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COMPARATIVE SMALL INTESTINAL HISTOMORPHOMETRY OF MUONG INDIGENOUS PIGS AND VIETNAMESE WILD BOARS

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ABSTRACT

The development of histomorphology structures of small intestine in pigs is affected by food consumptions. In the present study, twelve Muong indigenous pigs and nine Vietnamese wild boars were investigated to compare the morphology of small intestine. The tunica muscularis in duodenum of Muong indigenous pigs was significantly thicker than that in wild boars. In jejunum, the difference was observed in submucosa layer and the crypt depth. In the segment of ileum, the thickness of submucosa and inner mucosa layers of Muong indigenous pigs was significantly greater than that of the wild counterparts. Conversely, the villous height and the cript depth in the wild boars showed higher value. The ratio of villous height/crypt depth in duodenum and jejunum of Muong pigs was higher than that in wild boars but the contrary result was found in the ileum. The distribution of Goblet cells in duodenum of Muong indigenous pigs was 2.8 times more than that of Vietnamese wild boars. The results suggested that the difference in nutrient sources might be a factor to decipher the histomorphology structures of small intestine of pigs.

Keywords: Histomorphology, Muong indigenous pigs, small intestine, Vietnamese wild boars.

So sánh cấu trúc vi thể ruột non giữa lợn Mường và lợn rừng Việt Nam

TÓM TẮT

Sự phát triển cấu trúc vi thể ruột non của lợn chịu ảnh hưởng bởi thức ăn tiêu thụ. Chúng tôi tiến hành thí nghiệm trên 12 lợn Mường và 9 lợn rừng Việt Nam nhằm so sánh cấu trúc vi thể của ruột non. Lớp áo cơ đoạn tá tràng ruột non của lợn Mường dày hơn so với của lợn rừng. Ở đoạn không (hỗng) tràng, sự khác biệt có ý nghĩa ($p < 0,05$) được tìm thấy ở lớp tổ chức liên kết hạ niêm mạc và độ sâu của ống tuyến ruột. Tại hồi tràng, lớp hạ niêm mạc và niêm mạc của lợn Mường dày hơn so với ở lợn rừng, chiều cao lông nhung và độ sâu của ống tuyến ruột ở lợn rừng có giá trị cao hơn ($p < 0,05$). Tỷ lệ chiều cao lông nhung/độ sâu ống ruột tại tá tràng và không tràng của lợn Mường cao hơn so với tỷ lệ này của lợn rừng. Tuy nhiên, ở hồi tràng, tỷ lệ này ở lợn rừng lại đạt cao hơn. Số lượng tế bào Goblet tại tá tràng ruột lợn mường cao hơn 2,8 lần so với ở lợn rừng. Kết quả cho thấy sự khác biệt trong nguồn dinh dưỡng có thể là môt yếu tố quyết định sự khác biệt trong cấu trúc vi thể ruột non của hai nhóm lợn trong nghiên cứu này.

Từ khoá: Cấu trúc vi thể, lợn Mường, lợn rừng Việt Nam, ruột non.

1. INTRODUCTION

Small intestines are major site for digestion and nutrient absorption which are specially designed to accommodate enlarged surface areas (Herdt, 2007). Contributing to the digestive and absorptive abilities of small intestines are the architectural structures of mucosa including the large folds known as *plicae circulares*, fingerlike epithelial projection known as *villi* and brushlike surface membrane covering the villi known as *brush border* (Skrzypek *et al.,* 2007; Biagi *et al.,* 2007).

The digestive ability of small intestines is greatly influenced by quality of diet (Vicente *et al.,* 2009). Although the number and size of villi

are operated by wild genotype (Skryper *et al.,* 2007). Mitchaothai *et al.* (2010) have proven the effect of diet with high calcium on the height of villus. However, it is obviously that the quantity of feed compositions of the wild animal does not reflect individual preferred as well as special species' requirement but only the availability of food supply.

By comparing the morphology of small intestines, not only the individual response reflecting the level of adaptation can be defined but also the preferred feed profile for further conservation of Vietnamese indigenous and wild boars could be partially predicted.

2. MATERIALS AND METHODS

Twelve Muong indigenous pigs (7 M and 5 F; BW, 10.65 ± 2.3 kg; body length, BL, 58.83 ± 9.25 cms) have been randomly collected from Muong ethnic households in Hoabinh - a northwest mountainous province of Vietnam. Nine Vietnamese wild boars (7 M and 2 F; BW, 13.63 ± 0.57 kgs; BL, 70.28 ± 5.84 cms) were procured in Langson in far northern of Vietnam. Average age was estimated of 437.83 ± 102.62 d for Muong indigenous pigs but not available for Vietnamese wild boars (Biodata of animals were shown in appendix 1). Small intestine samples were taken immediately after death and separately cut from stomach and large intestine.

