ORIGINAL ARTICLE

Histomorphometric study of the effect of methionine on small intestine parameters in rat: an applied histologic study

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Background: Assessment of morphological changes has more often been used in the diagnosis and assessment of intestinal pathology and development. Since methionine is widely used in nutritional and sports supplements and also there is not enough information about the effect of this amino acid on the gastrointestinal histomorphometry, the aim of this study was to assess the effect of methionine on the small intestine histomorphometry.

Materials and methods: Thirty male Wistar rats were randomly divided to three equal groups. Two treatment groups received 100 and 200 mg/kg L-methionine solution respectively via intraperitoneal injection while the control group received normal saline. On day 21, all rats were euthanised and segments from three parts of small intestine were taken to histomorphometrical study. Paraffin sections were stained with haematoxylin and eosin, alcian blue (AB) and periodic acid Schiff (PAS) methods separately. In order to analyse histomorphometric features of each segment, villus height, width, area, crypt depth, villus height to crypt depth ratio, goblet cell number, and muscle layer thickness were measured.

Results and Conclusions: Obtained results revealed that methionine may change the histomorphometric parameters of small intestine. (Folia Morphol 2017; 76, 4: 620–629)

Key words: histomorphometry, small intestine, methionine, rat

INTRODUCTION

The small intestine is the long muscular tube of the gastrointestinal tract that is the major site for absorption and digestion of nutrients and drugs. It is made up of three structural segments: duodenum, jejunum and ileum [17–19]. All of the absorptive and also a part of the digestive capacity of the small intestine occur around and near villi and crypts [21]. Actually the crypt-villus is the main functional unit of absorption in the rat's small intestine. Various substances and also different physiological or pathological conditions may change the proliferation rate of enterocytes in the crypt and affect their migration rate to the top of the villi [46]. The relation between small intestine and nutritional factors is very complicated. Nutrients may directly cause changes or adaptations in the intestinal mucosa [4, 47].

Methionine (Met), a sulphur-containing essential amino acid, serves as the initiating amino acid in the synthesis of many proteins. It plays an important role in the synthesis of vital molecules such as cysteine, carnitine, taurine, lecithin, phosphotidyl choline and

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other phospholipids. It has a significant effect on improving cartilage formation and is therefore used in arthritis treatment [49].

Histomorphometric analysis is greatly used in studies about gastrointestinal pathophysiology. The availability of animal models and using morphometric analysis as a quantitative assessment have facilitated evaluating the morphological alterations of intestinal mucosa under various experimental conditions such as special diets and drugs [10].

The laboratory rat, as an animal model, has been used in several histological and histomorphometrical studies [1, 53]. Some of these studies have described the qualitative and quantitative changes that occur in the small intestine under various physiological and pathological conditions [10, 11, 13, 31, 32]. Many studies have investigated the effect of methionine supplementation on the skin [30], teeth [25], liver disorders [23], feather follicles [28], female reproductive system [3, 29] and neural tube in the embryos [24] but there is not enough information about its effect on the small intestine in the mammals.

Methionine is widely used in nutritional and sports supplements and on the other hand the effects of these supplements have not been investigated on the gastrointestinal histomorphometry. So the aim of this study was to assess the effect of injected methionine on histomorphometric aspects of small intestine in the rat.

MATERIALS AND METHODS

The experiment in this study was approved by the Animal Ethics Committee, a branch of the Research Council of the Veterinary School in Shahid Bahonar University, Kerman Province, Iran.

A total of thirty male Wistar rats weighing 190––210 g were used for this research. The rats were housed in clean plastic cages and kept under the following conditions: 12 h light/dark cycle, controlled temperature of 22–25ºC and humidity of 60–70%. The rats were allowed an adaptation period of 5 days with free access to commercial rodent diet and water *ad libitum*. The rats were randomly divided into three groups containing ten rats. All three groups received intraperitoneal injections for 20 consecutive days. Rats in the group A served as the control group and were administered with normal saline. Group B and C rats were treated with L-methionine (Merck, Germany) 100 mg/kg and 200 mg/kg body weight, respectively. Dose regimen, duration and route of administra-

