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DIETARY SELENIUM YEAST SUPPLEMENTATION IMPROVED SOME VILLI MORPHOLOGICAL CHARACTERISTICS IN DUODENUM AND JEJUNUM OF YOUNG GOATS

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ABSTRACT

The present study evaluated the effects of Selenium Yeast, (SY) on villi morphology in small intestine of young goats. Ten cross-bred goats of age 110-130 days and weighing 9.93 -10.71 kg, at the commencement of experiment, were used in present study. Animals were fed basal diet consisting of hay and concentrate. The concentrate (2 % BW) was provided into two equal portions at 0800 and 1700 h daily and the hay was fed at ad libitum. Water was provided at ad libitum. Animals were allocated into two dietary groups, consisting of basal diet (CON), or basal diet with Selenium Yeast, 0.3 mg/kg feed (SY). The results showed that the duodenal and jejunal masses (grams per kilogram empty body weight, g/kg EBW) in SY (3.2 ± 0.09 and 6.69 ± 0.17) increased significantly ($P < 0.05$) than in CON (2.88 ± 0.09 and 6.13 ± 0.26). Dietary treatments did not alter ($P > 0.05$) the lengths of intestine, duodenum, jejunum and ileum compared to CON. Mucosal weight (%) was higher ($P < 0.05$) in the duodenum (76.75 ± 0.68) and jejunum (76.3 ± 0.19) of SY goats than that of CON (73.53 ± 0.82 and 72.99 ± 0.85). In duodenum, the villus height (µm) increased ($P < 0.05$) in SY (559.98 ± 23.22) as compared to CON (468.32 ± 31.41). In jejunum, villus height and width increased ($P < 0.05$) in SY (578.06 ± 15.68 and 77.30 ± 2.56) compared to control (437.88 ± 29.12 and 63.84 ± 3.22). The improvement in villi dimensions (length and width) led to increase ($P < 0.05$) in villus surface area by 36.2 and 58.81 % in duodenum and jejunum of SY fed goats compared to that of CON. Dietary treatments did not alter villi morphology in ileum. It can be concluded from the present study that dietary SY supplementation produced positive effects on some villi morphological characteristics in both duodenum and jejunum of goats.

Key words: Selenium Yeast, Intestine, Goats, villi, Organic selenium

INTRODUCTION

Selenium (Se) one of the important microelements, is needed in adequate concentration in animal's diet for maintenance, growth and production. There are two main sources of Se i.e. inorganic and organic. Plants uptake inorganic forms of Se (selenate and selenite) mainly available in soil, and convert and store them in the organic form (selenomethionine and selenocystein) which makes selenoproteins (Finely, 2005 and Schrauzer, 2000). The availability of Se depends upon soil-plant-animal system. The Se status of soils in many areas of the world including Pakistan is low, yielding Se deficient plants, leading to deficiency of Se in animal's diet and eventually lead to lower Se concentration in animal products consumed by humans (Khan *et al*., 2008; Khan *et al*., 2006; Ahmad *et al*., 2009; Ramirez-Bribiesca *et al*., 2005). It is of great importance that the animals should be supplemented with proper amount of Se in the diet. Sodium selenite and selenium yeast are important source of inorganic and synthetic organic sources of Se, frequently used in animal's diet as supplements. The utilization and thus bioavailability of Se in animal body varies with the form of Se supplementation. Previous studies have shown increased absorption rate from gastrointestinal tract (GIT) and thus greater Se concentrations in various tissues of animals fed diet supplemented with organic Se compared to those fed inorganic Se (Ortman and Pehrson, 1999 and Boldizarova *et al*., 2005). Moreover, the utilization and bioavailability of Se differs greatly between ruminants and non-ruminants. Absorption of selenium in either form is much lower in ruminants than in non-ruminants (Wright and Bell, 1966) reported that absorption of supplemented organic Se was only 34% in sheep compared with 85% in swine. The low absorption of selenium in ruminants is attributed to reductive environment of rumen where the microorganisms convert selenium compound to elemental (insoluble) form and impairs its absorption in the intestine (Serra *et al*., 1994). Moreover, the inorganic Se is greatly reduced in the rumen and thus becomes less available for absorption than organic Se and thus the beneficial effects of organic selenium predominate over the inorganic selenium in ruminants (Gammelgaard *et al*., 2012).

