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Effects of subchronic methionine stimulation on oxidative status and morphological changes in the rat ileum

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Abstract. This study was conducted to explore the effects of sulfur containing amino acids on redox status and morphological parameters in the rat ileum tissue. Male *Wistar albino* rats were randomly divided into the following groups: K group (saline, 1 ml/day, i.p.), M group (methionine, 0.8 mmol/kg/day, i.p.), C group (methionine (0.8 mmol/kg/day) + L-cysteine (7 mg/kg/day), i.p.) and N group (methionine (0.8 mmol/kg/day) + N-acetyl-L-cysteine (50 mg/kg/day), i.p.). Activities of antioxidant enzymes in the ileum were analyzed to profile oxidative status. Morphometric analysis included measurement of villus height (μm), tunica mucosa thickness (μm), tunica muscularis thickness (μm), the total thickness of the ileal wall (μm) and the number of cells in the lamina propria (*per* 0.1 mm^2 of tissue). Results showed that methionine treatment reduced the activity of antioxidant enzymes (SOD, GPx, CAT) and the GSH content compared to the control group ($p > 0.05$). The application of methionine reduced the following parameters statistically significant compared to the control group: length of the ileal villi ($p < 0.01$), tunica mucosa thickness ($p < 0.01$), and ileal wall thickness ($p < 0.01$). We concluded that methionine induced the changes in the gut redox status, which implied oxidative stress occurrence. L-cysteine and N-acetyl-L-cysteine both exhibited antioxidant properties.

Key words: Rat — Ileum — Methionine — Oxidative status

Introduction

Methionine (Met) is an essential sulfur amino acid. Some of the numerous biological functions of methionine include: methylation, growth promotion, detoxification and synthesis of cysteine and glutathione (GSH) (Yen et al. 2002; Kim et al. 2006; Tesseraud et al. 2009).

L-Met is the biologically active form of Met and represents a precursor for protein synthesis (Zhang et al. 2018).

Despite the importance of methionine to physiological functions, it is also recognized as a toxic amino acid (Benevenga and Steele 1984). It is possible that the toxicity of methionine proceeds from the disulfides formation. The complexity of its metabolism, which leads to the hyperhomocysteinemia, is another reason for its potential harmful role. Hyperhomocysteinemia has been detected in patients

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with inflammatory bowel disease (IBD). Digestive diseases (IBD) in which oxidative stress play a major role are linked to the mucosal free radicals formation. Since methionine is an amino acid widely present in the diet, we were wondering what effects it has on the digestive tract.

Today, there is a large body of evidence indicating that dietary methionine restriction is associated with, increased longevity and decreased incidence of age-related disorders in mice and rats (Zimmerman et al. 2003; Miller et al. 2005; Malloy et al. 2006). Literature data indicates protective effects of methionine restriction such as; inhibition of colon carcinogenesis, reduction in visceral fat, glucose and blood lipids concentrations (Komninou et al. 2006; Malloy et al. 2006). Additionally, delayed onset of numerous aging impairments such as „reduction in immune function" is also a result of the methionine restriction diet (Miller et al. 2005). Literature data reported that the methionine restriction diet has potent anti-inflammatory effects in the mice model of colitis (Liu et al. 2017). Many potentially cellular mechanisms have been proposed, but there is little evidence to indicate a mechanism by which methionine restriction inhibits inflammation in the gut. One of the proposed mechanisms could be reduced production of reactive oxygen species (ROS) from numbers of inflammatory cells (eosinophils, neutrophils and macrophages) (Zhu and Li 2012). In truth, the amino acid methionine has a key role in the oxidative stress response, acting as a direct target of ROS, as its sulfur can be oxidized to sulfoxide (Campbell et al. 2016). Furthermore, methionine can act as a free radical scavenger and protect cells from oxidative stress (Luo and Levine 2009). Cysteine and glutathione (GSH) are products of the transsulfuration pathway in methionine metabolism, and can also be identified as ROS scavengers (Swennen et al. 2011).

On the other hand, methionine-rich diets have been associated with enhanced levels of free-radicals production and toxicity in rats and humans (Garlick 2006; Yalcinkaya et al. 2009; Gomez et al. 2011). Nevertheless, the latest research indicates that methionine diet supplementation in broilers have neutral impact on oxidative status (Zhang et al. 2018). The most commonly used supplemental sources of methionine in animals are DL-methionine (a mixture of the D-Met and L-Met) and DL-2-hydroxy-4-(methylthio) butanoic acid (DL-HMTBA).