tion in this study were based on a previous research that investigated the histomorphometric effect of methionine on the rat's skin [30]. At the end of the treatment period, all rats were euthanised. The abdomen was opened through a midline incision and the small intestine of each rat was gently removed. Then small segments of intestine, about 1 cm in size, were removed from the duodenum (below the pyloro-duodenal junction), jejunum (2 cm anterior to Meckel's diverticulum) and ileum (2 cm anterior to ileocecal junction). All intestinal portions were fixed in 10% neutral buffered formalin for 10 days [4, 36], dehydrated in graded anhydrous absolute ethanol and xylol, embedded in paraffin blocks and then cut into 5 μm sections. In each animal, one section that represented the best view of villus and crypts was selected to be analysed. The sections were stained using haematoxylin and eosin (H&E) (for standard histological evaluation), periodic acid Schiff (PAS) (in order to count the number of neutral goblet cells) and alcian blue (AB) (in order to count the number of acidic goblet cells) techniques. Slides were examined using an Olympus light microscope and photomicrographs were taken by an attached eyepiece camera (Dino-eye, AM-7023, 5Mp, Taiwan). The following intestinal histomorphometric parameters were evaluated in all sections stained with H&E method: villous height, villous width, crypt depth and the muscular layer thickness. These parameters were measured on 20 well-aligned villi and corresponding crypts from each section of all intestinal segments. The heights of the villi were defined from their tip to the base and the widths were measured at the half height point. Calculations using villous height and width at half height gave the villus surface area. The depth of intestinal crypts was measured as the distance from top of villus crypt to muscularis mucosa.

The number of goblet cells per villus was counted in ten well-oriented and adjacent crypt villus on each section stained by PAS and AB histochemical techniques. All measurements were made in μm, at ×100 magnification.

Statistical analysis

Results were expressed as mean values ± standard error. Data were analysed using one-way analysis of variance (ANOVA) followed by *post hoc*, Tukey's HSD test. Statistical analysis was performed using SPSS (SPSS for Windows, version 16.0, SPSS Inc., Chicago, Illinois). Differences were considered statistically significant when the calculated p value was less than 0.05.

Table 1. The effect of intraperitoneal administration of methionine on the histomorphometric parameters of rat duodenum (values are mean ± standard error)

	Control	Met 100	Met 200
VH [μm]	389.47 ± 33.84[a]	533.84 ± 23.05[b]	525.82 ± 14.61[b]
VW [μm]	69.47 ± 5.58[a]	103.22 ± 6.41[b]	106.66 ± 6.56[b]
VA [μm²]	27056.65 ± 2350.8[a]	55102.96 ± 2379.22[b]	56084.15 ± 1557.7[b]
CD [μm]	264.28 ± 13.74[a]	264.32 ± 32.31[a]	166.72 ± 9.79[b]
VH/CD	1.47 ± 0.13[a]	2.01 ± 0.09[b]	3.15 ± 0.09[c]
Goblet cells (AB)	8.67 ± 1.05[a]	15.83 ± 1.89[b]	12.57 ± 2.01[ab]
Goblet cells (PAS)	8.9 ± 0.86[a]	16.11 ± 1.43[b]	12.13 ± 1.11[ab]
Muscle layer thickness [μm]	77.94 ± 5.38[a]	101.26 ± 8.49[b]	157.17 ± 6.99[c]

[a, b, c] Means in the same row with no common superscript differ (p < 0.05).
AB — alcian blue; CD — crypt depth; PAS — periodic acid Schiff; VA — villus area; VH — villus height; VW — villus width

Table 2. The effect of intraperitoneal administration of methionine on the histomorphometric parameters of rat jejunum (values are mean ± standard error)

	Control	Met 100	Met 200
VH [μm]	353.04 ± 10.64[a]	405.53 ± 8.8[b]	423.16 ± 16.05[b]
VW [μm]	87.26 ± 5.78[a]	120.72 ± 2.79[b]	107.24 ± 5.54[b]
VA [μm²]	30406.09 ± 1101.46[a]	38287.1 ± 1782.05[b]	45380.1 ± 1721.66[c]
CD [μm]	214.61 ± 7.19[a]	208.15 ± 6.07[a]	161.86 ± 10.64[b]
VH/CD	1.64 ± 0.05[a]	1.52 ± 0.07[a]	2.61 ± 0.1[b]
Goblet cells (AB)	12 ± 1.21[a]	20 ± 1.37[b]	18.6 ± 1.63[b]
Goblet cells (PAS)	14 ± 1.31[a]	23.2 ± 1.88[b]	19.8 ± 0.86[b]
Muscle layer thickness [μm]	61.33 ± 5.12[a]	81.35 ± 5.01[b]	88.06 ± 6.09[b]