In ruminants, Se is required for biosynthesis of selenoproteins which make an integral part of antioxidation system in various tissues to prevent from toxic effects of oxidants such as hydrogen peroxide, produced as natural by-products during normal oxygen metabolism (Fairweather-Tait *et al*., 2010). The Se-dependent antioxidant enzymes such as glutathione peroxidase are normally found in various vital tissues of ruminant's body including liver, kidney, lungs, pancreas, spleen, skeletal muscles and gastrointestinal tract (Zhang *et al*., 2013; Chung *et al*., 2007; Van Ryssen *et al*., 1989) to ensure the protection from oxidative damage. The normal synthesis and thus activity of the antioxidant enzymes require availability of Se in these tissues which can only be ensured by its proper supplement in the diet.

GIT tract of ruminants is very important part of animal body which digests feedstuffs, convert them into diffusible form and then absorb for peripheral utilization. Intestinal mucosa, a demarcation between external and internal environments of the animal body is thus always under great oxidative stress due to presence of vary harmful toxins ingested through feed or formed during extensive metabolism in the intestine. The protection and therefore, normal growth and function of intestine needs certain important nutrients like Se to be provided in animals diet in adequate amount. The supplementation of Se in various forms in chickens and non-ruminants diet has shown positive effect on intestine. Read-Synder *et al*. (2009) reported advantageous effects of selenium yeast (SY) such as increased villus size and thus surface area in the intestine of normal broilers and protective effects on the intestine of broilers experimentally infected against retrovirus.

Though the Se plays an important role in ruminants but its effects on growth of GIT are less studied. Limited studies on pregnant sheep and adult steers have shown that Se supplementation improved mucosal cellularity characteristics in the small intestine (Neville *et al*., 2008; Reed *et al*., 2007; Soto-Navarro *et al*., 2004); however, the literature regarding the effects of dietary Se supplementation on the intestine of the young ruminants is scanty. Previous studies have shown reduction in growth performance of goats that received basal diet containing Se below 0.1 ppm than those fed basal diet supplemented with Se at optimal dose rate of 0.3 ppm (Yue *et al*., 2009, Shi *et al*., 2011). The increased growth performance in optimal Se fed goats at least in part, may be attributed to improvement in growth of small intestine. Therefore, the present study was designed to evaluate the effects of selenium yeast (SY) supplementation on small intestine of young goats.

MATERIALS AND METHODS

Animals and Feeding Management: Ten cross-bred goats of age 110-130 days and 9.93 – 10.71 kg body weight (BW), at the commencement of experiment, were used in the present research. Animals were given a period of four weeks to adapt the surroundings. After the adaptation period, animals were kept in individual pens of 2.5 × 4 Sq.ft area per pen. All animals were fed same basal diet consisting of guar hay and concentrate. The concentrate was fed at 2 % BW into two equal portions at 0800 and 1700 h daily and the hay was fed at ad libitum. Water was provided at ad libitum. The concentrate was composed of ground corn, cottonseed bran, wheat bran which contained 0.035 ppm Se. The Se concentration in feed samples was determined by using inductively coupled plasma-mass spectrometry (ICP-OES Optima 2100-DV, Perkin Elmer) as described by Taylor (2005). The metabolizable energy (ME) content (MJ/kg DM) was 14.87 and 8.76 for concentrate and hay respectively. Animals were divided into two groups, viz. Con and SY, with five animals in each group. Animals in Con. group received only basal diet and in SY, basal diet supplemented with organic selenium, i.e. Selenium Yeast, **SY** (Selemax™, Biorigin®, Lençóis Paulista, São Paulo, Brazil) at the dose rate of 0.3 mg/kg feed for a period of eight weeks.