DL-2-hydroxy-4-(methylthio) butanoic acid and D-Met must be converted to the physiologically active form of methionine which is L-met.

The dietary supplementation of methionine was found to improve intestinal integrity in weanling piglets (Chen et al. 2014). Additionally, the growth and development of the small intestine could be damaged by sulfur amino acid deficiency, as reported by the previous studies (Conde-Aguilera et al. 2016). Sulfur amino acid deficiency also decreased antioxidant capacity in piglets by reducing GSH and cysteine concentrations (Bauchart-Thevret et al. 2009).

L-cysteine was also found to attenuate oxidative stress in inflammatory bowel disease, *via* GSH synthesis (Oz et al. 2005). The small intestine serves as the principal site for the absorption of nutrients, water and electrolytes from the intestinal lumen. The intestinal mucosa expresses complex defence mechanisms and represent a key role in mucosal integrity (Verwelde and Jeurissen 1993; Hecht 2003; Ahmad et al. 2012). Methionine is absorbed from the diet with the highest fractional absorption rate among all proteinogenic amino acids, therefore, dietary methionine intake is considered very important for human and animal health benefits (Mastrototaro et al. 2016). Imbalance in redox regulation could damage the physiological functions of the intestine and contribute to the development of pathological processes. In addition, several studies with rats showed oxidative stress as a contributing factor for increased intestinal permeability (Sheth et al. 2003; Maeda et al. 2010). Moreover, it has been shown that the use of protective agents, such as L-cysteine, can alleviate oxidative stress and inflammation (Kim et al. 2009). Data analyzed N-acetylcysteine (NAC) administration, established that NAC protects tissue damage in ischemia-reperfusion injury (Cuzzocrea et al. 2000). This beneficial effect is explained due to its ability to scavenge free radicals. Furthermore, N-acetylcysteine reduces the inflammatory process by inhibiting the production of proinflammatory cytokines (Tsuji et al. 1999). Therefore, potential clinical use of L-cysteine and N-acetylcysteine should be considered.

However, to our knowledge, there is a lack of data about the effects of subchronic methionine administration in the rat intestine. Thus, the current study was conducted to investigate the effects of a three-week methionine application on the oxidative stress parameters and intestinal morphology in the rat ileum.

Materials and Methods

Animals and design

Wistar male albino rats (180–250 g) were used in our study. The age of rats at the beginning of the experiment was 15 to 20 days. They were acclimatized under standard laboratory conditions at 20 ± 2°C, 50 ± 15% relative humidity and normal photoperiod (12:12 light/dark cycle) for 7 days before the experiment. The rats were housed in transparent plexiglass cages and were provided with a commercial rat pellet diet and water *ad libitum* for 21 days.

After the acclimatization period, all the animals were randomly divided into 4 groups of 8 animals each, and

treated for 21 days by intraperitoneal (i.p.) injections: Group K (control group): rats received only normal saline (0.9% NaCl; 1 ml/day), Group M: rats were treated with methionine (0.8 mmol/kg/day); Group C: rats were treated with methionine (0.8 mmol/kg/day) + L-cysteine (7 mg/kg/day) (Liapi et al. 2009); Group N: rats were treated with methionine (0.8 mmol/kg/day) + N-acetyl-L-cysteine (50 mg/kg/day) (Akbulut et al. 2014). The doses of L-cysteine and N-acetyl-L-cysteine were selected based on earlier studies in which L-cysteine and N-acetyl-L-cysteine exhibited cytoprotective effects (Liapi et al. 2009; Akbulut et al. 2014).

After completion of the 21 day treatment, the animals were sacrificed by decapitation and the ileum tissue samples were taken for biochemical analysis and morphometric measurements.

All research procedures were performed in accordance with principles of good laboratory practice and the European Directive for Welfare of Laboratory Animals No: 2010/63/EU. The ethics protocol of the current study was approved by the Ethics Committee of the Medical Faculty, University of Belgrade, Serbia (No: 3307/2). Experimental procedures were in accordance with the ethical standards of the Medical Faculty, University of Belgrade.

Determination of oxidative stress parameters in the rat ileum by biochemical methods

For the assessment of oxidative stress biomarkers, weighted segments of rat ileum were homogenized in phosphate buffer saline (PBS) (10% weight/volume) using WiceTis HG-15D homogenizer. The homogenates were centrifuged at 10.000 × *g* for 10 minutes, using refrigerated centrifuge (Microcentrifuge MiniSpin, Eppendorf AG, Germany). Superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) activity, reduced glutathione (GSH) content and the index of lipid peroxidation (MDA) were analyzed. All parameters were measured spectrophotometrically using UV/VIS spectrophotometer (UV-2601, Rayleigh, Mainland, China). The method of Bradford was used for protein concentration measurement (Bradford 1976).

