Effect Of Dietary Organic Selenium Supplementation On Mass And Enzyme Activities Of Rumen Microbes Of Goat

Bismillah Khan[1], Jamila Soomro[1], Moolchand Malhi[1], Jahanzaib Khaliq[1]*, Tayyab Ahmad[2], Shua Namood[3], Muhammad Bilal Khan[4], Muhammad Arif[1], Muhammad Umar Farooq[5], Zaker Khan[6], Hamza Faiz[7], Shoaib Akhlaq[3], Wajid Amin[3], Irsa Mariam[8], and Maria Memon[6]

[1]Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam Pakistan.
[2]Institute of Pharmacy, Physiology, and Pharmacology of Veterinary Sciences, Agriculture University Faisalabad, Pakistan.
[3]Faculty 0f Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Pakistan.
[4]Departmant of Livestock and Dairy Development, Quetta, Balochistan.
[5]Department of Animal Products and Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Pakistan.
[6]Department of Veterinary Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Pakistan.
[7]Department of Veterinary Surgery, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan.
[8]Cholistan University of Veterinary and Animals Sciences, Bahawalpur, Pakistan.

* **Corresponding author**: jahanzaib Khaliq
* **Email**: jahanzaibvet@gmail.com

ABSTRACT
Eighteen cross-bred goats, 3–4 months old, weighing up to 10.5 kg and appearing to be in good condition, were randomly divided into three groups: LC, HC, and HC+Sey, with six goats in each group. The LC group received only a basal diet, while the HC group was provided a high concentrate diet enriched with organic selenium (Se) at 0.3 mg per kg in food, mixed into the morning feeding concentrate. All animals were given guar hay as the basal diet, and 150 g of concentrate was fed twice daily at 0800 and 1700 hours. Statistical analysis was conducted using one-way ANOVA, with significance set at $P < 0.05$. Results indicated that goats in the HC and HC-SeY groups had significantly higher ($P < 0.05$) molar concentrations of short-chain fatty acids (SCFA), such as acetic acid, propionate, and butyrate, in their rumen fluid compared to those in the LC group. The pH of the rumen fluid also significantly dropped in the HC and HC-SeY groups ($P < 0.05$), although no significant differences were observed between the two. Goats on the HC and HC-SeY diets also had a substantially higher protozoan and bacterial count ($P < 0.05$) compared to the LC group, with no significant differences between HC and HC-SeY groups. Enzyme activity in the rumen fluid was significantly higher ($P < 0.05$) in the HC and HC-SeY groups compared to LC, suggesting that high concentrate and selenium yeast diets promote a more robust microbial community in goats than a low concentrate diet alone.

Key words: Organic Selenium, Rumen Enzymes and Microbes, Short Chain Fatty Acids (SCFA)

INTRODUCTION

Selenium (Se) is a crucial microelement for all life forms and plays central role in livestock's output (Białek and Czauderna, 2019). Improved rumen efficiency, microbial populations and livestock production have been examined previously by addition of Se in diet (Shi *et al*., 2011). (Naziroglu *et al*., 1997) stated that 0.3 mg/kg of Se instantaneously and efficiently provokes the production of total volatile fatty acid (TVFA), acetic acid, propionic acid, and butyric acid in the rumen. Hence, biological Se in the form of SeY has a suitable application viewpoint as it is nontoxic and extra effective than inorganic Se (Kišidayová *et al*., 2014).Rumen, a gastrointestinal organ that distinguishes ruminants from other vertebrate, is very active natural fermentation chamber harboring a variety of microorganisms which (Dai *et al*., 2015; Ogimoto & Imai, 1981) ferment and reduce plant fibers and transform them into consumable proteins and short chain fatty acids (SCFAs, the main energy resource for ruminants) for assimilation and absorption (Dai *et al*., 2015). Rumen microorganisms are important for ruminant nourishment as they are openly related with the animal's diet (Puniya *et al*., 2015). The rumen microbiome mainly contains bacteria, along with fungi, protozoan, and a slight amount of phages (Miller et al., 2012), with every microbe in a dynamic hormany of struggle and coordination. Previous research have demonstrated that ruminal microbes are firmly associated to the livestock yield proficiency and offer the host with approximately 65–75% of its energy need via anaerobic fermentation (Jewell et al., 2015; Chen *et al*., 2020). The structure of rumen's microbome is influenced by different elements like host variety, well-being, and nutrition, and accordingly, it differs through diverse areas and seasons (Yáñez-Ruiz *et al*., 2015).A high-

fiber intake and a constant microbiota are fundamental for protection of ruminants. Over feeding of grain diets in dairy animals lowers rumen's pH because of raised production of short chain fatty acids (SCFA) increase the threat of sub-acute rumen acidosis (SARA).(Boerman *et al*., 2015). (Plaizier *et al*.,2012; Ye *et al*.,2016)**.**At low pH significant amount of bacterial endotoxins Lipopolysaccharide, (LPS) are produced which disturb the rumen epithelial activity(Gozho *et al*., 2005; Emmanuel *et al*., 2007)and commonly come with ruminal microbiota dysbiosis. A disturbed ruminal ecosystem results surge of harmful effects on animal wellbeing and efficiency (Sirohi SK *et al*., 2012).A close interdependent relationship exist between gut health,
supplement with trace element and gut microbiome (Zhang *et al*., 2015;Biscarini et al., 2018; Ishaq *et al*., 2019).Previously it has been shown that ruminal microbiom produces their protein and cell membrane by incorporating Se from diet (Hidiroglou Heaney & Jenkins, 1968), and increase resistance of cell wall against oxidative injury by supplementation of diet with Se (Cobanova *et al*., 2017; Mihalikova *et al*., 2005).Improved muscle strength and growth rate was observed in sheep under heat-stressed by addition of Se at supranutritional level (Chauhan *et al*., 2014 , 2015 & 2016). Taking into consideration the valuable role of additional dose of Se particularly in stressful circumstances, we hypothesize that in HC diet increasing Se level would improve HC diet-induced ruminal pH, microbial count and enzymes. Hence, the current study was planned to determine selenium yeast (SY) effect against HC diet induced fermentation pattern, microbial count and enzymes in rumen of goat.