Slaughtering, Samples Collection and Measurements: Animals were slaughtered at the end of 8thweek. Immediately after the slaughter, the abdominal cavity was opened and the small intestine was isolated from rest of the gastrointestinal tract. Intestinal segments (duodenum, jejunum, and ileum) were identified and isolated as described by Neville *et al*. (2008). Briefly, the duodenum was identified as the segment from the pylorus to a point directly adjacent to the entry of the gastro splenic vein into the mesenteric vein. The jejunum was the segment from the caudal end of the duodenum to the junction of jejunum and ileum. This junction was determined by measuring 15 cm up the mesenteric vein from the junction of the mesenteric and ileocecal veins and then up the mesenteric arcade to the point of intestinal joining. From this point, a 15 cm measurement was made caudally down the small intestine, which was identified as the terminal end of the jejunum and the beginning of the ileum. The ileum measurement was terminated at the ileocecal junction. Duodenum, jejunum and ileum were gently and very carefully separated from mesenteries and fat and laid straight on the cleaned surgical table and length was measured. The luminal content (digesta) from each section was removed gently and weighed. After washing with phosphate buffer solution (PBS), the empty weight of each intestinal section was recorded.

After rinsing with PBS intestinal tissues were collected for two purposes. Firstly, a segment (5 cm^2) of intestine from the middle of each section of small intestine viz. duodenum, jejunum and ileum, was taken and carefully rinsed and cleaned with PBS. The layer of epithelium was isolated from underlying muscle layer

through gentle sliding of glass slide on opened intestinal wall and collected in empty and weighed eppendorf tube. The eppendorf tube with epithelial content was reweighed and the weight of intestinal epithelium was calculated as: weight of eppendorf tube filled with epithelial content minus weight of empty eppendorf tube. The epithelial (mucosal) weight expressed in percentage (%) was calculated as epithelial weight / whole weight of intestinal segment × 100. The weight was recorded through digital weighing balance (AE Adams, AAA 250L, China). Secondly, small pieces of intestine tissues (1 cm^2) were collected from duodenum, jejunum and ileum and fixed in 4% paraformaldehyde solution until analyzed for histomorphometry. Histomorphology of each intestinal section was evaluated by method as described by Moolchand *et al*. (2013). Briefly, samples were fixed in 4% paraformaldehyde overnight, dehydrated, cleared, and embedded in paraffin. Sections of 5 to 7 µm in thickness were cut and stained by the standard hematoxylin and eosin (H&E) procedure. The slides were observed under light microscope at 40 x magnification. Five villi were determined for each goat. The villus surface area was determined as height × width. The morphometric procedure was carried out with a computerized image analysis program (DigiPro 4.0, Labomed, USA).

Statistical Analysis: Data were presented as Mean ± SEM. Differences considered significant at $P < 0.05$ were determined by Student's t test using statistical software SPSS12.0 (StatSoft, Tulsa, OK, USA).

RESULTS

The weights of small intestine, duodenum, jejunum and ileum are expressed as grams per kilogram of empty body weight (g/kg.EBW). The EBW of an animal is equal to BW minus total digesta weight.. Weights of small intestine and ileum were not altered ($P > 0.05$) between the groups; however, dietary Selenium Yeast (SY) supplementation increased ($P < 0.05$) duodenal and jejunal weights (3.2 ± 0.09 and 6.69 ± 0.17) compared to control (2.88 ± 0.09 and 6.13 ± 0.26). Dietary SY supplementation did not alter ($P > 0.05$) the lengths of small intestine, duodenum, jejunum and ileum (centimeters per kilogram of empty body weight, cm/kg.EBW) compared to Con (Table 1).

Figure 1 shows the effects of dietary SY supplementation on mucosal weight (%) in duodenum, jejunum and ileum of goats. Mucosal weight increased ($P < 0.05$) in the duodenum (76.75 ± 0.68) and jejunum (76.3 ± 0.19) of goats in SY group compared to that of control (73.53 ± 0.82 and 72.99 ± 0.85); however, no difference on mucosal weight in ileum was found between the groups.

Histomorphology showed that in duodenum, the villus height (µm) and surface area (μm^2) increased ($P < 0.05$) in SY (559.98 ± 23.22 and 89877 ± 8685.95 μm^2) as compared to control (468.32 ± 31.41 and 65991 ± 7843.1). Crypt depth and villus: crypt ratio in duodenum were not different ($P > 0.05$) between the groups (Table 2). In jejunum, villus height and width increased ($P < 0.05$) in SY (578.06 ± 15.68 and 77.30 ± 2.56) compared to control (437.88 ± 29.12 and 63.84 ± 3.22). The increase in villi dimensions (length and width) led to increase ($P < 0.05$) in villus surface area (44754±2279) in SY compared to control (28180 ± 2809). Crypt depth and villus: crypt ratio in jejunum were not different ($P > 0.05$) between the groups (Table 3). In ileum, the morphometric characteristics of intestinal wall such as villus hight and width, crypt depth and villus: crypt ratio in jejunum were not different ($P > 0.05$) between the groups (Table 5).