SOD activity

The activity of total SOD was measured, as the change of extinction in time (10 min) at 480 nm, kinetically. The SOD activity was determined as the percent of inhibition of epinephrine autooxidation under base conditions by tissue sampling. The reaction mixture contained epinephrine (0.5 mmol/l), Na-bicarbonate buffer (pH 10.2) and 0.1 ml of the sample; the reaction started by adding 0.1 ml of epinephrine solution (0.01 M into 0.01 M HCl). The activity of SOD was presented as U/mg of protein (Sun and Zigman 1987).

GPx activity

The activity of GPx in the rat ileum was determined using the method of Günzler (Günzler et al. 1974). Determination was based on oxidation of reduced GSH with GPx using NADPH. Glutathione reductase (GR) catalyzed the reaction. The decrease of absorbance at 340 nm was measured as a GPx activity, represented as U/mg of protein.

CAT activity

CAT activity was determined according to the method of Aebi (1984), in which the disappearance of hydrogen peroxide is followed at 240 nm. The reaction medium contained 30 mM H_2O_2, 50 mmol/l potassium phosphate buffer pH 7.0, and 0.1 ml sample. CAT activity was expressed as U/mg protein.

GSH content

The Ellman's reagent was used for GSH content determination (Ellman 1959). It creates the yellow color anion, reacting with the aliphatic thiol compounds. The concentration of GSH was determined by reading the extinction on a spectrophotometer at 412 nm. The content of GSH was expressed as μmol GSH/mg protein.

Index of lipid peroxidation

The index of lipid peroxidation was determined according to the method of Draper and Hadley (Draper and Hadley 1990), based on detection of malondialdehyde (MDA) concentration in ileum tissue samples homogenates. MDA, one of the final products of lipid peroxidation, reacts with thiobarbituric acid (TBA). At high temperatures, MDA participates with the TBA, to produce the red color conjugate, with the maximal absorption at 532 nm. The intensity of induced lipid peroxidation was determined by adding ferric ions and ascorbic acid. The amount of generated MDA was measured at 533 nm.

Intestinal morphology analysis

The ileal samples were fixed in 10% neutral buffered formalin and processed. Paraplast, and tissue sections (3–5 μm thick) were routinely stained with hematoxylin and eosin. Morphometric measurements were performed using an optical microscope Opton Photomicroscope III (Carl Zeiss AG, Oberkochen, Germany). The tissue elements of ileal mucosa, hand labeled on microphotographs were acquired with an

Olympus C3030-Z digital camera (Olympus Deutschland GmbH, Hamburg, Germany), projected on the monitor and then measured using a computer-aided image analysis software called Fiji (Schindelin et al. 2012). Measurements were performed on two sections for each tissue sample. All elements of the ileal mucosa were measured: villus height (μm), tunica mucosa thickness (μm), tunica muscularis thickness (μm), as well as the total thickness of the ileal wall (μm) using tissue samples with strictly longitudinal sections. The number of nucleated cells in the lamina propria (*per* 0.1 mm^2 of tissue) was assessed on cross sections of the ileal mucosa.

All chemicals and reagents were obtained from Sigma (Sigma Chemical Co, St. Louis, MO, USA).

Statistical analysis

All results were expressed as means ± standard error, representing eight rats in each group. The differences between means were assessed, and values of $p < 0.05$ and $p < 0.01$, $p < 0.001$ were considered significant and highly significant, respectively. Groups of data were compared with an analysis of variance followed by the Bonferroni *post-hoc* test. A comparison was also performed using Student's *t*-test. Data were analyzed using Graph-Pad Prism 6 statistical software (GraphPad software; San Diego, CA).

Results

Oxidative status parameters

SOD activity

As presented in Figure 1A, three-week methionine treatment decreased the SOD activity by 73.04%. Administration of both L-cysteine and N-acetyl-L-cysteine increased the SOD activity by 388.28% ($p < 0.01$), and by 342.03% ($p < 0.05$), respectively, compared to the Group M.

Glutathione peroxidase activity

Activity of the GPx in the methionine-treated group decreased compared to the control values (by 17.74%). A statistically significant increase appeared after L-cysteine treatment (by 234.68%, $p < 0.001$), as well as after N-acetyl-L-cysteine application (by 123.58%, $p < 0.01$) (Fig. 1B).