[a, b, c] Means in the same column with no common superscript differ (p < 0.05).
AB — alcian blue; CD — crypt depth; PAS — periodic acid Schiff; VA — villus area; VH — villus height; VW — villus width

Table 3. The effect of intraperitoneal administration of methionine on the histomorphometric parameters of rat ileum (values are mean ± standard error)

	Control	Met 100	Met 200
VH [μm]	199.47 ± 5.11[a]	272.2 ± 7.46[b]	265.56 ± 2.78[b]
VW [μm]	94.53 ± 2.51[a]	130.73 ± 3.15[b]	118.58 ± 4.5[b]
VA [μm²]	18856.89 ± 482.22[a]	33811.26 ± 2356.84[b]	31490.1 ± 329.83[b]
CD [μm]	221.72 ± 5.01[a]	186.87 ± 3.66[b]	206.68 ± 0.73[a]
VH/CD	0.9 ± 0.02[a]	1.45 ± 0.04[b]	1.28 ± 0.01[c]
Goblet cells (AB)	19.8 ± 2.08[a]	27.67 ± 1.48[b]	25 ± 1.63[ab]
Goblet cells (PAS)	24.86 ± 1.35[a]	31 ± 1.68[b]	24.56 ± 1.55[a]
Muscle layer thickness [μm]	55.13 ± 4.54[a]	66.85 ± 3.94[b]	72.66 ± 4.15[b]

[a, b, c] Means in the same column with no common superscript differ (p < 0.05).