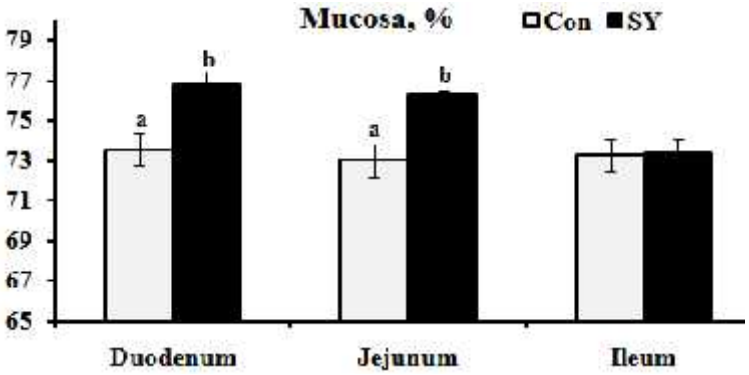

Fig. 1. Effects of dietary organic selenium supplementation on mucosal weight (%) in various sections of small intestine of goats. Con = control; SY = Selenium Yeast (0.3 mg/kg.feed); [a, b]Values (Mean ± SE) with different scripts within columns differ significantly at $P < 0.05$

Table 1. Effect of dietary organic selenium supplementation on empty weights and lengths of various sections of small intestine of goats.

Items	Groups		
	Con	SY	P-Value
Weight, g/kg.EBW			
Intestine	24.64 ± 1.03	24.88 ± 0.81	NS
Duodenum	2.88 ± 0.09	3.2 ± 0.09	0.028
Jejunum	6.13 ± 0.26	6.69 ± 0.17	0.047
Ileum	15.56 ± 0.71	14.99 ± 0.59	NS
Length, cm/kg.EBW			
Duodenum	17.06 ± 0.88	16.54 ± 0.62	NS
Jejunum	5.05 ± 0.37	5.31 ± 0.23	NS
Ileum	90.61 ± 5.06	81.59 ± 1.61	NS
Small intestine	137.78 ± 7	126.96 ± 3.06	NS

Con = Control; SY = Selenium Yeast (0.3 mg/kg.feed); NS = Non-significant. Values are Mean ± SE, Significance level P < 0.05.

Table 2. Effect of SY supplementation on villi histomorphometry in duodenum of goats

Items	Groups		
	Con	SY	P-Value
Villus height, µm	468.32 ± 31.41	559.98 ± 23.22	0.016
Villus width, µm	69.66 ± 4.86	80.14 ± 7.13	NS
Crypt depth, µm	213.3 ± 18.30	245.7 ± 15.96	NS
Villi surface, μm^2	65991 ± 7843.1	89877 ± 8685.95	0.043
Villus:Crypt	2.28 ± 16.86	2.36 ± 17.36	NS

Con = Control; SY = Selenium Yeast (0.3 mg/kg.feed); NS = Non-significant. Values are Mean ± SE, Significance level P < 0.05.

Table 3. Effect of SY supplementation on villi histomorphometry in jejunum of goats

Items	Groups		
	Con	SY	P-Value
Villi height	437.88 ±29.12	578.06±15.68	0.001
Villi width	63.84±3.22	77.30±2.56	0.033
Crypt depth	166.64±6.80	210.34±7.22	NS
Villi surface	28180±2809	44754±2279	0.001
Villus:Crypt	2.63±0.13	2.77±0.15	NS

Con = Control; SY = Selenium Yeast (0.3 mg/kg.feed); NS = Non-significant. Values are Mean ± SE, Significance level P < 0.05.