CAT activity

CAT activity that was decreased following methionine application (by 16.78%, $p > 0.05$) was not statistically significant compared to the control. On the other hand, CAT activity

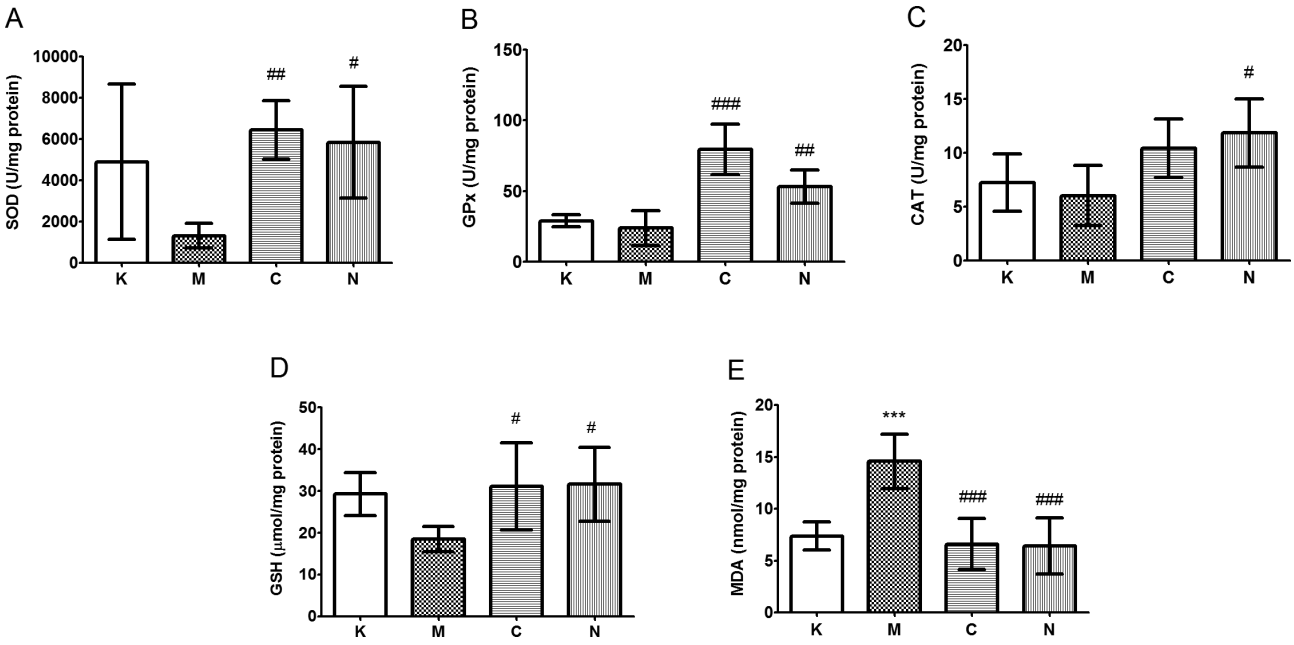

Figure 1. SOD activity (**A**), GPx activity (**B**), CAT activity (**C**), GSH content (**D**) and MDA concentration (**E**) in the ileum tissue homogenate after 21 day of treatment with sulfur amino acids. K, control group; M, methionine group; C, methionine + L-cysteine group; D, methionine + N-acetyl-L-cysteine group. ## $p < 0.01$ C *vs.* M, # $p < 0.05$ N *vs.* M (A); ### $p < 0.001$ C *vs.* M, ## $p < 0.01$ N *vs.* M (B); # $p < 0.05$ N *vs.* M (C); # $p < 0.05$ C *vs.* M, N *vs.* M (D); *** $p < 0.001$ M *vs.* K, ### $p < 0.001$ C *vs.* M, N *vs.* M (E).