All the contents were gently removed under tap water. Samples were selected (3x4 cm - longitudinal) from 3 segments including Duodenum, Jejunum and Ileum; and fixed in 10% buffered formalin for further histomorphological studies.

Tissue selected from fixed area was trimmed (≤ 3mm thick) and refixed in neutral buffered formalin up to 48 hours before processing. Samples were processed 18 hours then embedded in melted paraffin.

Paraffin blocks were sectioned in 3 µm using microtome then floated on 37°C water bath. The most intact sections were chosen and placed onto the surface of clean glass slides. The slides were left on warming block (up to 4 hours) for drying and the wax starting to melt.

Different segments of the small intestine including duodenum, jejunum and ileum from each individual sample were sectioned into 3 µm for H&E staining and 5 µm for Alcian blue staining. A slight modification of the Orcein-Alcian blue staining method described by Singh and Gorton (1989) was employed for differentiation of sulphated mucins and sialomucins.

Tissue sections were examined at the X5 and X20 magnifications for histometry and goblet cells enumeration under a light microscope (Leica, Japan).

Each sample was measured in triplicates for muscularis externa, submucosa, inner mucosa, villous height and crypt depth. Goblet cells were counted on 20 different fields on each duodenum slide that has been stained with Alcian blue.

Diagram for measurement of studied parameters is described below.

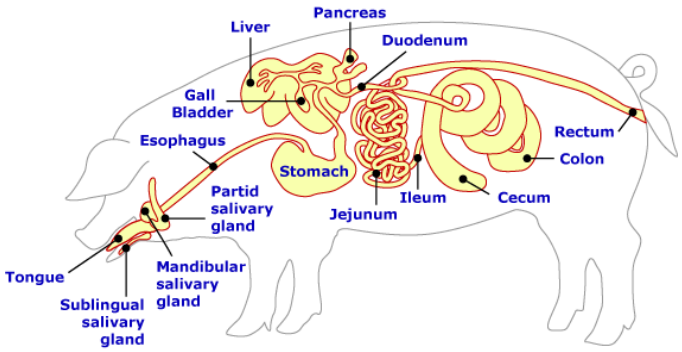
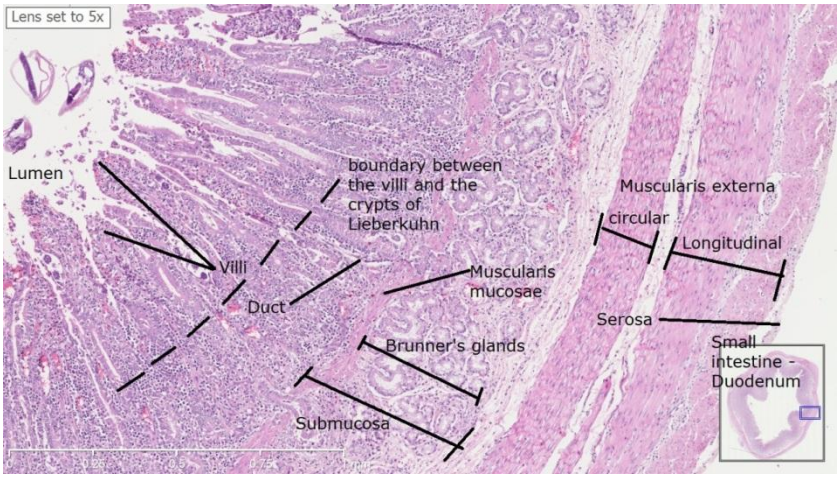


Diagram for small intestinal necropsies

Source: https://courses.ecampus.oregonstate.edu/ans312/ten/swine_1.htm



Source: http://www.onlineveterinaryanatomy.net/content/equine-duodenum-histology-0

Table 1. Comparative histometry of the duodenum (µm; Mean ± S.E.M)

	Muong indigenous pigs	Vietnamese wild boars
Parameter	n = 12	n = 9
Muscularis externa	408.58 ± 22[a]	372.83 ± 9[b]
Submucosa	164.61 ± 15	164.42 ± 7
Inner mucosa	687.84 ± 19	697.28 ± 9
Villus height	349.99 ± 14	353.41 ± 14
Crypts depth	249.25 ± 7	262.95 ± 9