Table 4. Effect of SY supplementation on villi histomorphometry in ileum of goats

Items	Groups		
	Con	SY	P-Value
Villi height	376.92 ± 16.97	393.94 ± 12.82	NS
Villi width	58.29 ± 2.37	55.73 ± 2.18	NS
Crypt depth	138.24 ± 1.97	136.73 ± 2.48	NS
Villi surface	22034 ± 1579.48	22038 ± 1459.19	NS
Villus:Crypt	2.73 ± 0.15	2.88 ± 0.09	NS

Con = Control; SY = Selenium Yeast (0.3 mg/kg.feed); NS = Non-significant. Values are Mean ± SE, Significance level P < 0.05.

DISCUSSION

In the present study, dietary selenium yeast (SY) supplementation did not alter intestinal and ileal weights. However, when expressed as gram per kilogram of empty body weight (g/kg.EBW); **SY** increased both duodenal and jejunal weights. Previous studies have shown that supplementation of organic Se as high-Se wheat in steers did not affect intestinal, duodenal and ileal weights but increased jejunal weight (Soto-Navarro *et al*., 2004).

The increase in duodenal mass in present study may be explained by the reason SY supplementation improved activities of certain pancreatic enzymes in pigs and increased nutrient digestibility in pigs and cattle

(Adkins and Ewan, 1984 and Wang *et al*., 2009). This suggests an increase functional load on duodenum, which might have been reflected by increase in its weight. In the current study, dietary SY supplementation did not alter the lengths of intestine, duodenum, jejunum and ileum, however, the proportional mucosal weight increased in SY treated goats compared to control. Previous studies have shown similar findings in jejunal mucosa of steers fed high-Se wheat (Soto-Navarro *et al*., 2004).

Mucosal epithelium is lined by villi which provide surface area for nutrient absorption in the intestine. The histometric analysis revealed that the length and width of villi increased in duodenum and jejunum of SY fed goats compared to control but the crypt depth was not changed between the groups. The overall results show that Se supplementation improved some morphometric characteristics of villi in duodenum and jejunum. The increase in epithelial tissue mass results from either in increase of cell size (hypertrophy) or cell number (hyperplasia). Amount of DNA content in the tissue represents increased cell number or hyperplasia (Reynolds *et al*., 1990) and Se treatment increased DNA content in jejunal mucosa of steers and ewes, independent of its chemical nature and source. These data suggest that dietary Se induces hyperplasia in duodenal and jejunal mucosa which was further evidenced by higher number of proliferating cells in intestinal crypts of Se fed steers and sheep (Neville *et al*., 2008 and Soto-Navarro *et al*., 2004). Besides proliferative effect, Se might have, at least in part, protective effects on villi to increase its size. Previous studies have shown that mice fed high Se diet have higher enzyme activities which help in detoxification including glutathione peroxidase (GSH-Px) (Rao *et al*., 2001). Selenium has been shown to exert protective effect on intestine experimentally infected with retrovirus in chicken (Read-Synder *et al*., 2009).

Intestine transfers nutrients required for growth, maintenance and production in animals. The peripheral flow of nutrients from intestine depends upon the surface area provided by the villi lining the interior intestinal wall. In the current study, increase in villi length and width led to increase in mucosal surface area by 36.2 and 62.48 % in duodenum and jejunum of **SY** fed goats compared to control. Consistent with our findings, previous studies have shown Se feeding increased villi size and thus surface area in the small intestine (Read-Synder *et al*., 2009 and Sara *et al*., 2011). The increase in mucosal surface area in duodenum and jejunum suggests an increase in absorption rate of nutrients from the small intestine. Moreover, the proportional mucosal weight is an index of energy consumption and thus flow of nutrients to the periphery. Higher the proportion of mucosa to muscle higher would be the rate of nutrient flow because most of the energy is consumed by epithelial cells rather than muscle cells (Johnson *et al*., 1990; Baldwin, 1999; Malhi *et al*., 2013). In the current study, the mucosal weight in duodenum and jejunum increased in SY fed goats. Furthermore, the increase in peripheral flow of nutrients from intestine would, result in increased visceral organ masses and improve growth performance of animal. Previous studies have shown that Se supplementation in diet increased visceral organs mass and improved growth characteristic in sheep (Neville *et al*., 2008 and Reed *et al*., 2007).

It can be concluded from the present study that dietary SY supplementation in diet improved some morphological characteristics of villi in the duodenum and jejunum of goats; however the ileum was not affected by SY supplementation.

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