MATERIALS AND METHODS

Animals and adaptation period

Eighteen (18) evidently sound cross-reproduced goats having 3-4 months old enough and 10.5 kg body weight (BW), were bought from a local goat market of Hyderabad region and were brought at Livestock Trial Station (LTS), at Sindh Agriculture University (SAU). Goats were given a base time of 4-5 weeks to adjust the environmental factors during which they were fed by support diet. During variation period, all goats were ear tagged for recognizable proof, all goats were dewormed with Fendox plus (oxfendazol) and were infused subcutaneously with Ivermectin against helminthes and other parasitic pervasions, Creatures were immunized against a few normal irresistible illnesses like pleuropneumonia, goat pox and enterotoxemia. Creatures were regularly inspected for any sort of anomaly. All the while, the unsuitable creatures were separated and were supplanted by solid ones.

Experimental design and feeding management:

Goats (N=18) were randomly separated into three groups, viz., LC, HC and HC+Sey, with six goats in each group (Table.I).Goats in group LC, got just basal nutrition routine and in group HC high concentrate nutrition was given and HC+Sey, basal eating routine enhanced with natural selenium at the portion pace of 0.3 mg/kg in feed, blended in amass at early daytime taking care of. The wellsprings of Se for natural structure (selenium yeast, Selemax™, Biorigin®, 19 LençóisPaulista, São Paulo, Brazil) and all creatures were taken care of same basal eating regimen comprising of guar feed. The concentrate (150 g) was given into two equivalent bits at 0800 and 1700 h everyday and the roughage was taken care of at not indispensable. Water was given at not indispensable. The compound structure of diet is yielded (Table.II).

Table I. Grouping of Animals

Groups	Low concentrate(LC)	High concentrate(HC)	High concentrate + selenium(HC+Sey)
No. of Animals	N=6	N=6	N=6
Dose of Se	Nil	Nil	0.3mg/kg

Table II. Chemical composition of experimental ration feed to goats (% on DM basis)

Chemical Composition	Concentrate	Hay
Moisture	12.98	10.85
Salt (NaCl) %	0.38	0.23
Crude protein, % DM	16.66	10.12
Crude fat, % DM	10.75	6.70
Crude fiber, % DM	9.23	19.82
Crude ash, % DM	8.15	11.06
Gross Energy (Kcal)	4053.09	3446.35

Table III. Preparation of 0.1 M Phosphate Buffer Solution (PBS, pH 7)

Chemical Compounds	Concentration (g/l)
NaCl	0.8
KCl	0.2
Na2HPO412H2O	3.4
KH2PO4	0.2

Table IV.Preparation of Neutral Buffered Formalin (NBF, pH 7) Chemical

Compounds	Concentration (ml/l)
Formalin 40%	100

Table V. Preparation of 5% HgCl2

Chemical Compounds	Concentration (gl/l)
HgCl2	50

Table VI. Preparation of Formaline Saline Solution.

Compound	Concentration
Formaline (37.8%)	1 part
Saline solution (0.9%)	2 part

Slaughtering and Rumen fluid sample collection

Toward the finish of trial period, the goats were slaughtered at Research Trail Station (RTS), following the Islamic Halal technique. Following the butcher, the stomach depression was pricked and opened . Entire stomach was segregated from rest of the viscera and was gathered in a tub. Reticulo-rumen (rumen), omasum and stomach were segregated. An aliquot of 20-30 ml of ruminal liquid example was gathered by stressing the rumen digest through two layers of cheesecloth. The pH of rumen tests were recoreded with computerized pH meter. Following recording of pH, the stressed ruminal liquid examples were gathered for two purposes. One piece was put away at - 200C after expansion of formalin-saline arrangement which was ready by blending of one piece of formaldehyde (37.8%) with two pieces of saline (0.9%) solution(Yang and Vagra, 1989)for investigation of short chain unsaturated fats (SCFA). One more piece was centrifuged to gather microbial mass was handled through cell fractionation and the concentrate was gathered for microbial chemicals investigation.

Figure. I

Figure. II

Analysis of Ruminal Fluid for SCFA Determination

Sample preparation

Frozen rumen fluid samples were thawed and 5 ml was taken into centrifuge tube. 1 mL of 25% met phosphoric acid was added and mixed it thoroughly. Samples were allowed to stand for 30 minutes and centrifuged at 2,000 rpm for 10 minutes as described by Supernatant was used to determine the SCFAs concentration as described below.

Determination of ruminal SCFA concentration

Rumen SCFAs for example acetic acid, propionic acid and butyric acid set on by chromatograph HP6890N as portrayed by (Yang *et al*., 2012). Pure Nitrogen was utilized as transporter gas with a consistent stream pace of 2.8ml/min. what's more, a split proportion of 1:30. Fine section temperature was set to 140°C for 4 min and afterward raised at 25°C/min. to 240°C. The temperature of the infusion port and the FID (Fire Ionization Locator) were set to 180°C and 250°C. Tiglic corrosive was utilized as an inside norm.