AB — alcian blue; CD — crypt depth; PAS — periodic acid Schiff; VA — villus area; VH — villus height; VW — villus width

RESULTS

According to our findings, the villus height and width in the duodenum (Table 1, Fig. 1) jejunum (Table 2, Fig. 2) and ileum (Table 3, Fig. 3) were significantly increased in both treatment groups compared with controls (p < 0.05). However, there was not any
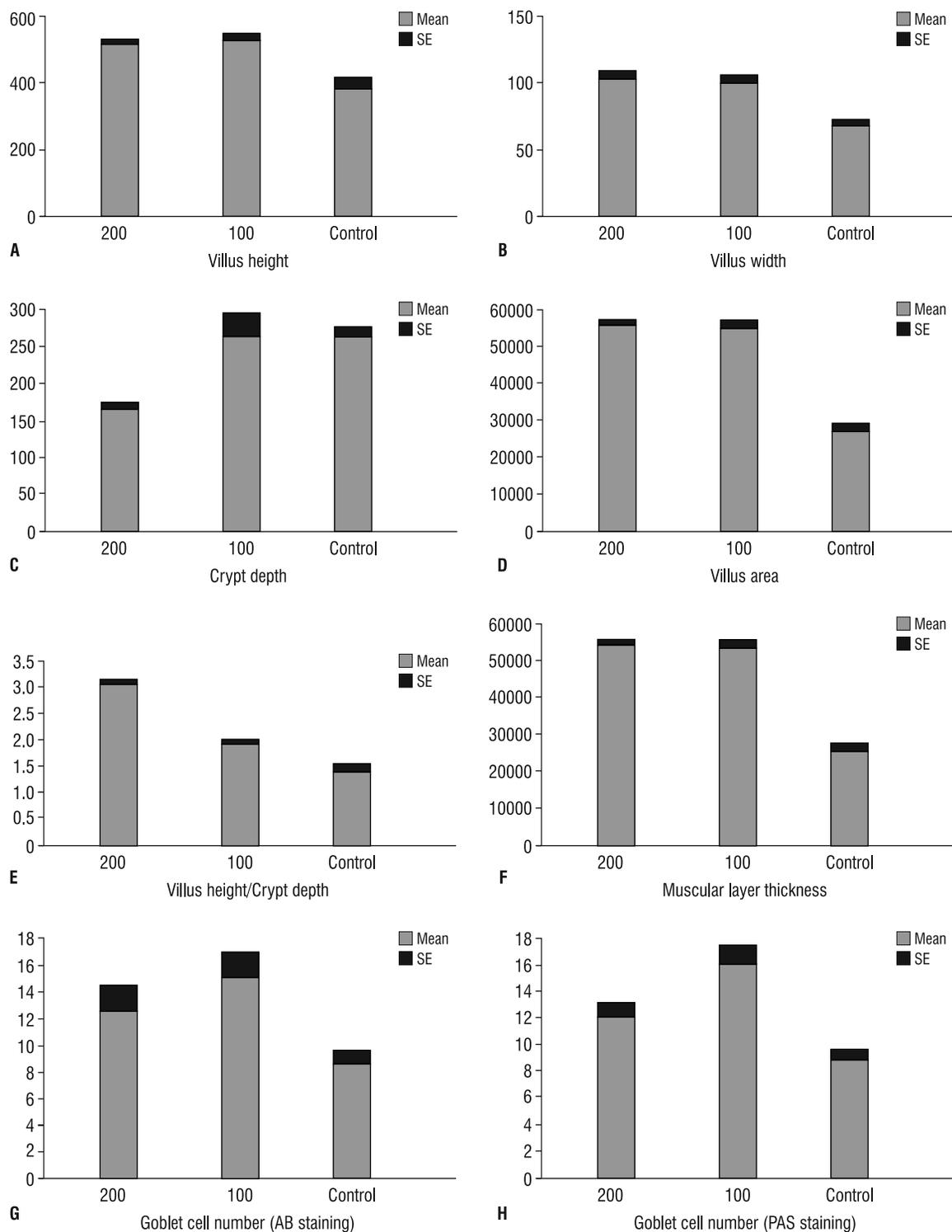
Figure 1. Histomorphometric measurements of the duodenum of control (Control) and treatment groups: 100 (Met 100) and 200 (Met 200). Bars are mean ± standard error (SE). The unit of villus height, villus width, crypt depth and muscular layer thickness is μm. The unit of villus area is μm^2; **A.** Villus height; **B.** Villus width; **C.** Crypt depth; **D.** Villus area; **E.** Villus height/crypt depth; **F.** Muscular layer thickness; **G.** Goblet cell number (AB staining); **H.** Goblet cell number (PAS staining).