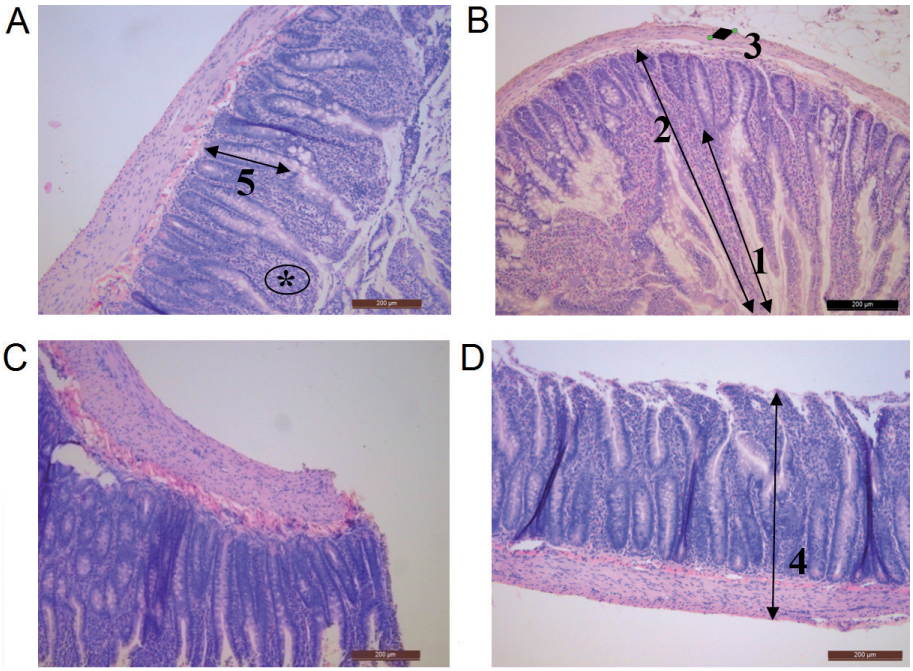

Figure 2. Ileum tissue samples in control conditions (**A**), during methionine loading (**B**) and after the administration of L-cysteine (**C**) or N-acetyl-L-cysteine (**D**) under methionine loading conditions. Staining with hematoxylin and eosin; original magnification: 200×; 1, length of ileal villi; 2, tunica mucosa thickness; 3, tunica muscularis thickness; 4, total wall thickness; 5, crypt depth; asterisk, number of cells in lamina propria /0.1mm^2.

was increased in Group C (by 72.60%, $p > 0.05$), and in Group N as well (by 96.62%, $p < 0.05$), compared to the Group M (Fig. 1C).

Reduced glutathione content

Reduced glutathione concentration was lower in the Group M compared to the control (by 36.83%, $p > 0.05$). However, when applied simultaneously with methionine, L-cysteine and N-acetyl-L-cysteine increased the GSH concentration by 68.29% and by 70.93% ($p < 0.05$), respectively, in a statistically significant manner, in comparison to the Group M (Fig. 1D).

Index of lipid peroxidation

Index of lipid peroxidation, measured as MDA concentration, was increased in the Group M compared to the control values (by 97.62%, $p < 0.001$). Contrary to this, the concentration of MDA was decreased in the Group C (by 54.83%, $p < 0.001$) and in the Group N (by 56.09%, $p < 0.001$), compared to the Group M (Fig. 1E).

Morphometry parameters in the rat ileum

As presented in Figure 2, morphometry analysis was performed on the histological preparations of the rat ileum. Each parameter was tested in Group K, M, C and N. Analysis included determination of the following parameters: length of the ileal villi, tunica mucosa thickness, tunica muscularis thickness, ileal wall thickness and number of cells *per* 0.1 mm^2. The numerical values in parentheses were expressed as means ± standard error for each parameter.

Length of the ileal villi

Methionine load was statistically highly significant and decreased the length of the ileal villi (698.1 ± 31.75 μm) compared to the control value (863.8 ± 34.17 μm) – decrease by 19.18% ($p < 0.01$). In both of the Group C and N, the length of the ileal villi was decreased compared to the Group M ($p < 0.001$). The decrease following L-cysteine (372.3 ± 15.23 μm) application was by 46.67% and following N-acetyl-L-cysteine (321.1 ± 20.67 μm) by 54.00% (Fig. 3A; Table 1).

Tunica mucosa thickness

Tunica mucosa thickness was statistically significant ($p < 0.01$) decreased following methionine application (742.6 ± 28.88 μm) – decrease by 17.51% compared to the control (900.2 ± 38.19 μm). Both L-cysteine and N-acetyl-L-cysteine statistically highly significant ($p < 0.001$) decreased mucosal thickness, compared to the Group M. The decrease in Group C (438.8 ±7.10 μm) was by 40.91%, and in Group N (367.0 ± 13.66 μm) was by 50.58% (Fig. 3B; Table 1).