Determination of Microbial Mass

Protozoa count from slide

Rumen protozoa was counted by method describe by (Brar *et al*., 2000). Briefly 9 ml of lugol's solution was added in 1 ml of strained rumen fluid and mixed well. After mixing, 0.1 ml of sample was transferred to clean glass slide and spread over the known area (24x60mm) and counting was done on 30-50 fields in a zigzag manner under low power. Total No.of protozoan/mililiter of rumen liquid was counted by the following formula:

Total |No.of protozoan/mililiter of rumen liquid = Average protozoa counted/field X 1000X 100.

Protozoa count from fresh rumen fluid

A drop of new strainrd rumen liquid was put on slide and inspected promptly under low power for counting of various sizes (little medium and enormous) of protozoa. Normal number of protozoa were made by counting from 3 distinct fields (Brar *et al*., 2000; Kiro, 2017).

Determination of ruminal bacterial count

Ruminal bacteria were counted through method describe by (Brar *et al*., 2000). Brifly strained rumen liquid was centrifuged at 300rpm for 5 minutes. 10% formaline was mixed to make ratio of 1:1 to kill bacteria and mixture was strained. In 2 ml of this mixture 8 ml of distilled water was added for obtaining 1:10 dilution and in a serial dilution finally we made dilution upto $1X10^4$ with distilled water. From this final dilution we placed 0.01ml on clean glass slide and a loop ful of nigrosine stain solution was mixed with sample and spread over the slide. After air drying 10 different fields were examined under oil immersion. Bacteria were counted which appeared colorless against dark background.
Total number of bacteria/ml of rumen fluid was counted by the following formula:
Total number of bacteria/ml of rumen fluid = Average number of bacteria/field X1000000X 1000

Determination of rumen enzymes

Urease (urea amino hydrolase)

The examine technique of urease activity was completed by pathak method (Pathak *et al*., 1996). The protein activity was done by estimating how much alkali delivered during the brooding of the catalyst test with urea. In such manner, 0.25 mL test, 0.25 mL ammonia solution (15 mg ammonia and 8 mg EDTA in 25 mL phosphate buffer) and 0.5 mL phosphate buffer was blend in a glass cylinder and incubated this solution for 15 min at 37 °C. Ammonium solution and refined water were utilized as a control and blank, separately. After incubation, 5 mL phenol mixture and 5 mL NaOH mixture was added to the cylinder and stired enthusiastically. The cylinder was incubated for 15 min at 37 °C for color's advancement. At last, the absorbance at 635 nm was recorded against the clear. An adjustment bend was utilized to decide how much smelling salts nitrogen delivered in each tube (Pathak *et al*., 1996). The catalyst action was characterized as mg salt nitrogen delivered min/mL test.

Cellulase

The activity of a filter paper addresses complete lactase action. The movement of catalyst was resolved colorimeterically by estimating how much decreasing glucose was delivered during the incubation of protein test with filter paper (Pathak *et al*.,1996). In such manner, 1 mL solution, 1 mL orthphosphate buffer, and 1 mL refined water were blend in a cylinder, which contained 50 mg strip Whatman filter paper and was brooded for 1 hour at 39 °C. After this time 3 mL dinitrosalycylic corrosive reagent was putted into the cylinder and kept in a hot water tube for 10 min. Then, at that point, a 1 mL pottasium sodium tartrate mixture was putted into the cylinder and afterward cooled under running water. At long last, the mixture was made up to a volume of 20 mL with refined water. Catalyst movement was communicated as mg lessening glucose delivered h/mL solution (Pathak *et al*.,1996).

Protease

The examine system depended on the (Blackburn et al., 1968) technique. the protein lysis movement was characterized as how much catalyst that would solubilize the same of 1.0 mg tyrosine in 1 min.

Amylase (1,4 α-D-glucanohydrolase)

By monitoring the rate at which reducing sugars were released while the enzyme was incubating with starch, the activity of a-amylase was ascertained. This was done by combining 0.25 mL of material, 0.25 mL of starch solution, and 0.5 mL of phosphate buffer in a container and incubating it for 15 min at 39 °C. Following this, a DNS approach was employed for color development as previously mentioned in the Fpase technique. The remaining procedures were the same as those used to measure the glucose content in Fpase. mg of reducing sugars (glucose) produced per minute per milliliter of material was used to quantify enzyme activity.

Lipase

Cherry and Crandall technique of titration of palmitic acid delivered by the lysis of hydrogen explained lipase activity, (Oser *et al*.,1965). Chemical examples were incubted with an olive oil emulsion and palmitic acid delivered were titrated with sodium hydroxide 0.05. Units of lipase action per miliLiter of protein test were determined as mL NaOH for titration of the palmitic acid delivered by hydrolysis.

STATISTICAL ANALYSIS

Information are shown as means ± SE. contrasts with a P-valve of < 0.05 were thought of as critical. One-way ANOVA test was utilized to analyze information between gatherings. All measurable investigations were performed by utilizing SPSS programming

RESULTS

Determination of ruminal SCFA concentration and pH

We have analyzed rumen liquid of the goats to decide the impact of HC diet and selenium on SCFA concentrations. The molar concentration of acetic acid, propionic acid, butyric acid and complete SCFA altogether expanded ($P < 0.05$) in ruminal liquid of goats eaten HC and HC+SeY eats less contrasted and those who eaten LC feed (Table.VII). Simultaneously, the pH of ruminal liquid altogether decreases ($P < 0.05$) in HC and HC+SeY contrasted with LC On the other hand, no massive contrasts ($P > 0.05$) were found in the SCFA focus and pH among HC and HC-SeY medicines.