significant difference between treatment groups in all intestinal segments (Tables 1–3). It was observed that in all small intestine portions, the groups treated with methionine showed significant increase in villus area compared with the control groups (p < 0.05). It was noticeable that the difference in the villus area
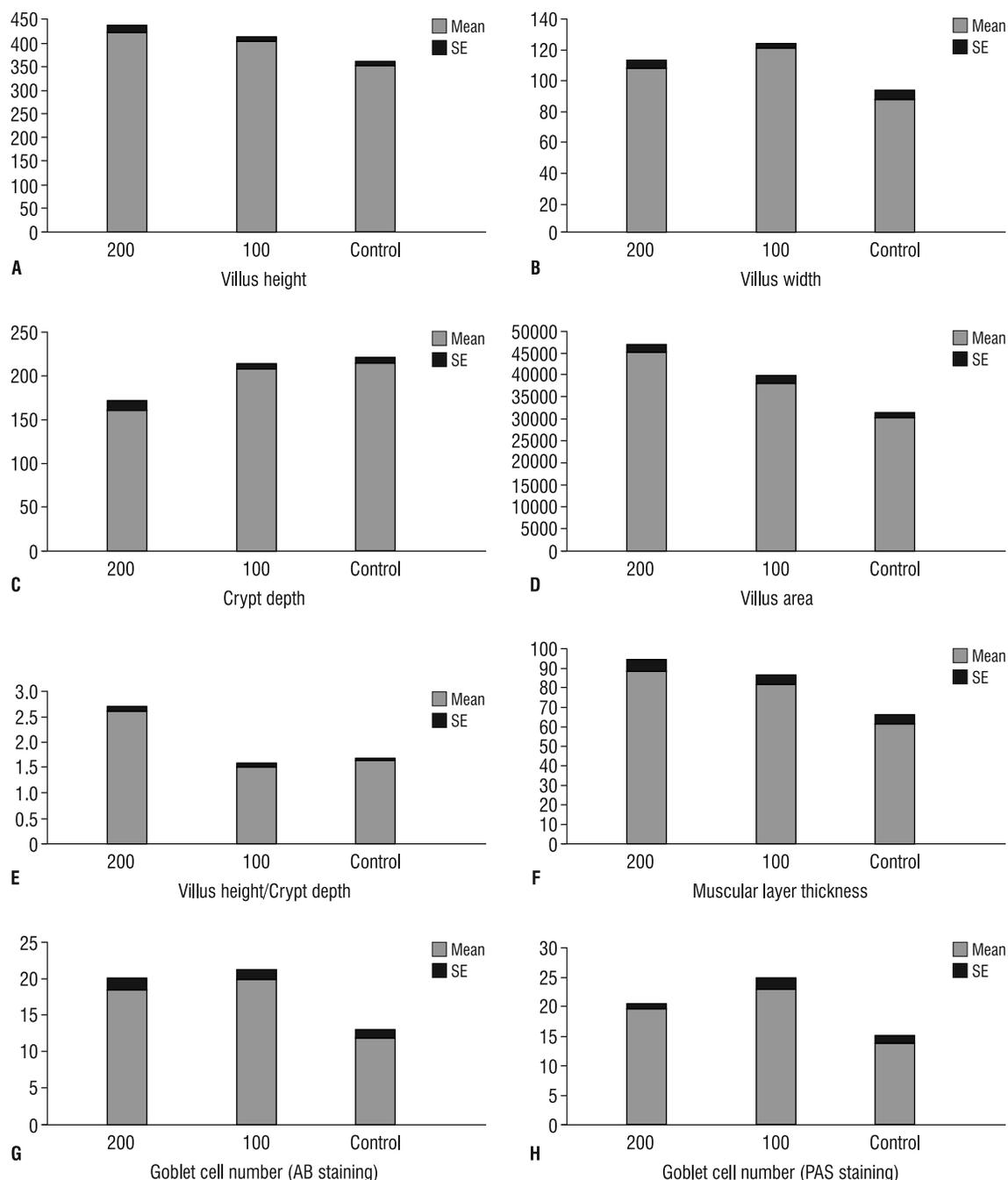
Figure 2. Histomorphometric measurements of the jejunum of control (Control) and treatment groups: 100 (Met 100) and 200 (Met 200). Bars are mean ± standard error (SE). The unit of villus height, villus width, crypt depth and muscular layer thickness is μm. The unit of villus area is μm2; **A.** Villus height; **B.** Villus width; **C.** Crypt depth; **D.** Villus area; **E.** Villus height/crypt depth; **F.** Muscular layer thickness; **G.** Goblet cell number (AB staining); **H.** Goblet cell number (PAS staining).

