Pakistan J. Zool., vol. 52(2), pp 565-571, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190205120240

Influence of Dietary Selenium Yeast Supplementation on Fermentation Pattern, Papillae Morphology and Antioxidant Status in Rumen of Goat

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Article Information
Received 05 February 2019
Revised 25 April 2019
Accepted 19 April 2019
Available online 20 January 2020

Authors' Contribution
MM conceived and designed the study. ABS executed the experiment and wrote the manuscript. SAS and MGS supervised the study and helped in data analysis. NHK and SPS helped in sample analysis. RM and MAS helped in collection and analysis of data and manuscript revision.

Key words
Anti-oxidation, Glutathione peroxidase, Hyperplasia, Organic selenium, Papillae, Rumen, Selenium yeast.

ABSTRACT

The present study evaluated the influence of selenium yeast (SY) on fermentation pattern, papillae morphology and antioxidant status in rumen of goats. A total of ten goats were randomly divided into two groups; control (C, n=5) and selenium yeast (SY, n=5). Animals were fed concentrate (2 % BW) as basal diet without (C) or with selenium yeast (SY, @ 0.3 mg.Se.kg^{-1}.diet) supplementation. Hay and water was provided ad libitum. The results revealed that the molar concentrations of propionate and the total short chain fatty acid (SCFA) increased ($P < 0.005$) and the pH decreased ($P < 0.05$) in the ruminal fluid of SY goats compared to C. The morphometry showed that the length, width and the density of papillae in the atrium ruminis, ventral rumen and ventral blind sac of rumen increased ($P < 0.05$) which led to an increase ($P < 0.005$) in the surface area of these 3 regions in SY group compared to C. The epithelial thickness in rumen papillae wall increased ($P < 0.05$) which was associated with higher ($P < 0.05$) number of cell layers in stratum germinativum in SY group compared to C. The glutathione peroxidase (GSH-Px) activity increased ($P < 0.05$) in ruminal epithelium of SY goats compared to C. The present study demonstrated an improvement in ruminal fermentation, and hyperplasia and enhanced GSH-Px activity in the ruminial epithelium of goats fed SY supplemented diet compared to control that were fed diet without SY supplementation.

INTRODUCTION

Selenium (Se) an essential trace mineral found in both organic and inorganic forms in nature, has a specific place among the nutrients in animal feed because of its wide biological role in animal body (Mehdi *et al*., 2016). Selenium forms several seleno-enzymes such as glutathione peroxidase, which play an important role in antioxidation and prevents the cellular damage from the free radicals produced by oxidative metabolism (Saha *et al*., 2016; Mehdi *et al*., 2016). Animals receive organic Se as selenoamino acids e.g. selenocystine, selenocysteine and selenomethionine mainly from plants feed (Schrauzer, 2000) which depend upon the ability for Se uptake of plants and Se concentration in the soil. The Se level in soils and forages in various regions of Pakistan are below critical level, leading to Se deficiency in the animal (Khan *et al*., 2008; Saha *et al*., 2016) which can be overcome by supplementing Se in animal's diet. Selenium yeast (SY) is the synthetic form of organic Se used as supplement in the diet. Rumen is a large pre-gastric fermentation chamber in ruminants, which produces short chain fatty acids (SCFA) mainly acetate, propionate and butyrate to fulfill ~80% of the total energy requirements of ruminants (Bergman, 1990). The rumen wall is lined with multi-layered stratified squamous epithelium consisting of papillae, which increase the absorptive surface area (Malhi *et al*.,