Table VII.Determination of ruminal SCFA concentration and pH

Items	Treatments			
	LC	HC	HC+SeY	LSD(0.05)
Acetate (mmol)	43.52 ±0.46^{b}	58.26± 1.05^{a}	57.63 ±0.69^{a}	2.168
Propionate (mmol)	20.16±0 5^{b}	29.60±0 .73^{a}	28.75±0 .77^{a}	2.253
Butyrate (mmol)	8.78 ±0.26^{b}	13.02±0.54^{a}	11.94±0 .39^{a}	1.396
TSCFA(mmol)	72.47± 1.03^{b}	100.25 ±0.06^{a}	99.40±0 .30^{a}	2.175
Ph	6.93 ±0.03^{a}	5.96±0 .11^{b}	6.03 ±0.06^{b}	0.280

Protozoa count from fresh rumen fluid

Result of protozoan count from fresh rumen fluid is shown in table 4.2 Under the low power we counted different sizes of protozoa (large, medium and small) were meaningfully increased ($P < 0.05$) in rumen liquid of goats eaten HC and HC+SeY diets compared with those eaten LC diet. Among HC and HC+SY groups the higher number of protozoa was noted in HC-SeY(146.17± 5.64) but not significantly ($P > 0.05$) different from HC group(141.05± 3.98)

Table VIII.Population of large, medium and small protozoa in fresh rumen fluid

Items	Treatments			
	LC	HC	HC+SeY	LSD(0.05)
Large	2.83±0.16^{b}	11.27± 0.47^{a}	10.60 ±0.25^{a}	0.901
Medium	9.93± 0.21^{b}	66.61 ± 6.43^{a}	56.02±3.24^{a}	10.87
Small	113.61± 1.52^{b}	141.05± 3.98^{a}	146.17± 5.64^{a}	10.84

Protozoa count from slide

Table.IX shows the result of HC diet and selenium effect on protozoan count in goats. The protozoan count in rumen liquid of goats eaten HC and HC+SeY diets was significantly increased ($P < 0.05$) compared with those eaten LC diet whereas no major alteration ($P > 0.05$) between HC and HC+SeY groups.

Table IX.Population of small, medium and large protozoa count from slide

Items	Treatments			
	LC	HC	HC+SeY	LSD(0.05)
Protozoa	483094±29611^{b}	654191±5773.5^{a}	740289±38116^{a}	92363

Ruminal bacterial count

Table.X shows the effect of HC diet and selenium effect on bacterial count in goats. The bacterial count in rumen liquid of goats eaten HC and HC+SeY diets was significantly increased ($P < 0.05$) compared with those eaten LC diet whereas no major alteration ($P > 0.05$) between HC and HC+SeY groups.

Table X. Ruminal bacterial count

Items	Treatments			
	LC	HC	HC+Sey	LSD(0.05)
Bacteria	62×10^{10}±0.8786^{b}	82×10^{10}±0.5367^{a}	83×10^{10}±0.3183^{a}	1.9851

Ruminal Enzymatic Activities

Table.XI shows the events of different enzymes in rumen liquid of goat. HC diet and selenium significantly increased ($P < 0.05$) the activities of tested all enzyme in rumen liquid of goat of HC and HC+SeY group parallel to LC goats.

Table XI.Ruminal Enzymatic Activities

Items	Treatments			
	LC	HC	HC+SeY	LSD(0.05)
Urease	7.02±0.05^{b}	7.35±0.02^{a}	7.40±0.05^{a}	0.1130
Cellulase	415.03±5.92^{b}	431.37±0.62^{a}	433.53±0.33^{a}	10.342
Protease	0.18±0.27^{b}	0.25±0.89^{a}	0.26±0.87^{a}	0.018
Amylase	139.65±2.50^{b}	145.25±0.34^{a}	145.88±0.15^{a}	4.390
Lipase	0.98±0.04^{b}	1.13±0.03^{a}	1.20±0.23^{a}	0.086

DISCUSSION

A well-known biologically significant trace element called selenium is a crucial component of glutathione peroxidase, an essential component of the body's anti-oxidation defense mechanism. (Fairweather-Tait *et al*., 2010). Previous studies in milking cows and lambs have shown beneficial effects of selenium supplementation on production efficiency, nutrient absorption, glutathione peroxidase, superoxide dismutase activity, and ruminal microbial development. (Hidiroglou *et al*., 1968; Wang *et al*., 2009). Previous studies showed that ruminal microbes integrate selenium (Se) from the food to create their protein and cell wall component (Hidiroglou Heaney & Jenkins, 1968), and that Se addition increased the cell membrane's resilience to oxidative damage. (Cobanova et al., 2017; Mihalikova *et al*., 2005).