in the jejunum was significant between two treatment groups (Fig. 2, Table 2). The results of this study showed that the depth of the crypts in duodenum and jejunum were significantly decreased in the Met 200 group as compared to both Met 100 and control groups (p < 0.05), but there was no significant difference between the Met 100 and control groups.

However in the ileum, the depth of the crypts of Met 100 group were decreased compared to the other groups (p < 0.05) (Fig. 4; Tables 1–3).

On histological observation, in the duodenum, significantly increased villus height to crypt depth ratio was observed in methionine treated groups as compared to the control group (p < 0.05). It should
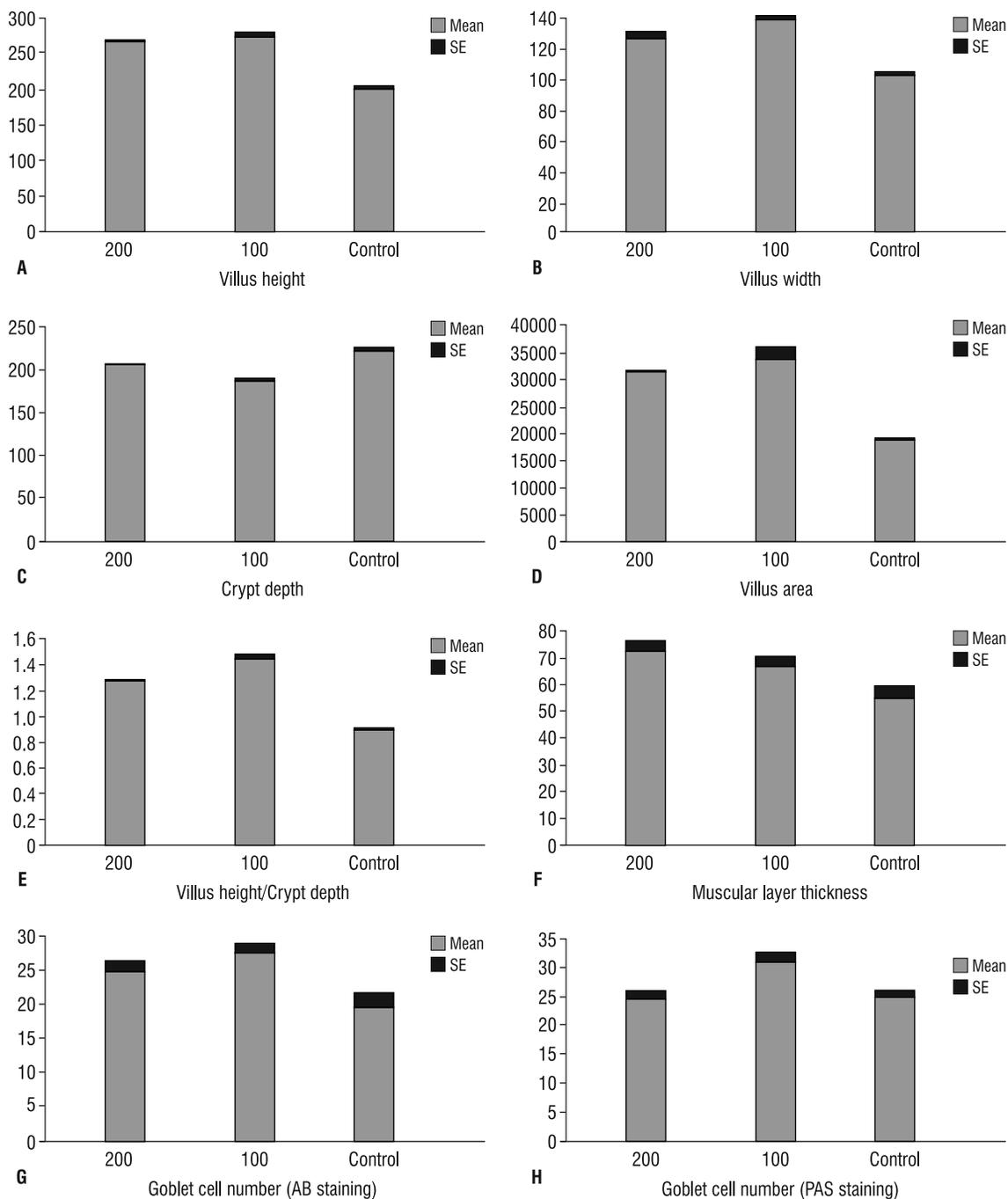
Figure 3. Histomorphometric measurements of the ileum of control (Control) and treatment groups: 100 (Met 100) and 200 (Met 200). Bars are mean ± standard error (SE). The unit of villus height, villus width, crypt depth and muscular layer thickness is μm. The unit of villus area is μm^2; **A.** Villus height; **B.** Villus width; **C.** Crypt depth; **D.** Villus area; **E.** Villus height/crypt depth; **F.** Muscular layer thickness; **G.** Goblet cell number (AB staining); **H.** Goblet cell number (PAS staining).

also be noted that a significant increase in villus height to crypt depth ratio was observed in the Met 200 group compared to Met 100 group (Table 1). In the jejunum, this ratio was greater in the Met 200 group compared with the other groups (p < 0.05). No significant difference was observed the Met 100 and