Note: [a,b]Values within row with different superscripts differ at p <0.05

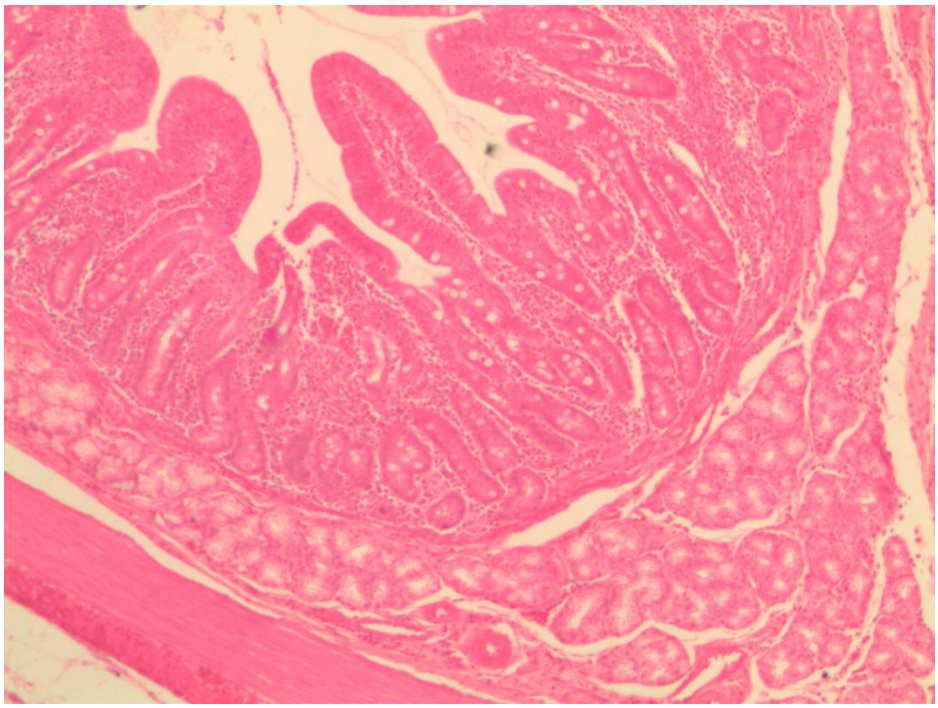


Figure 1. Photomicrograph of the duodenum of Vietnamese wild pig (H&E, X20)

One way ANOVA has been employed for statistical analysis and only data with *p-value* less than 0.05 was considered significant.

3. RESULTS

3.1. Duodenal histometry

The results of morphology measurement of duodenum are presented in Table 1. Two studied subjects revealed similarities of duodenal structure with four out of five measured elements being comparable ($p \geq 0.05$). In this segment of small intestines, only the thickness of tunica muscularis of Muong pigs was significantly ($p < 0.05$) greater than that of the Vietnamese wild boars (408.58 ± 22 vs. 372.83 ± 9).

3.2. Jejunal histometry

The results of morphology measurement of the middle segment of the jejunum are presented in Table 2. In this portion, the submucosa layer of the Muong pig was significantly ($p < 0.05$) thicker than that in the wild pig counterpart. On the other hand, the crypts in the Vietnamese wild boars revealed significantly greater depth than those of the Muong pigs (227 µm vs. 214 µm).

Although the layers of tunica muscular and mucosa of the Vietnamese wild boars were slightly greater than that of the Muong pig, the difference was not significant ($p \geq 0.05$).

3.3. Ileal histometry

The mean values of ileal morphometry are given in Table 3. Among the three structural layers of ileum, the Muong pigs presented an elevation of the thickness of both submucosa and tunica mucosa compared to that of the Vietnamese wild boars ($p < 0.05$). Conversely, the height of ileal villi and the cript depth of Vietnamese wild boars ileum were significantly ($p < 0.05$) greater than that of Muong pigs (291.29 ± 14 vs. 266 ± 21 and 204.46 ± 6 vs. 191.17 ± 12, respectively).

Table 2. Comparative histometry of the jejunum (µm; Mean ± S.E.M)

	Muong indigenous pigs	Vietnamese wild boars
Parameter	n = 12	n = 9
Muscularis externa	411.41 ± 46	450.73 ± 35
Submucosa	338.29 ± 71[a]	188.46 ± 9[b]
Inner mucosa	630.15 ± 26	637.81 ± 28
Villus height	288.23 ± 25	282.92 ± 18
Crypts depth	213.75 ± 13[a]	226.99 ± 8[b]

Note: [a,b]Values within a row with different superscripts differ at p < 0.05

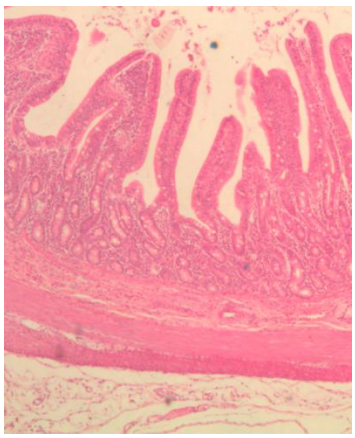


Figure 2. Photomicrograph of the Jejunum of Muong indigenous pig (H&E, X10)

Table 3. Comparative histometry of the ileum (µm; Mean ± S.E.M)