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2013; Shen *et al*., 2005). The surface area and thus SCFA absorption depends upon the size and number of papillae, which vary as a result of dynamic process influenced by the effect of nutrients mediated through SCFA concentration and pH within the rumen (Malhi *et al*., 2013; Yan *et al*., 2014). Trace elements including Se in ruminant's diet have important role in morpho-functional adaptation of ruminal papillae (Wang *et al*., 2009; Černík *et al*., 2013). It has been demonstrated that the Se supplementation improves rumen fermentation, which would positively affect the papillae growth. Furthermore, ruminal epithelium is highly active tissue and thus always predisposed to oxidative stress due to oxidants produced endogenously by active oxidative metabolism (Steele *et al*., 2011). The excess oxidants in ruminal epithelium are removed by a complex system of antioxidant enzymes (Schogor *et al*., 2013). Glutathione peroxidase (GSH-Px) is Se containing antioxidant enzyme found in gastrointestinal tract including rumen, which protects the tissue from damage (Al-Gubory *et al*., 2008; Abbasi *et al*., 2018). The synthesis of GSH-Px in tissues depends upon the availability of Se which is directly related with the amount of Se intake by an animal. A considerable research has been carried out previously to evaluate the effects of dietary selenium supplementation in chicken and non-ruminants but limited work has been done in ruminants. Moreover, the previous literature mainly focused on metabolism of Se, growth performance and carcass parameters in ruminants and very few studies evaluated the effect of dietary Se on Se metabolism in rumen and ruminal fermentation pattern (Juniper *et al*., 2008; Shi *et al*., 2011) but its effect on papillae morphology has not been studied yet. The present study was therefore, designed to assess the effects of dietary SY supplementation on tissue GSH-Px activity and morpho-functional adaptation in ruminal papillae.

MATERIALS AND METHODS

Animals and feeding management

The present study was carried out on ten crossbred goats having age of 110-130 days and weighing 9.93-10.71 kg. After the adaptation period of four weeks, the animals were kept in individual pens and were randomly divided into two groups; control (C, n=5) and selenium yeast (SY, n=5). Animals were fed concentrate (2 % BW) as basal diet without (C) or with the supplementation of selenium yeast (SY, Selemax™, Biorigin®, Lençóis Paulista, São Paulo, Brazil @0.3 mg.Se.kg^{-1}.diet). Hay and water was provided ad libitum. The ingredients of concentrate included ground corn, cottonseed bran and wheat bran. The contents of dry matter (DM, %) and metabolizable energy (ME, MJ/kg.DM) were 88.36 and 14.87 in concentrate, and 90.28 and 8.76 in hay respectively. On the (% of DM) basis, the contents of crude protein (CP), crude fat (CF) and crude fiber (CF) was 18.67, 4.32 and 8.67 in concentrate, and 9.12, 3.4 and 27.82 in hay, respectively. The Se content in concentrate diet was 0.035 mg.kg^{-1}.diet, whereas the Se was not detected in hay 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 experiment lasted for a period of eight weeks.

Slaughtering of animals, sampling and measurements

The animals were slaughtered at the end of experiment and immediately the abdominal cavity was opened and the complex stomach was separated from rest of the viscera and collected in a clean tub. Reticulo-rumen (rumen), omasum and abomasum were identified and isolated carefully, their contents were removed and after washing in cold phosphate buffer solution (PBS), their empty weights were recorded with digital weighing balance (QUA® 810, China). The contents (digesta) from rumen were collected in a clean container to collect ruminal fluid for future analysis.

Sample collection

Ruminal digesta was strained through 2-layered cheesecloth to collect 20-30 ml of ruminal fluid sample, after measuring the pH on portable pH meter (Hanna Instruments, 211, Romania); the ruminal fluid (preserved with 5% $HgCl_2$, v/v, 1/20) was stored at –20 ^{0}C for future analysis of short chain fatty acids (SCFA). A piece of ruminal wall (5 cm^2) was taken from atrium ruminis, rumen epithelium was isolated from underlying muscle layer, weighed (AE Adams, AAA 250L, China) and then stored frozen until analysis of GSH-Px activity. Rumen tissue samples (1 cm^2) each from atrium ruminis (AR), ventral ruminis (VR) and ventral blind sac (VBS) were collected to measure density and dimensions (length and width) of papillae. Another rumen tissue sample (1 cm^2) was collected from AR and fixed in 4% paraformaldehyde solution until analyzed for histomorphometry.