the control groups (Table 2). In the ileum, the villus height to crypt depth ratio was significantly increased in treatment groups compared to the control group and also the villus height to crypt depth ratio was greater in Met 100 group compared with Met 200 group (Table 3).
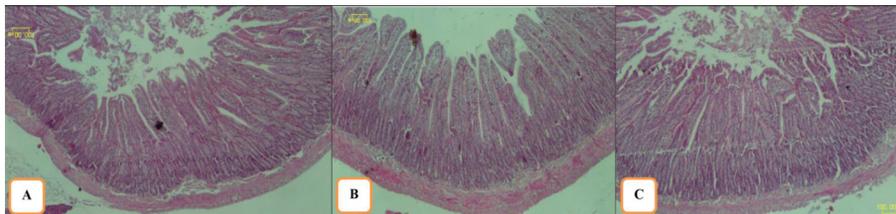
Figure 4. Histological features of the jejunum of control (**A**), Met 100 (**B**) and Met 200 (**C**) groups. Based on these figures, villi heigh and width in both Met 100 and Met 200 are increased compare to the control group; haematoxylin and eosin (H&E); 40×.
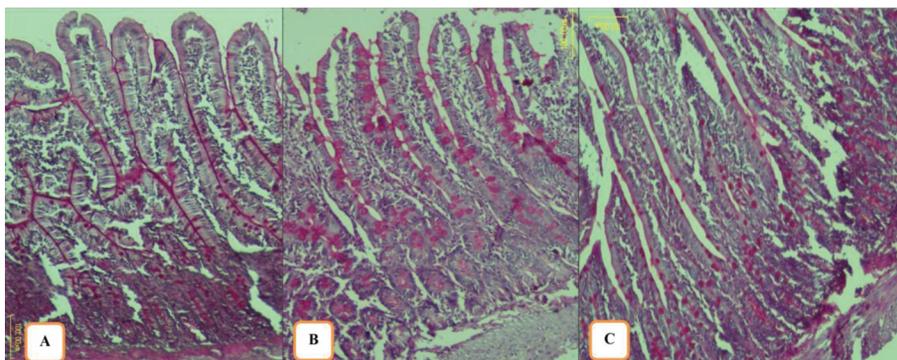
Figure 5. Periodic acid Schiff (PAS) staining of the jejunum of control (**A**), Met 100 (**B**) and Met 200 (**C**) groups. The greatest number of PAS-positive goblet cells are visible in the Met 100 group (**B**); PAS; 40×.
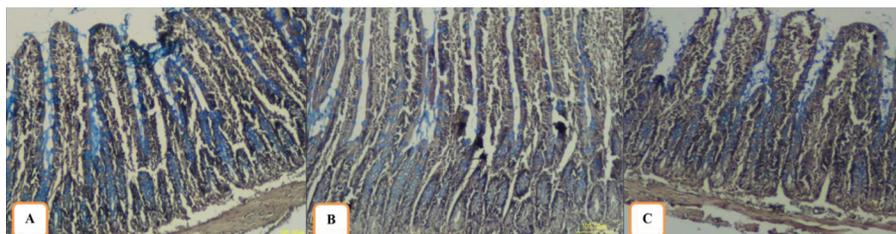
Figure 6. Alcian blue (AB) staining shows an increase in the acidic goblet cells of the jejunum of control (**A**), Met 100 (**B**) and Met 200 (**C**) groups. The greatest number of AB-positive goblet cells are visible in the Met 200 group (**C**); AB; 40×.

In all small intestine segments, the thickness of the muscular layer was greater in the methionine treated groups than in the controls ($p < 0.05$). Also, in the duodenum the Met 200 groups had thicker muscular layer compared with the Met 100 group ($p < 0.05$) (Tables 1–3).

In the duodenum, jejunum and ileum the count of goblet cells that were stained by AB and PAS methods, showed a growth in the treatment groups compared to controls. In all small intestine parts, goblet cells in the Met 100 group were more numerous than in the other groups and this increase was significant in comparison with the control groups ($p < 0.05$) (Figs. 5, 6).

All data are shown in Tables 1–3 and Figures 1–3.

DISCUSSION

The morphology of small intestine is considered as the main indicator of normal gut histology [22]. Absorption of nutrients and drugs is facilitated through the enormous surface area available in this organ [18]. A part of the functional status of the small intestine is defined by villus height and crypt depth [22]. The villi and crypts of the absorptive epithelium play an important role in the final stage of nutrient digestion and assimilation [54]. Alterations in intestinal morphology may affect nutrient metabolisability and performance [22]. For instance, an increase in villi height may result in a growth in total luminal villus absorptive area and leads to satisfactory digestive

enzyme action and higher transport of nutrients at the villus surface [48].

The development of small intestine can be evaluated through measurements of the crypt depth, villus height and surface area to define the available area for digestion and absorption [9, 44].

Nowadays methionine supplements are widely used for the treatment of some skin and hair diseases [45], arthritis [49], tooth growth [25] and also protect the kidney from some drugs' nephrotoxicity [5]. Due to the important side effects of methionine [29, 35, 37, 38], the consumption of this amino acid must be under controlled conditions. This is the first study to investigate the effect of different methionine dosages on the histomorphometric parameters of the small intestine of rat. Also to the authors' knowledge, there have been no studies done on the effects of amino acids on the histology and morphology of the rat's small intestine.