Rumen Protozoan count

In this research, it was found that goats given a high concentrate diet along with selenium yeast had a larger microbial community than goats fed a high or low concentrate diet alone. These results are consistent with a research by Moss & Newbold *et al*. (2000) in which they noted a favorable reaction of the microbial community to the addition of Se. Similar findings were reported by (Cobanova *et al*., 2017), who found that adding 0.4 mg/kg DM of selenium to sheep's food boosted the activity of ruminal bacteria and protozoa. Similar to this, lambs given a purified meal deficient in Se showed higher ruminal microbial concentration with Se addition (Hidiroglou *et al*., 1968). (Mihalikova *et al*., 2005) discovered that feeding calves 0.3 mg of selenium per kilogram of dry matter (DM) increased ruminal protozoa growth. The results of this research corroborate the notion that dietary se supplementation promotes ruminal microbial development (Kim & Combs *et al*., 1997). Additionally, it has been noted that the addition of Se boosted ruminal microbial content in ewes given a purified low Se diet and aided protozoa development in calves (Hidiroglou *et al*., 1968). (Mihalikova *et al*., 2005).

Ruminal SCFA concentration and pH

In the current research, we found that goats fed high concentrate and high concentrate plus selenium yeast diets had significantly higher ruminal SCFA concentrations than goats fed low concentrate diets. The ruminal pH is decreased concurrently with a rise in SCFA levels. With the inclusion of Se yeast, dairy cows' ruminal total volatile fatty acid (VFA) content and nutrient digestion in the entire system increased (Wang *et al*., 2009). The overall concentration of SCFAs in the rumen ranges from 60 to 150 mmol l-1, though this fluctuates greatly depending on the food consumed. Grain consumption has a higher ruminal SCFA content than grass consumption. Exceptionally high values, such as 200 mmol l-1, can be attained when animals feed on new vegetation or are given meals high in starch. (Bergman, 1990). When compared to two daily feeds, feeding lactating cows a set diet of hay and high-cereal concentrates in similar quantities throughout the day decreases the diurnal variance in ruminal SCFA concentrations and pH. (Sutton *et al*., 1986).

Ruminal Enzymatic Activities

In the results presented, we found that goats fed the HC + HC+Sey diet had substantially higher ruminal enzyme activities than goats fed the high and low concentrate diets alone. The rumen microorganisms engaged in feed digestion are qualitatively reflected in the rumen microbial enzyme activities. The varied enzyme activities that have been proven to occur in the rumen include amylases, proteases, phytases, and those that break down particular plant poisons (Pathak *et al*., 1996). (e.g., tannases). The diversity of the microbial population and the number of fibrolytic enzymes generated by specific microorganisms are both factors in the variety of enzymes found in the rumen. (Ali *et al*., 1995). The concerted actions of these enzymes are necessary for efficient breakdown of complicated substrates in the rumen.
(Cowan *et al*., 2004; Hess *et al*., 2011, Roumpeka *et al*., 2017) Rumen bacteria are an abundant source of enzymes for plant biomass degradation for use in ethanol production, and manipulation of the rumen microbiota provides chances to lower the cost of food production. (Huws *et al*., 2018).In comparison to ruminal bacteria, ruminal fungi generate a wide variety of enzymes and typically break down a broader variety of substrates. The most tough plant cell wall polymers can also be broken down by ruminal fungi (Wubah *et al*., 1993; Trinci *et al*., 1994), and the cellulases and xylanases they generate are some of the most potent fibrolytic enzymes known to date. (Trinci *et al*., 1994). The rumen protozoal community exhibits activity for each of the key fibrolytic enzymes. The current research generally indicates that supplementing with HC+SeY improves the ruminal acidic environment caused by HC diet.

CONCLUSIONS

In this study we determined increased microbial population was in goats fed on high concentrate + selenium yeast diet compared to goat fed on high and low concentrate diet alone. Ruminal SCFA concentrations were improved significantly in goats fed on high concentrate and high concentrate + selenium yeast diet parallel to low concentrate diet. Ruminal enzymatic activities were improved significantly in goats fed on high concentrate + selenium yeast diet parallel to high and low concentrate food alone.

ACKNOWLEDGEMENTS

The research work was accomplished by utilizing budget of Department of Veterinary Physiology and Biochemistry, Sindh Agriculture University Tandojam, Pakistan.

Statement of conflict of interest

The mentioned authors have declared no conflict of interest.

CONTRIBUTION

BK, JS, MM and JK conceived and designed the experiments .BK performed the experiments. TA, SN, MBK and MA analyzed the data. MUF, ZK, MF, SA and MM revised the manuscript .BK, WA and IM wrote the manuscript.