Histo-morphometric analysis of papillae

Rumen tissue from three rumen regions was rinsed and the papillae were and counted to determine the density (number /cm^2). At least fifteen papillae were used from each sample to measure length and width by using sliding caliper. Total surface area of papillae (mm^2/cm^2) was determined as: length × width × density × 2.

For histomorphology the ruminal tissue samples from atrium ruminis were fixed in 4% paraformalin solution for

24 hours and then dehydrated, cleared, and embedded in paraffin. Sections of 5-7 µm thickness were cut and stained by standard hematoxylin and eosin (H/E) procedure and then morphological characteristics were analyzed by using DigiPro 4.0 (Labomed, USA). Thickness of stratum corneum (SC) and stratum germinativum (SGv) was determined; the latter consists of stratum granulosum (SG) and stratum spinosum (SS).

Analysis of short chain fatty acids (SCFA)

The ruminal fluid samples were prepared and the concentrations of acetate, propionate and butyrate were determined by using GC HP6890N (Agilent Technologies, Delaware, USA) as described by Yang *et al*. (2012). Nitrogen (99.99%) was used as carrier gas with a constant flow rate of 2. 8 ml /min and a split ratio of 1:30. The temperatures of capillary column and injection port and the FID were set to 140°C, 180°C and 250°C, respectively. The capillary column temperature was then gradually raised to 240°C. Tiglic acid was used as an internal standard.

Analysis of glutathione peroxidase (GSH-Px) activity

After homogenization of the ruminal tissue sample in Tris buffer (pH 7.4), the GSH-Px activity was measured by using GSH-Px assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) based on coupled reduction reaction using cumen hydroperoxide as a substrate. The oxidation of NADPH by glutathione reductase was monitored in a spectrophotometer with the decrease in absorbance at 340 nm. One unit of enzymatic activity was defined as the amount of protein that oxidizes 1 µm of NADPH per minute, expressed as units per mg protein (U/mg protein). The protein content in the homogenate was determined by the bicinchoninic acid assay (BCA assay, Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Statistical analysis

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

RESULTS

Table I shows ruminal fermentation pattern in the goats of two groups. The molar concentrations of acetate and butyrate were not changed ($P > 0.05$) between the groups, however, the molar concentration of propionate and total short chain fatty acid significantly increased ($P < 0.05$) in SY (26.01 ± 1.04 and 87.76 ± 1.84) compared to C (20.69 ± 0.43 and 81.54 ± 1.14) respectively. The pH of ruminal fluid significantly decreased ($P < 0.05$) in SY (5.81 ± 0.11) compared to C (6.27 ± 0.12) (Table I).

Table I. Influence of dietary selenium yeast (SY) supplementation on the molar concentrations of short chain fatty acids (SCFA) and the pH of the ruminal fluid of goats.

Items	Groups		P-value
	C	SY	
Acetate (mmol)	52.34 ±0.98	53.44 ±0.89	0.427
Propionate (mmol)	20.69 ±0.43	26.01 ±1.04	0.001
Butyrate (mmol)	8.50 ± 0.42	8.30 ± 0.39	0.732
TSCFA (mmol)	81.54 ±1.14	87.76 ±1.84	0.021
pH	6.27 ± 0.12	5.81 ± 0.11	0.025

Goats were fed basal diet without SY supplementation (C, n=5) and Basal diet + SY supplementation @ 0.3 mg.Se.kg^{-1}. diet (SY, n=5) for 8 weeks. Values (mean ± SE) differ at $P < 0.05$.

Table II. Influence of dietary selenium yeast (SY) supplementation on empty weights of different parts of whole stomach.