Based on the results of this study, administration of methionine caused an increase in villus height, villus width and finally villus area of all intestinal segments. It has been suggested that increased villi height is correlated with improved normal gut histology and may increase performance by improving nutrient absorption [2, 50]. The measurement of the villus area correlates very well with the total number of epithelial cells in the villus [12]. Therefore, it seems that following the injection of methionine could lead to an increase in digestion and absorption of the nutrients in all small intestine parts. The duodenum has the major role in nutrient absorption and the decrease in villi size from the duodenum to ileum is due to the lower absorptive capacity in the last portion of small intestine [41]. This is consistent with our findings in this study that showed the villi height in the duodenum was greater than those in the jejunum and ileum. It is also noticeable that the villi width was increased from the duodenum, through the jejunum to the ileum. Depth of the crypts was decreased in methionine treated rats compared to the controls. However, the crypt depth of the duodenum in the Met 100 group was not decreased as compared to the control group. Kelly et al. [20] reported that decrease in the crypt depth caused an increase in the enzymatic activity of the small intestine of pigs. So we suggest that methionine supplementation may increase the intestinal digestion activity. Following the administration of methionine, the ratio of villus height to crypt depth was increased. This parameter

was not increased in the jejunum of Met 100 group compared to the control group. A higher villus height to crypt depth ratio results in a decreased turnover of the intestinal mucosa. A slower turnover rate of the intestinal epithelium leads to a lower maintenance requirement and finally can result in a higher growth efficiency of the animal [52]. Overall it can be said that villus height, crypt depth and the ratio of villus height to crypt depth are considered as a criterion to reflect the small intestine morphology and absorption capacity [27]. Therefore, an increase in villus height, villus height to crypt depth ratio or decrease in the crypt depth is correlated with an improvement in the digestion and absorption of nutrients [14–16, 55].

Villus height to crypt depth ratio is one of the most important histomorphometric parameters of the small intestine. Based on the obtained results in the duodenum and jejunum, the villus height to crypt depth ratio was significantly increased in Met 200 group compared to Met 100 group. Since the duodenum and jejunum are the most important absorptive parts of the small intestine, it may be concluded that the 200 mg/kg dosage of methionine has a better effect on the absorption of the nutrients than the 100 mg/kg dosage. So it can be interpreted that 200 mg/kg dosage does not have toxic effects on the small intestine. This finding is in contrast with a previous study conducted by Nazem et al. [29] which showed that 200 mg/kg methionine has more toxic effects than 100 mg/kg dosage in rat's uterus.

Unlike the obtained results in duodenum and jejunum, the villus height to crypt depth ratio was greater in Met 100 compare to Met 200 in the ileum. It may be due to an imbalance in amount of amino acids in this small intestine part. The similar pattern between the histomorphometry of the ileum and the two other parts of small intestine was also reported in the pregnant rats [36].

The epithelial cells of the small intestine are covered by a protective mucus blanket composed of high molecular weight glycoproteins known as mucins [34]. Mucins which are synthesised and secreted from goblet cells act as the first line of defence against intestinal pathogens and play a significant role in the maintenance of mucosal homeostasis [6, 26, 43]. The mucus layer acts as a medium in which digestion and absorption processes happen close to the brush-border membrane [42]. Several studies have reported the modulation of goblet cell number and

mucin secretion under different conditions such as varied diet, surgery and altered microbiota [7, 33, 39, 40, 51]. Obtained results in our study showed that in all intestinal segments, the number of goblet cells that were stained by PAS and AB methods were increased in methionine treated rats compared to controls, which may be indicative of an increase in mucin secretion. The increase in mucin secretion may favour movement of the digesta [8]. Also any changes in the thickness of the mucus layer may influence nutrient digestibility processes [42]. So it seems that administration of methionine may affect digestion by an increase in mucin secretion in the small intestine.

Muscular layer thickness was also increased after methionine administration. A previous study conducted by Sabet Sarvestani et al. [36] showed that the duodenum has gained more capacity to digest food intake during pregnancy by increasing villi length and muscular layer thickness. A positive effect of methionine supplementation on skeletal muscle growth has been also reported in the chickens [45]. In this study, the tunica muscularis was thicker in all intestinal segments in methionine treated rats, which may increase the contact between the intestinal content and the mucosa. We proposed that methionine may have a positive effect on the growth of the tunica muscularis layer and therefore improves digestion.

CONCLUSIONS

In conclusion we have shown that administration of methionine as the first limiting amino acid may affect the digestion and absorption of nutrients in all small intestine segments by an increase in villi length, villi width, villi area, number of goblet cells and finally the muscular layer thickness. Although injection of methionine caused an alteration in small intestine histomorphometric parameters, defining the optimal dose of methionine in rat needs further studies. It is noticeable that there is not a direct relation between increased methionine dosages and their effects on some tissues in rats [30].

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