Table 1. Morphometric parameters of the rat ileum in control group, during methionine load and after application of L-cysteine and N-acetyl-L-cysteine in methionine load conditions

	Group			
	K	M	C	N
Length of the ileal villi (μm)	863.8 ± 34.17	698.1 ± 31.75[a]	372.3 ± 15.23[b]	321.1 ± 20.67[b]
Tunica mucosa thickness (μm)	900.2 ± 38.19	742.6 ± 28.88[a]	438.8 ± 7.10[b]	367.0 ± 13.66[b]
Tunica muscularis thickness (μm)	77.15 ± 5.52	92.91 ± 10.19	118.9 ± 7.21	100.9 ± 6.79
Ileal wall thickness (μm)	994.4 ± 44.89	825.8 ± 31.82[a]	529.3 ± 14.35[b]	545.4 ± 15.95[b]
Number of cells *per* 0.1 mm^2	1347 ± 36.89	1511 ± 39.97	1432 ± 103.6	1428 ± 91.30
Crypt depth (μm)	288.8 ± 19.41	159.3 ± 24.5[c]	83.3 ± 14.70[d]	81.23 ±13.47[d]
Villus height/crypt depth ratio	3.1 ± 0.14	5.4 ± 0.67[e]	4.6 ± 0.44	4.1 ± 0.45

The values are presented as the mean ± standard error for 8 animals *per* group. [a] $p < 0.01$ statistically highly significant difference in comparison to the Group K, [b] $p < 0.001$ statistically highly significant compared to the Group M, [c] $p < 0.001$ statistically highly significant difference iin comparison to the Group K. K, control group; M, methionine group; C, methionine + L-cysteine group; D, methionine + N-acetyl-L-cysteine group.

Tunica muscularis thickness

Methionine application increased tunica muscularis thickness (92.91 ± 10.19 μm) compared to the control group (77.15 ± 5.52 μm) – increase by 20.43%. Tunica muscularis thickness was increased also in the Group C and N, compared to the Group M. There was an increase following L-cysteine application (118.9 ± 7.21 μm) by 27.97%, and following N-acetyl-L-cysteine application (100.9 ± 6.79 μm) by 8.60%. None of the changes were statistically significant (Fig. 3C; Table 1).

Ileal wall thickness

Methionine load induced a statistically highly significant ($p < 0.01$) decrease of the ileal wall thickness (825.8 ± 31.82 μm) compared to the control value (994.4 ± 44.89 μm) – decrease by 16.95%. Application of L-cysteine and N-acetyl-L-cysteine in the methionine load conditions produced a statistically highly significant decrease of the ileal wall thickness ($p < 0.001$). Compared to the Group M, decrease following L-cysteine administration (529.3 ± 14.35 μm) was by 35.90%, and following N-acetyl-L-cysteine (545.4 ± 15.95 μm) was by 33.95% (Fig. 3D; Table 1).

Number of cells per 0.1 mm^2

The number of cells in lamina propria tunicae mucosae increased by 12.17% in the Group M (1511 ± 39.97 cells/mm^2) compared to the control (1347 ± 36.89 cells/mm^2) and was not statistically significant. Both L-cysteine and N-acetyl-L-cysteine induced a decrease in the number of cells compared to the Group M, which was not statistically significant. The decrease following L-cysteine administration (1432 ± 103.6 cells/mm^2) was by 5.23%, and following N-acetyl-L-cysteine (1428 ± 91.30 cells/mm^2) was by 5.49% (Fig. 4A; Table 1).

Crypt depth and the villus height/crypt depth (VH/CD) ratio

Crypt depth was statistically highly significant ($p < 0.001$) decreased after methionine application (159.3 ± 24.5 μm) – decrease by 44.84% compared to the control (288.8 ± 19.41 μm). Both L-cysteine and N-acetyl-L-cysteine statistically significant ($p < 0.05$) decreased crypt depth compared to the Group M. The decrease in Group C (83.3 ± 14.7 μm) was by 47.71%, and in Group N (81.23 ± 13.47 μm) was by 49.01% (Fig. 4B; Table 1).

Methionine load induced a statistically significant ($p < 0.05$) increase of the VH/CD ratio (5.4 ± 0.67) compared to the control (3.1 ± 0.14) – increase by 74.19%. Villus height/crypt depth ratio was decreased in the Group C and N, compared to the Group M. There was the decrease following L-cysteine application (4.6 ± 0.44) by 14.81%, and following N-acetyl-L-cysteine application (4.1 ± 0.45) by 24.07%. None of the changes were statistically significant (Fig. 4C; Table 1).

Discussion

The literature data indicate that methionine induces the oxidative status changes in the intestine. Furthermore, it has been reported that methionine acts as a key protector of the intestinal mucosa, as an oxygen species scavenger (Luo and Levine 2009). Therefore, we examined the effects of subchronic methionine application on the oxidative stress parameters in the rat ileum tissue.