	Muong indigenous pigs	Vietnamese wild boars
Parameter	n = 12	n = 9
Muscularis externa	450.31 ± 50	446.92 ± 25
Submucosa	505.68 ± 57[a]	429.79 ± 33[b]
Inner mucosa	616.27 ± 32[a]	585.05 ± 21[b]
Villus height	266.30 ± 21[a]	291.29 ± 14[b]
Crypts depth	191.17 ± 12[a]	204.46 ± 6[b]

Note: [a,b]Means (± S.E.M) within a row with different superscripts indicate significant differences at $p < 0.05$

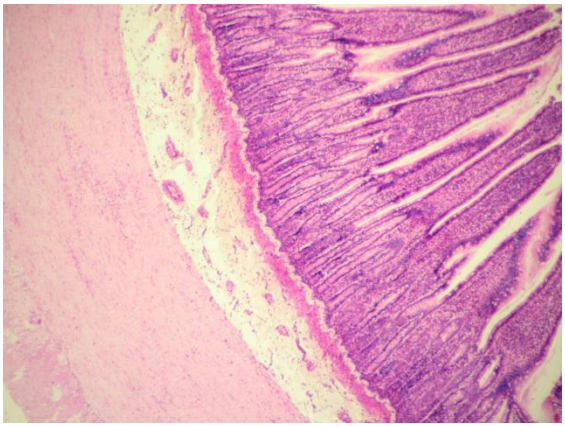


Figure 3. Photomicrograph of the Ileum of Vietnamese wild pig (H&E, 10X)

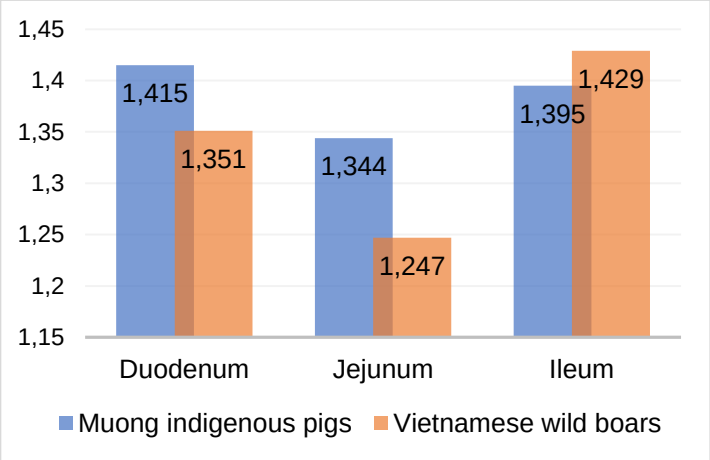


Figure 4. Comparative vilus/crypts of small intestine

3.4. Comparative ratio of villous height and crypt depth of small intestines

Figure 4 shows the comparison between the ratio of villus height and crypt depth of the small intestine in the three different regions.

In the two first segments, i.e., the duodenum and jejunum, the villus/crypt ratio of Muong indigenous pigs was higher than that of the Vietnamese wild boars. However, at the ileal end, this ratio was greater in the Vietnamese wild boars (1.429 ± 0.216 vs. 1.395 ± 0.187).

3.5. Quantities of duodenal goblet cells

The total number of duodenal goblet cells enumerated in both species is shown in Figure 4.

The analysis revealed that the number of Goblet cells was significantly ($p < 0.05$) higher in the Muong indigenous pig as compared to that of the Vietnamese wild pig (790.56 vs. 274.14).

Figure 6 and Figure 7 show the duodenal histology of the Muong indigenous and Vietnamese wild boars, respectively. It is evident that the goblet cells in the Muong indigenous pig were evenly distributed in all layers (Figure 3). However, fewer goblet cells were seen in the Vietnamese wild boars which also almost completely disappeared on the epithelial layer of villus (Figure 4).

Figures 8 - 10 show the histology of the duodenum of both pig breeds being stained by Orcein and AB to demonstrate the different type of mucus producing cells, i.e., sulphated and carboxylated mucins, respectively. The sulphated and carboxylated mucins are seen as brown to black and light to slight blue colour, respectively.