Items	Groups		P-Value
	C	SY	
Empty weights (g)			
Rumen	433.75±63.41	473.2±45.31	0.576
Omasum	57.50 ± 11.92	46.50 ± 3.12	0.35
Abomasum	101.25±18.65	91.25 ± 9.60	0.602
Whole stomach	592.50±84.18	611.0±45.97	0.831
% EBW			
Rumen	3.32 ± 0.44	3.66 ± 0.37	0.511
Omasum	0.43 ± 0.07	0.36 ± 0.02	0.328
Abomasum	0.78 ± 0.15	0.71 ± 0.07	0.613
Whole stomach	4.53 ± 0.59	4.72 ± 0.39	0.756
% WS			
Rumen	73.20 ± 2.80	77.14 ± 0.91	0.018
Omasum	9.50 ± 1.04	7.65 ± 0.394	0.107
Abomasum	17.30 ± 2.08	15.20 ± 1.99	0.426

EBW=empty body weight, WS=whole stomach. Goats were fed basal diet without SY supplementation (C, n=5) and Basal diet + SY supplementation @ 0.3 mg.Se.kg^{-1}.diet (SY, n=5) for 8 weeks. Values (mean ± SE) differ at $P < 0.05$.

The empty weights of different parts of whole stomach are depicted in Table II. The results revealed that empty weights of rumen, omasum, abomasum and the

whole stomach expressed either in grams (g) or percent of empty body weight (EBW) were not different between the groups. However, the ruminal weight expressed as percent of whole stomach significantly increased ($P < 0.05$) in SY (77.14 ± 0.91) compared to C (73.20 ± 2.80).

Table III shows the influence of dietary SY supplementation on papillae morphology in different rumen regions of goats. The length, width and density of papillae in the atrium ruminis, ventral rumen and ventral blind sac significantly increased ($P < 0.05$) in rumen of SY goats compared to C. The increases in papillae dimensions and densities led to an increase in the surface area of these 3 regions by 163, 112 and 122% in SY compared to C ($P < 0.005$; Table III).

Table III. Influence of dietary selenium yeast (SY) supplementation on papillae morphology in various rumen regions of goats.

Items	Groups		P-value
	C	SY	
Atrium ruminis			
Length, mm	4.12 ± 0.20	5.76 ± 0.07	0.016
Width, mm	1.42 ± 0.07	2.31 ± 0.05	0.034
Density, No./cm^2	71 ± 1.3	82 ± 2.96	0.001
Surface area mm^2/cm^2	831 ±59.78	2182±116.7	0.001
Ventral rumen			
Length, mm	3.59 ± 0.20	4.62 ± 0.15	0.011
Width, mm	1.26 ± 0.09	1.82 ± 0.10	0.012
Density, No./cm^2	77 ± 4.37	88 ± 3.12	0.003
Surface area mm2/cm^2	697 ±91.15	1480±135.6	0.001
Ventral blind sac			
Length, mm	2.67 ± 0.23	3.82 ± 0.08	0.001
Width, mm	1.13 ± 0.01	1.58 ± 0.08	0.038
Density, No./cm^2	82 ± 3.12	91 ± 3.06	0.042
Surface area mm^2/cm^2	495 ±52.21	1098±41.88	0.003

Goats were fed basal diet without SY supplementation (C, n=5) and Basal diet + SY supplementation @ 0.3 mg.Se.kg^{-1}.diet (SY, n=5) for 8 weeks. Values (mean ± SE) differ at $P < 0.05$.

Papillae in rumen of goats are shown in Table IV. The results showed an increase in the papillae height (2603.24 ± 48.3 vs 2412.45 ± 19.05 µm, $P < 0.005$) and decrease in the mean distance between two papillae (783.66 ±18.46 vs 960.26 ± 21.35 µm, $P < 0.005$) in SY compared to C. The total wall thickness of papillae was not significantly different ($P > 0.05$) between the groups; however, the thickness of epithelium increased ($P < 0.05$) in SY compared to C (211.63±3.42 vs 187.58 ± 4.32). The number of cell layers forming stratum corneum (SC) showed no significant difference ($P > 0.05$) between the groups; however, the number of cell layers in stratum germinativum (SGv) was significantly higher (5.6 ± 0.22 vs 4.4 ± 0.24, $P < 0.05$) in SY compared to C.