REFERENCES

1. Ali, B. R., Zhou, L., Graves, F. M., Freedman, R. B., Black, G. W., Gilbert, H. J., & Hazlewood, G. P. (1995). Cellulases and hemicellulases of the anaerobic fungus Piromyces constitute a multiprotein cellulose-binding complex and are encoded by multigene families. *FEMS Microbiology letters*, *125*(1), 15-21.
2. Beam TM, TC Jenkins, PJ Moate, RA Kohn, DL Palmquist. (2000). Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J Dairy Sci 83, 2564-2573.
3. Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological reviews*, *70*(2), 567-590.
4. Bernalier A, G Fonty, F Bonnemoy, P Gouet. (1992). Degradation and fermentation of cellulose by the rumen anaerobic fungi in axenic cultures or in association with cellulolytic bacteria. Current Microbiology 25, 143-148
5. Białek, M., and Czauderna, M. (2019). Composition of rumen-surrounding fat and fatty acid profile in selected tissues of lambs fed diets supplemented with fish and rapeseed oils, carnosic acid, and different chemical forms of selenium. Livestock Science 226, 122–132.
6. Biscarini, F., Palazzo, F., Castellani, F., Masetti, G., Grotta, L., Cichelli, A., et al. (2018). Rumen microbiome in dairy calves fed copper and grape-pomace dietary supplementations: composition and predicted functional profile. PLoS ONE. 13:e0205670.
7. Blackburn T.H., (1968) Protease production by Bacteroides amylophilus strain H18, J. Gen. Microbiol. 53 27–36
8. Boerman, J. P., Potts, S. B., VandeHaar, M. J., Allen, M. S., & Lock, A. L. (2015). Milk production responses to a change in dietary starch concentration vary by production level in dairy cattle. *Journal of dairy science*, *98*(7), 4698-4706.
9. Brar, R. S., Sandhu, H. S., & Singh, A. (2002). *Veterinary clinical diagnosis by laboratory methods*. Kalyani Publishers.
10. Brock FM, CW Forsberg, JG Buchanan-Smith. (1982). Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. Applied Enviromental Microbiology 44, 561-569.
11. Brown MS, CH Ponce, R Pulikanti. (2006). Adaptation of beef cattle to high-concentrate diets: performance and ruminal metabolism. Journal of Animal Science 84, 25-33
12. Chauhan SS, Celi P, Leury BJ, Clarke IJ, Dunshea FR. (2014) Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. Journal of Animal Science; 92:3364–74.
13. Chauhan S, Celi P, Leury B, Dunshea F. (2015) High dietary selenium and vitamin E supplementation ameliorates the impacts of heat load on oxidative status and acid-base balance in sheep. Journal of Animal Science; 93:3342–54.
14. Chauhan S, Ponnampalam E, Celi P, Hopkins D, Leury B, Dunshea F. (2016) High dietary vitamin E and selenium improves feed intake and weight gain of finisher lambs and maintains redox homeostasis under hot conditions. Small Ruminant Research; 137:17–23.
15. Chaucheyras-Durand, F., & Ossa, F. (2014). The rumen microbiome: composition, abundance, diversity, and new investigative tools. The Professional Animal Scientist, *30*(1), 1-12.
16. Chen, J., Harstad, O. M., Mcallister, T., Drsch, P., and Holo, H. (2020). Propionic acid bacteria enhance ruminal feed degradation and reduce methane production in vitro. Active Agriculture Scandell and Animal Science 69, 169–175.
17. Čobanová, K., Faix, Š., Plachá, I., Mihaliková, K., Váradyová, Z., Kišidayová, S., &Grešáková, Ľ., (2017). Effects of different dietary selenium sources on antioxidant status and blood phagocytic activity in sheep. *Biological trace element research*, *175*(2), 339-346.
18. Colville T, Bassert JM: (2016). Clinical anatomy & Physiology for veterinary Technicians.3rdedition.Chapter 16, Digestive system,Sabrina M. Tiperman,Pp:396-397.ISBN: 978-0-323-22793-3.
19. Cotta MA, RB Hespell. (1986). Proteolytic activity of the ruminal bacterium Butyrivibrio fibrisolvens. Applied Enviromental Microbiology 52, 51-58.
20. Counotte GH, RA Prins, RH Janssen, MJ Debie. (1981). Role of Megasphaera elsdenii in the Fermentation of dl-[2-C]lactate in the Rumen of Dairy Cattle. Applied Enviromental Microbiology 42, 649-655.
21. Cowan, D. A., Arslanoglu, A., Burton, S. G., Baker, G. C., Cameron, R. A., Smith, J. J., & Meyer Q., (2004). Metagenomics, gene discovery and the ideal biocatalyst. Biochemical Society Transactions, 32(2), 298-302.
22. Dai, X., Tian, Y., Li, J., Luo, Y., Liu, D., Zheng, H., et al. (2015). Metatranscriptomic analyses of plant cell wall polysaccharide degradation by microorganisms in the cow rumen. Applied Enviromental Microbiology. 81, 1375–1386.
23. Dehority BA. (1969). Pectin-fermenting bacteria isolated from the bovine rumen. Journal of Bacteriol 99, 189-196.
24. Denman SE, MJ Nicholson, JL Brookman, MK Theodorou, CS McSweeney. (2008). Detection and monitoring of anaerobic rumen fungi using an ARISA method. Letter Applied Microbiology 47, 492-499.
25. Dijkstra, J., &Tamminga, S. (1995). Simulation of the effects of diet on the contribution of rumen protozoa to degradation of fibre in the rumen. *British Journal of Nutrition*, *74*(5), 617-634.

