutrient absorption and economic parameters.

In conclusion, this is the first mathematical model for accurately measuring the surface area of abnormal villus (ridge-like shape). Demonstrating the difference in villus surface due to the villus shape could help in explaining the nutritional phenomena and the changes in economic parameters due to testing new diets, pharmaceutical formulations or in the case of infection studies. Further validation studies should be conducted for testing the model on other animal species.

Author's Contribution

Mohamed El Sabry designed the protocol, performed the study and shared in collection of data, writing and revision of the manuscript. Wafaa A. Abd El-Ghany shared in collection of data, along with writing, revision and submission of the manuscript. All authors approved the final version of the manuscript.

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