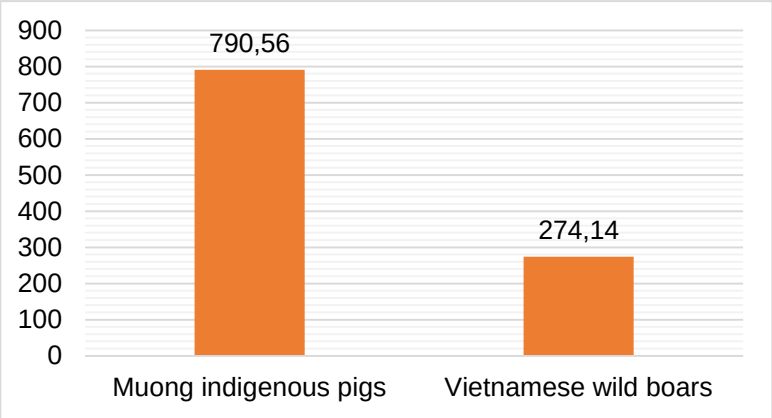


Figure 5. Comparative quantities of duodenal goblet cells

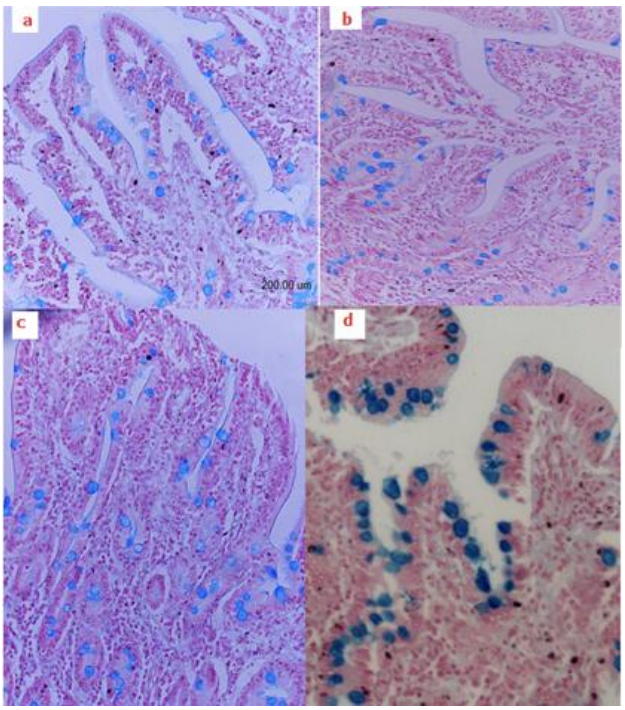


Figure 6. Photomicrograph of the duodenum of the Muong indigenous pig

Note: The goblet cells (blue color) is evident at almost all layers (Alcian blue staining, 400X)

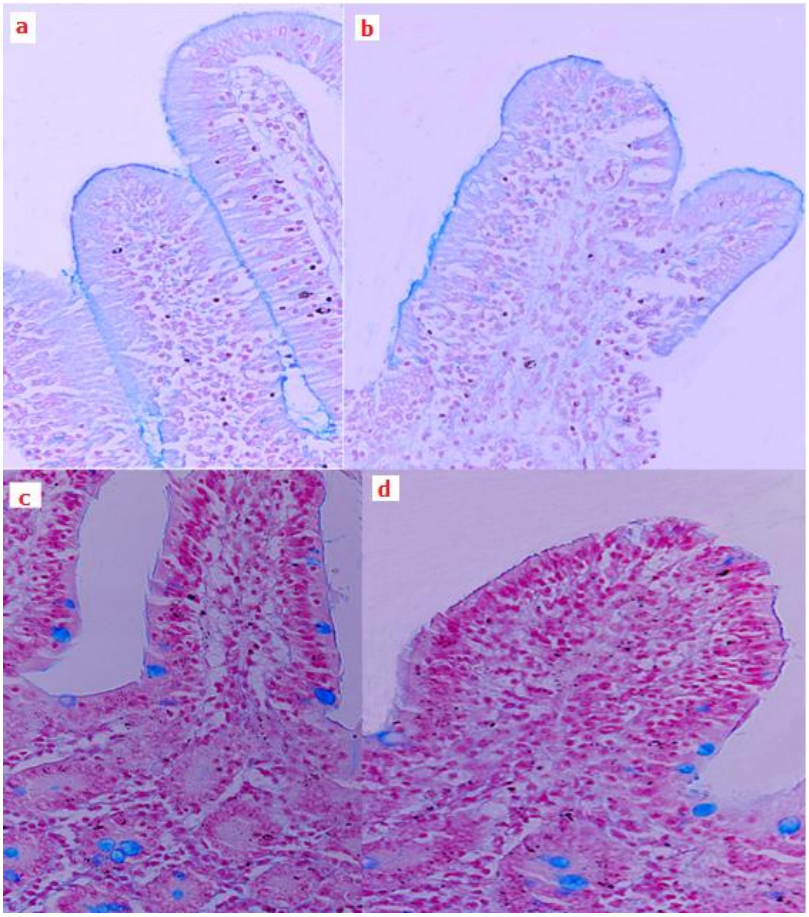


Figure 7. Photomicrograph of goblet cells (blue color) in duodenum of the Vietnamese wild boars

Note: Not only they are scarcely seen but disappear at the epithelial surface (Alcian blue staining, 400X)