Table IV. Influence of dietary selenium yeast (SY) supplementation on histomorphometric parameters of papillae in the atrium ruminis of goats

Items	Groups		P-value
	C	SY	
Papillae thickness, µm	397.28 ±27.28	378.53±16.37	0.384
Inter-papillae distance, µm	960.26 ±21.35	783.66±18.46	0.001
Epithelial thickness, µm	187.58 ± 4.32	211.63±3.42	0.013
Thickness of epithelial strata (No. of cell layers)			
Stratum corneum	3.2 ± 0.20	2.8 ± 0.20	0.417
Stratum germinativum	4.4 ± 0.24	5.6 ± 0.22	0.049

Goats were fed basal diet without SY supplementation (C, n=5) and Basal diet + SY supplementation @ 0.3 mg.Se.kg^{-1}.diet (SY, n=5) for 8 weeks. Values (mean ± SE) differ at $P < 0.05$.

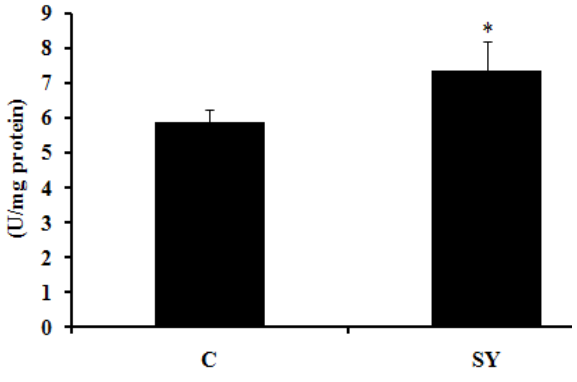

Fig. I. Influence of dietary selenium yeast (SY) supplementation on glutathione peroxidase (GSH-Px) activity in ruminal epithelium of goats. Goats were fed basal diet without SY supplementation (C, n=5) and Basal diet + SY supplementation @ 0.3 mg.Se.kg^{-1}.diet (SY, n=5) for 8 weeks. Values (mean ± SE) differ at $P < 0.05$.

Figure 1 shows the influence of dietary SY supplementation on glutathione peroxidase (GSH-Px) activity in ruminal epithelial tissue of goats. The GSH-Px activity increased ($P < 0.05$) in SY compared to C.

DISCUSSION

The present study determined the changes in ruminal fermentation and the morpho-functional adaptation in the ruminal papillae of goats fed diet supplemented with selenium yeast (SY). In the current study, the molar concentration of propionate and total short chain fatty acid (SCFA) increased by 25.71% and 7.63% and the pH decreased by 0.46 units in the ruminal fluid of goats supplemented with selenium yeast (SY) compared to control. The reduction in pH of ruminal fluid may be due to increase in total SCFA concentration, which resulted from an increase in propionate concentration with SY supplementation. Consistent with our results, Wang *et al*. (2009) and Liu *et al*. (2007) reported increased propionate and total SCFA concentrations and decreased pH in ruminal fluid of cattle fed SY at the dose rate of 0.3 mg Se/kg.DM. The increased SCFA concentrations suggest that SY improved microbial fermentation rate in the rumen. Previous studies have shown the supplementation of selenium yeast altered microbial count and improved fermentation rate in rumen of sheep and goats (Mihaliková *et al*., 2005; Faixová *et al*., 2016; Abbasi *et al*., 2018). The improved fermentation rate suggests an increase in rumen efficiency that increases workload that is reflected by an increase in its mass (Johnson *et al*., 1990). Moreover, the changes in growth pattern of an organ is best indicated by measuring its relative or proportional weight (McLeod and Baldwin, 2000). In the present study, we did not find any difference in the intact weights of forestomaches, however, relative to whole stomach, the weight of rumen was higher in SY fed goats compared to control. Neville *et al*. (2008) and Soto-Navarro *et al*. (2004) observed no difference in intact organ weights of rumen, reticulum, omasum and abomasum of steers and ewes fed organic selenium in the form of high Se-wheat compared with their controls, however, they did not calculate proportional weights.