26. Duskova D, M Marounek. (2001). Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rumen bacterium Lachnospira multiparus. Letter Applied Microbiology 33, 159-163.
27. Dyce, K. M., Sack, W. O., &Wensing, C. J. G. (2010). Chapter 28: The Abdomen of the Ruminant. Textbook of Veterinary Anatomy. 4th Ed. Missouri: Saunders Elsevier, 677-679.
28. Emmanuel, D. G. V., Madsen, K. L., Churchill, T. A., Dunn, S. M., &Ametaj, B. N. (2007). Acidosis and lipopolysaccharide from Escherichia coli B: 055 cause hyperpermeability of rumen and colon tissues. Journal of Dairy Science, 90(12), 5552-5557.
29. Fairweather-Tait, S. J., Collings, R., & Hurst, R. (2010). Selenium bioavailability: current knowledge and future research requirements. *The American journal of clinical nutrition*, *91*(5), 1484S-1491S.
30. Finch, J. M., & Turner, R. J. (1996). Effects of selenium and vitamin E on the immune responses of domestic animals. *Research in veterinary science*, *60*(2), 97-106.
31. Finley, J. W. (2005). Selenium accumulation in plant foods. *Nutrition reviews*, *63*(6), 196-202.
32. Fondevila M, BA Dehority. (1996). Interactions between Fibrobacter succinogenes, Prevotella ruminicola, and Ruminococcus flavefaciens in the digestion of cellulose from forages. Journal of Animal Science 74, 678-684.
33. Fondevila M, BA Dehority. (2001). In vitro growth and starch digestion by Entodinium exiguum as influenced by the presence or absence of live bacteria. Journal of Animal Science 79, 2465-2471.
34. Fuentes MC, S Calsamiglia, PW Cardozo, B Vlaeminck. (2009). Effect of pH and level of concentrate in the diet on the production of biohydrogenation intermediates in a dual-flow continuous culture. Journal of Dairy Science 92, 4456-4466
35. Galfi, P., Neogrady, S., & Sakata, T. (1991). Effects of volatile fatty acids on the epithelial cell proliferation of the digestive tract and its hormonal mediation. In *Physiological aspects of digestion and metabolism in ruminants* (pp. 49-59). Academic Press.
36. Goiri, I., Oregui, L. M., & Garcia-Rodriguez, A. (2010). Use of chitosans to modulate ruminal fermentation of a 50: 50 forage-to-concentrate diet in sheep. *Journal of animal science*, *88*(2), 749-755.
37. Gozho, G. N., Plaizier, J. C., Krause, D. O., Kennedy, A. D., & Wittenberg, K. M. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. Journal of dairy science, 88(4), 1399-1403.
38. Graham, C., & Simmons, N. L. (2005). Functional organization of the bovine rumen epithelium. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 288(1), R173-R181.
39. Grenet E, A Breton, P Barry, G Fonty. (1989). Rumen anaerobic fungi and plant substrate colonization as affected by diet composition. Animal Feed Science Technology 26, 55-70.
40. Gressley TF, MB Hall, LE Armentano. (2011). Ruminant nutrition symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. J Animal Science 89, 1120-1130
41. Hess, M., Sczyrba, A., Egan, R., Kim, T. W., Chokhawala, H., Schroth, G., ... & Rubin, E. M. (2011). Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. *Science*, *331*(6016), 463-467.
42. Hidiroglou, M., Heaney, D. P., & Jenkins, K. J. (1968). Metabolism of inorganic selenium in rumen bacteria. Canadian journal of physiology and pharmacology, 46(2), 229-232.
43. Hook SE, J Dijkstra, ADG Wright, BW McBride, J France. (2012). Modeling the distribution of ciliate protozoa in the reticulo-rumen using linear programming. Journal of Dairy Science 95, 255-265.
44. Huber, J. T. (1969). Development of the digestive and metabolic apparatus of the calf. *Journal of Dairy Science*, *52*(8), 1303-1315.
45. Huhn, K., Müller, F., Honscha, K. U., Pfannkuche, H., &Gäbel, G. (2003). Molecular and functional evidence for a Na+–HCO 3−-cotransporter in sheep ruminal epithelium. *Journal of Comparative Physiology B*, *173*(4), 277-284.
46. Huws, S. A., Creevey, C. J., Oyama, L. B., Mizrahi, I., Denman, S. E., Popova, M &Morgavi, D. P. (2018). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. Frontiers in microbiology, 9, 2161.
47. Ishaq, S. L., Page, C. M., Yeoman, C. J., Murphy, T. W., Emon, M. L. V., and Stewart, W. C. (2019). Zinc AA supplementation alters yearling ram rumen bacterial communities but zinc sulfate supplementation does not. Journal of Animal Science; 97, 687–697.
48. Jenkins TC, RJ Wallace, PJ Moate, EE Mosley. (2008). Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. Journal of Animal Science 86, 397-412.
49. Jewell, K. A., McCormick, C. A., Odt, C. L., Weimer, P. J., and Suen, G. (2015). Ruminal bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. Applied Enviromental Microbiology; 81, 4697–4710.
50. Kehoe, S. I., Dechow, C. D., & Heinrichs, A. J. (2007). Effects of weaning age and milk feeding frequency on dairy calf growth, health and rumen parameters. *Livestock Science*, *110*(3) 267-272.
51. Khan, Z. I., Hussain, A., Ashraf, M., & McDowell, L. R. (2006). Mineral status of soils and forages in South western Punjab-Pakistan: Micro-minerals. *Asian-Australasian Journal of Animal Sciences*, *19*(8), 1139-1147.
52. Khan, Z. I., Hussain, A., Ashraf, M., & McDowell, L. R. (2008). Assessment and comparison of blood plasma mineral concentrations of different classes of grazing sheep. *Trace Elements & Electrolytes*, *25*(1).
53. Kim, J., Van Soest, P. J., & Combs, G. F. (1997). Studies on the effects of selenium on rumen microbial fermentation in vitro. Biological trace element research, 56(2), 203-213.