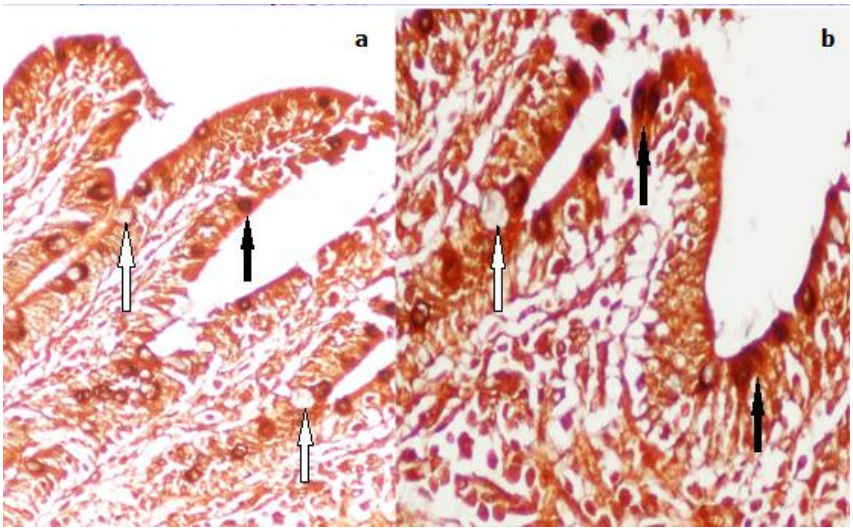


Figure 8. Photomicrograph of the duodenum of the Muong indigenous pigs

Note: It predominantly composes of sulphated mucins (brown to black - black arrow) compared to very few sialomucins (slight blue - white arrow) on epithelium of duodenum (Orcein-AB; a: 200X; b: 400X)

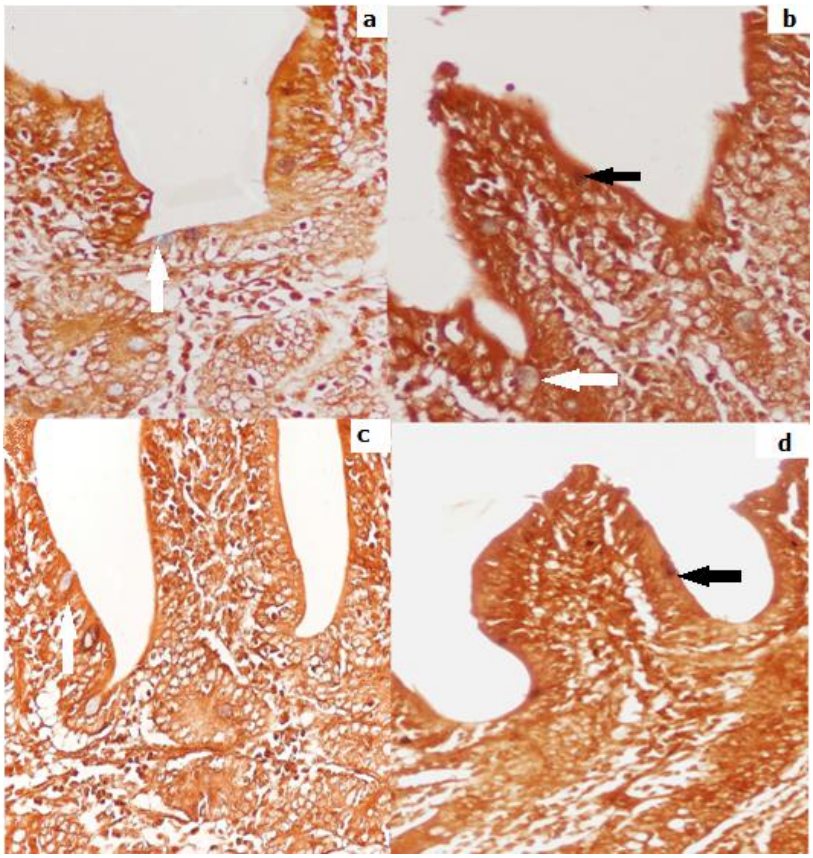


Figure 9. Photomicrograph of the duodenum of the Vietnamese wild boars. Note the scarcity of goblet cells population containing sulphated mucins (brown to black - black arrow) and sialomucins (slight blue - white arrow). (Orcein-AB; a and b: 400×; c and d: 200×)