Oxidative stress is characterized by an imbalance between the antioxidative defence system and ROS production

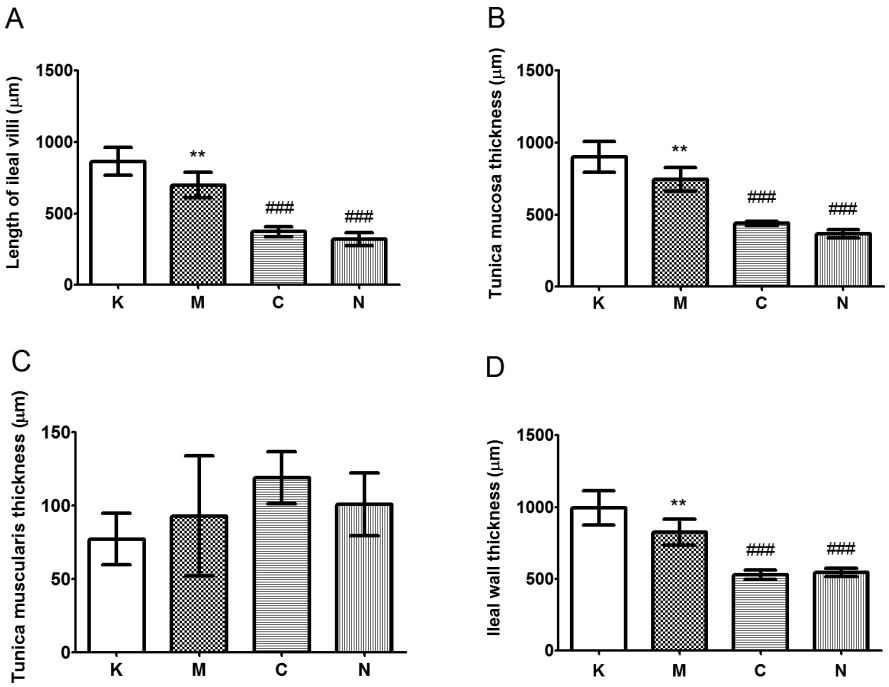

Figure 3. Length of ileal villi (**A**), tunica mucosa thickness (**B**), tunica muscularis thickness (**C**), and ileal wall thickness (**D**) in control group (K), during methionine application (M) and after the administration of L-cysteine (C) or N-acetyl-L-cysteine (N) under methionine loading conditions. ** $p < 0.01$ M *vs.* K; ### $p < 0.001$ C *vs.* M, N *vs.* M (A, B, D).

(Carden and Granger 2000). MDA, as the final product of lipid peroxidation, represents a good indicator of oxidative injury and intestine function impairment. Our results demonstrated increased levels of MDA concentration in the ileum tissue homogenate, after a three-week methionine treatment, compared to the control group. These results are consistent with our previous research, which included determination of the oxidative stress markers in the colon and liver tisssue homogenates, following methionine application (Stojanović et al. 2018a, 2018b). These effects could be explained by the prooxidative properties of the methionine. A contrary, recent study, has demonstrated that methionine diet supplementation for 28 days decreases the MDA level in the ileum of the piglets, which indicates its beneficial effects (Su et al. 2018). Additionally, contrary to our results, study of Ruan et al. showed that methionine deficiency led to oxidative stress in the small intestine of broilers (Ruan et al. 2018). The explanation for the protective effect of methionine lies in the fact that methionine residues may have antioxidant properties, protecting the molecules where they are located. Further investigations would be needed to clarify the exact mechanism by which methionine affects intestine MDA concentration.

Nevertheless, harmful effects of lipid peroxidation in cells, could be, at least in part, alleviated by antioxidant activity of cysteine and glutathione (Swennen et al. 2011). Glutathione plays a key role in protecting the cells against free radicals. Although, there is a lack of information about the parenteral route of methionine application on oxida-