The interior of rumen is lined with stratified epithelium consisting of papillae which provide surface area for SCFA absorption and it has been shown that the size and number and the surface area of papillae are influenced by nutrients and the concentrations of SCFA to which they are exposed (Malhi *et al*., 2013). In the current study, length, width and density of papillae and thereby surface area increased in the atrium ruminis, ventral rumen and ventral blind sac of rumen in SY goats compared to control. To our knowledge, the influence of dietary SY supplementation on papillae morphology has not been reported before. However, in the previous study we have shown that dietary SY supplementation increased gut performance, improved villi morphology in the small intestine of goats (Ahmad *et al*., 2016; Malhi *et al*., 2017; Samo *et al*., 2018). The positive effects of SY on papillae morphology could be indirectly mediated through the propionate which was increased in the present study. Increasing ruminal propionate concentration through exogenous treatment increased the size and thus surface area of papillae in the rumen of sheep (Mentschel *et al*., 2001).

Ruminal epithelium is highly active tissue and thus always predisposed to oxidative stress due to oxidants produced endogenously by active oxidative metabolism (Steele *et al*., 2011). The excess oxidants in ruminal epithelium are removed by a complex system of antioxidant enzymes (Schogor *et al*., 2013). Glutathione peroxidase (GSH-Px) is Se containing antioxidant enzyme found in gastrointestinal tract including rumen, which improves antioxidant status and protects the tissue from damage (Al-Gubory *et al*., 2008; Yue *et al*., 2009). In the present study, we observed an increase in GSH-Px activity by 25.6% in SY goats compared to control. This suggests that in addition to trophic effect by increasing propionate concentration, SY improved epithelial morphology by enhancing the protective mechanism through increased GSH-Px activity. No previous studies have measured the effect of SY on GSH-Px activity in rumen, however, SY supplementation at the dose rates between 0.3-0.5 mg.Se. kg^{-1}.diet elevated SOD and GSH-Px activities in blood, duodenal, ileal and colonic mucosae, and liver of sheep and goats (Yue *et al*., 2009; Čobanová *et al*., 2017; Abassi *et al*., 2018). It is believed that the synthesis and secretion of GSH-Px is directly related with the bioavailability of Se in particular tissue (Rao *et al*., 2001; Ahmed *et al*., 2016). Rumen epithelium retains considerable amount of Se depending upon form and dosage supplemented in diet (Čobanová *et al*., 2017). The alteration in papillae size is associated with changes in its tissue mass (Moolchand *et al*., 2013). Similarly, we found that thickness of ruminal epithelium increased in goats fed SY diet compared to control. In addition, the increase in epithelial thickness was associated with higher number of cell layers forming stratum germinativum (SGv). This suggests hyperplastic effects of Se on epithelial tissue in rumen. Increased mucosal thickness and hyperplasia has been reported in colon of goat fed SY supplemented diet (Abbasi *et al*., 2018). Moreover, irrespective of its chemical nature and source, Se increased DNA content in jejunal mucosa (Neville *et al*., 2008) and number of proliferating cells in

intestinal crypts (Soto-Navarro *et al*., 2004) of steers and ewes.

CONCLUSION

In conclusion, the present data demonstrated that the dietary SY supplementation improved ruminal fermentation pattern, induced ruminal epithelial growth and increased GSH-Px activity in ruminal epithelium of goats.

Statement of conflict of interest

Authors have declared no conflict of interest.

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