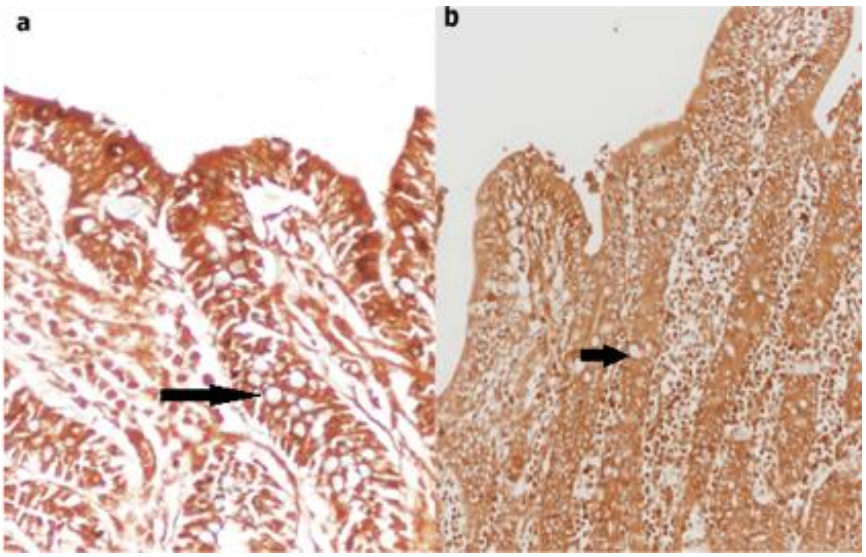


Figure 10. Photomicrograph of the duodenum of the Muong indigenous (a) and Vietnamese wild boars (b). Although it is evident that there is different quality and quantity of goblet cells on the epithelium, the carboxylated mucins producing cells in the crypt are still of majority (light to slight blue - black arrow; Orcein-Alcian blue; a: 400×; b: 200×)

4. DISCUSSION

In general, there exists vast similarity in the morphometry of the duodenum in Vietnamese wild boars and Muong indigenous pigs especially with regards to the thickness of submucosa and tunica mucosa with the exception of a thicker muscularis externa in the Muong indigenous pigs. This discrepancy occurs probably due to physiological activity since it is the main site for digestion, in particular mixing of feed with digestive secretions (Herdt, 2007). With reference to digestive function, Uhr (1995) has proven that the influence of genetic improvement during domestication rather than originated modification led to an increase in small intestinal dimensions. Uhr (1995) stated that in the domestic pig as seen in the Muong indigenous breed in this study, the greater development of tissue at the site where digestion took place resulted from the correlation between high rate of digestion and high protein biosynthesis as compared to that of the Vietnamese wild boars. In the wild boars, the diet is usually those of low digestibility and protein.

The increased thickness of the small intestine of the Muong pigs could have arisen due to the existence of the Peyer's patches. The appearance of Peyer's patches can be considered as genetically fixed in submucosa of digestive tract (Uhr, 1995). Furthermore, it is especially abundant in ileum and is affected by the stimulation of microbes immigrating from colon (Driessen *et al.,* 2002; Fayed *et al.,* 2010). It is likely that the manner of being reared under an "intensive" farming system would have created stress to the pigs. Thus, the gut environment of Muong pigs is supposedly to be more sensitive to disease leading to much more lymphatic tissue (Peyer's patches) to confer a better immune system (Uhr, 1995).

However, this finding is in contrast to those reported elsewhere documenting wild boars to harbor greater number of Peyer's patches (Skrzypek *et al.*, 2007). Nevertheless, in that study the pigs used were crossbred which would have assumed the conformity towards what is seen in the Muong pigs which were reared intensively as opposed to that of the "purebred" Vietnamese wild boars used in this study. Likewise, several findings in this study bear resemblance to those of regulating of shallower of transversal furrows in duodenum, longer of villi in jejunum of Polish landrace/Pietrain in comparison with Duroc/Hampshire/wild boar crossbreed piglets (Skrzypek *et al.*, 2007).

Although GI morphology was greatly influenced by feed composition, frequency of food intake, body shape and size (Kararli, 1995), the ileum still remains conserved as it is the main site for absorption (Gourevitch D. 2005). Therefore, it is not surprising or unexpected for the presence of statistical difference in ileal morphology between the Muong indigenous and Vietnamese wild boars. In this study, due to the continuously changing and different living conditions between both breeds, ileal histomorphometry are highly differentiated (Uhr, 1995).

On the contrary with the middle layer, the tunica mucosa diminished towards the end of the length of small bowel and conformed to the typical morphology of small intestines (Gourevitch, 2005; Budiño *et al.*, 2005). Such trend was also seen in the in rats (Vigueras *et al.*, 1999; Hosoyamada and Sakai, 2005), geriatric dogs (Kuzmuk *et al.*, 2005) and geese (Liu *et al.,* 2010).

In considering the ratio of villus height and crypts depth (VH/CD ratio), the influence of quality and quantity of food intake must be considered. The present study yielded lower ratios than those observed in weaned and monitored feed-intake commercial pig breeds (Tang *et al.,* 1999; Brundige *et al.*, 2008) but higher ratios than in dietary restricted and malnourished pigs (Nunez *et al.*, 1996). This discrepancy could have arisen from the different sampling time and also nature of feeding in the two breeds studied.