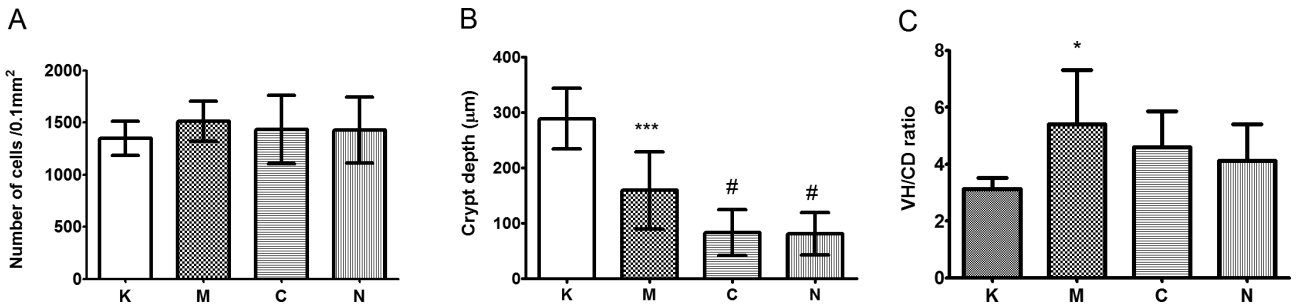

Figure 4. Number of cells per 0.1 mm^2 (**A**), crypt depth (**B**) and villus height/crypt depth (VH/CD) ratio (**C**) in control group (K), during methionine application (M) and after the administration of L-cysteine (C) or N-acetyl-L-cysteine (N) under methionine loading conditions. *** $p < 0.001$ M *vs.* K; # $p < 0.05$ C *vs.* M, N *vs.* M (B); * $p < 0.05$ M *vs.* K (C).

tive status, literature data indicate that sulfur amino acid supplementation in diet influence the GSH content in the cell (Chen et al. 2013). Having that in mind, we observed the reduced glutathione concentration in the ileum tissue homogenates, after three-weeks of intaperitoneal methionine administration. Our results showed lower amounts of GSH in rats that received methionine i.p, compared to the control group. Therefore, in combination with our previous investigations (Stojanović et al. 2018a, 2018b), we demonstrated lowering of the reduced glutathione content in the liver and colon tissue homogenates after methionine treatment. We are free to conclude that methionine application compromised antioxidant status in these organs. Further, simultaneous application of methionine with L-cysteine (Group C) and N-acetyl-L-cysteine (Group N) was associated with increased GSH concentrations compared to the Group M. In accordance with our data, several studies have demonstrated that N-acetylcysteine increases GSH levels in the liver, kidneys and lungs (Albelda et al. 1994; Sehirli et al. 2003; Sener et al. 2003). Overall, these findings indicate prooxidant properties of methionine and its potential harmful effects in the intestine, while L-cysteine and N-acetyl-L-cysteine exhibit antioxidant effects. The cause of these effects of sulfur amino acids on GSH concentration are up until now still unknown. The suggested mechanism may involve alterations in enzymes included in the glutathione cycle – GSH reductase and GSH peroxidase.

Additionally, in our study, administration of L-cysteine and N-acetyl-L-cysteine in the methionine loading conditions increased the activity of several key antioxidant enzymes: SOD, GPx and CAT, compared to the Group M. On the other hand, methionine reduced all the mentioned parameters in comparison with the control values, indicating its prooxidant properties. Likewise, N-acetylcysteine protects small intestine functions in piglets that are challenged with lipopolysaccharide, by incresed SOD and CAT activity (Hou et al. 2012). These protective effects could be, in part, explained by antioxidant properties of L-cysteine and N-acetyl-L-cysteine, achieved *via* regulating NF- κB and Nrf signaling pathway (Song et al. 2016). Since the accumulating evidence has demonstrated that oxidative stress plays a critical role in inflammatory bowel disease, these results indicate that L-cysteine and N-acetyl-L-cysteine may have beneficial effects on the small intestine *via* oxidative stress reduction, as potential therapeutic agents (Alzoghaibi 2013).

In this study, methionine intervention decreased villus height, tunica mucosa thickness and total thickness of the ileal wall, compared to the control. Recent evidence to support our findings has indicated that there is lower crypt depth in the duodenum of weanling piglets, following methionine supplementation. (Chen et al. 2014). It is possible that methionine with its prooxidant properties leads to damage of the intestinal wall. Additionally, according to our results, the number of cells in lamina propria tunicae mucosae was increased following methionine application. This is consistent with our previous investigation which included colon tissue samples of the rat (Stojanović et al. 2018a). Similarly, recent studies have demonstrated that sulfur amino acid deficiency decreased the number of cells in the ileum of the piglets (Bauchart-Thevret et al. 2009). It is possible that methionine proinflammatory properties are responsible for the increasing of the lamina propria cellular infiltration.

In conclusion, the results of this study demonstrate harmful effects of methionine due to its prooxidant ability, as well as potential antioxidant properties of L-cysteine and N-acetyl-L cysteine. Our findings provided the evidence that, gut function may be potentially improved by the modulation of the redox state by antioxidants. Further investigations are needed, to clarify the precise relationship between methionine metabolism and reactive oxygen species production. Finally, the results of this study may be helpful in the development of new therapeutic strategies for inflammatory bowel disease.

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Conflict of interest. The authors declare that they have no conflict of interest.

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