Although some authors considered VH:CD ratios as the standard index of absorptive abilities (Pluske *et al.*, 1996; Pluske *et al.*, 1997; Montagne *et al.*, 2003). However, the response of these ratios maybe reversed by a variety of factors. Hyperplasia of crypt taht are not accompanied with the increasing villous height may lead to a

decrease of VH:CD ratio (Kien *et al*., 2007). The influence of microflora and external environment on the enterocyte turnover rate has been proven to result in shorter villi and deeper crypt (Kelly *et al*., 1994).

Conversely, the VH:CD ratio may not be unchanged even though the diminishing factors of the both element are obvious. Fasting condition reduces cell division in the crypts which might led to villous atrophy (Nunez *et al.,* 1996; Fernandez-Estivariz *et al.,* 2003). The high calcium diet may promote the VH:CD ratio in wild boars mainly by diminished depth of crypts but there was no affection of this dietary component on the villous height (Mitchaothai *et al*., 2010). Therefore, the increase/decrease of both villous height and crypt depth influenced by different factors must be sifted when making conclusion of absorptive abilities of small intestines based on VH:CD ratio. Sifting of these factors is almost impossible in this study as its main aim was mainly the histomorphometry and further studies should be carried out to determine the factors governing VH:CD in both breeds.

The total number of duodenal goblet cells in this study did not agree with previous researches. This may due to different methodology since almost researchers used villi as a standard unit (Fernandez-Estivariz *et al*., 2003; Brundige *et al*., 2008) instead of scoring total number of cells on field and also different species did not have the same quantities of Goblet cells on epithelium (Paulini *et al*., 1987; Ito *et al*., 2009).

The influence of fiber diet on mucus production of gastrointestinal tract has been documented. Ito *et al.* (2009) have found the relation of soluble fiber and small intestinal mucins activity where higher molecular weight fiber resulted in the greater number of goblet cells. The similar results were obtained by Satchithanandam *et al.* (1990) when they compared the mucin reactivity to 5% guar gum, 5% citrus fiber and fiber-free control diets. The results indicated that statistically greater reaction has been found in both luminal and tissue level of 5% citrus fiber feeding group.

The modeling study carried out by Paulus *et al.* (1993) stated the same origin stem cell of goblet and columnar cells and the shift to goblet cell took place at specific stage in the development of epithelial columnar cells. Therefore, factors affecting turnover proliferation of small intestines and crypt cell division in particularly such as daily food restriction (Nunez *et al*., 1996; Fernandez-Estivariz *et al.,* 2003), prolonged protein depletion or lower nutrition requirement of protein-energy (Sherman *et al*., 1985) are all responsible for the decrease of the mucin production in small intestines. The Vietnamese wild boars are destined to have lower number of goblet cells as their diet albeit fibrous but likely to be of lower digestibility apart from prolonged protein depletion or low protein diet. However, Muong indigenous pigs owing being managed under a much more intensive and commercial system (better feed quality in terms of fiber and protein) have much higher goblet cell population.

In general, the occupying of sulphomucin containing cells on the villi and the predominant of sialomucin producing cells in both deeper areas of the crypt and Brunner' gland in both breeds were in agreement with the earlier observations (Poddlar and Jacob, 1979; Singh and Gorton, 1989).

5. CONCLUSION

The differences of morphometry of small intestine were mainly observed in jejunum and ileum which were the most influenced by the dietary feed intake and also the modify abilities of the absorptive structure to the hash and continuingly changing of nurture condition.

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Appendix 1. Biodata of animals

No.	Code	Body weight (kg)	Body length (cm)	Sex	Estimated age (day)
1	HB1	9.5	50.6	Female	350
2	HB2	8.0	42.5	Male	330
3	HB3	8.2	43.1	Male	330
4	HB4	8.5	45.2	Female	350
5	HB5	8.0	43.0	Male	330
6	HB6	9.3	49.4	Female	350
7	HB7	14.0	67.2	Female	547
8	HB8	12.5	60.2	Male	530
9	HB9	13.8	66.2	Male	547
10	HB10	12.0	57.6	Male	530
11	HB11	12.5	59.8	Male	530
12	HB12	11.5	61.1	Female	530
13	LS1	12.0	61.4	Male	n.d
14	LS2	13.5	69.2	Female	n.d
15	LS3	12.5	66.4	Male	n.d
16	LS4	14.0	73.2	Male	n.d
17	LS5	14.5	75.8	Male	n.d
18	LS6	13.5	64.6	Male	n.d
19	LS7	14.0	71.7	Female	n.d
20	LS8	13.5	69.7	Male	n.d
21	LS9	15.2	80.5	Male	n.d

Note: HB, LS: Hoa Binh, Lang Son province; n.d: Not detected