54. Kiro R P.(2017) Assessment of the Rumen Fluid of a Bovine Patient. Dairy and Veterinary Science; 2(3): 555588.
55. Kišidayová, S., Mihaliková, K., Siroka, P., Cobanová, K., and Váradyová,Z. (2014). Effects of inorganic and organic selenium on the fatty acid composition of rumen contents of sheep and the rumen bacteria and ciliated protozoa. Animal Feeding Science Technology. 193, 51–57.
56. Kluck, R. M., Bossy-Wetzel, E., Green, D. R., & Newmeyer, D. D. (1997). The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science, 275(5303), 1132-1136.
57. König, H. E., &Liebich, H. G. (Eds.). (2020). Veterinary anatomy of domestic animals: Textbook and colour atlas.
58. Langbein L., Grund, C., Kuhn, C., Praetzel, S., Kartenbeck, J., Brandner, J. M., & Franke, W. W. (2002). Tight junctions and compositionally related junctional structures in mammalian stratified epithelia and cell cultures derived therefrom. *European journal of cell biology*, *81*(8), 419-435.
59. Leighton, B., Nicholas, A. R., & Pogson, C. I. (1983). The pathway of ketogenesis in rumen epithelium of the sheep. *Biochemical Journal*, *216*(3), 769-772.
60. Lettat, A., Nozière, P., Silberberg, M., Morgavi, D. P., Berger, C., & Martin, C. (2010). Experimental feed induction of ruminal lactic, propionic, or butyric acidosis in sheep. *Journal of animal science*, *88*(9), 3041-3046.
61. Liu K, J Wang, D Bu, S Zhao, C McSweeney, P Yu, D Li. (2009). Isolation and biochemical characterization of two lipases from a metagenomics Library of China Holstein cow rumen. Biochemistry and Biophysiology Research Communication 385, 605-611.
62. Liu Y, WB Whitman. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Academy Science 1125, 171-189.
63. Mackie RI, S Heath. (1979). Enumeration and isolation of lactate-utilizing bacteria from the rumen of sheep. Applied Environmental Microbiology 38, 416-421.
64. Mahan, D. C., Cline, T. R., & Richert, B. (1999). Effects of dietary levels of selenium-enriched yeast and sodium selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum glutathione peroxidase activity, carcass characteristics, and loin quality. *Journal of animal science*, *77*(8), 2172-2179.
65. Maia MR, LC Chaudhary, CS Bestwick, AJ Richardson, N McKain, TR Larson, IA Graham, RJ Wallace. (2010). Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiology 10, 52.
66. Michalet-Doreau B, I Fernandez, C Peyron, L Millet, G Fonty. (2001). Fibrolytic activities and cellulolytic bacterial community structure in the solid and liquid phases of rumen contents. Reproduction Nutrition Dev 41, 187-194.
67. Michalet-Doreau B, I Fernandez, G Fonty. (2002). A comparison of enzymatic and molecular approaches to characterize the cellulolytic microbial ecosystems of the rumen and the cecum. Journal of Animal Science 80, 790-796.
68. Mihaliková, K., Boldižárová, K., Faix, Š., &Kišidayová, S. (2005). The effects of organic selenium supplementation on the rumen ciliate population in sheep. Folia microbiologica, *50*(4), 353-356.
69. Miller, M. E. B., Yeoman, C. J., Chia, N., Tringe, S. G., Angly, F. E., Edwards, R. A., et al. (2012). Phage–bacteria relationships and crispr elements revealed by a metagenomic survey of the rumen microbiome. Enviromental Microbiology; 14, 207–227.
70. Moharrery, A., & Das, T. K. (2001). Correlation between microbial enzyme activities in the rumen fluid of sheep under different treatments. *Reproduction Nutrition*, *41*(6), 513-529.
71. Morales MS, BA Dehority. (2009). Ionized calcium requirement of rumen cellulolytic bacteria. Journal of Dairy Science 92, 5079-5091.
72. Moss, A. R., Jouany, J. P., & Newbold, J. (2000, May). Methane production by ruminants: its contribution to global warming. In *Annales de zootechnie* (Vol. 49, No. 3, pp. 231-253). EDP Sciences.
73. Naziroglu, M., Aksakal, M., Cay, M., and Celik, S. (1997). Effects of vitamin E and selenium on some rumen parameters in lambs. Active Veterinary Hung. 45, 447–456.
74. Nozière, P., Steinberg, W., Silberberg, M., &Morgavi, D. P. (2014). Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. *Journal of Dairy Science*, *97*(4), 2319-2328.
75. Ogimoto K, Imai S (1981). Atlas of rumen microbiology. Tokyo, Japan: Scientific Society Press; 1–232p.
76. Oser B.L., (1965) Hawk's Physiologyical Chemistry, 14th edition, TATA McGraw-hill Publishing Co. LTD, New Delhi, p. 1123.
77. Pandir, D., Bekdemir, F. O., Doğanyiğit, Z., & Per, S. (2017). Protective Effects of Sodium Selenite and Vitamin-E on LPS Induced Endotoxemia of Rats. *ARC Journal of Nutritional Growth*, *2*, 19-25.
78. Pathak N.N., Kamra D.N., Jakhmola R.C., (1996) Analytical Techniques in Animal Nutrition Research, International Book Distributing Co., India, p. 201
79. Pitta DW, E Pinchak, SE Dowd, J Osterstock, V Gontcharova, E Youn, K Dorton, I Yoon, BR Min, JD Fulford, TA Wickersham, DP Malinowski (2010). Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. Microbiology and Ecology 59, 511-522.
80. Plaizier, J. C., Khafipour, E., Li, S., Gozho, G. N., & Krause, D. O. (2012). Subacute ruminal acidosis (SARA), endotoxins and health consequences. Animal Feed Science and Technology, 172(1-2), 9-21.
81. Plaizier J, Khafipour E, Li S, Gozho G, Krause D. (2016) Subacute ruminal acidosis (SARA), endotoxins and health consequences. Animal Feed Science Technology; 172:9–21.

82. Puniya, A. K., Singh, R., and Kamra, D. N. (2015). Rumen Microbiology: From Evolution to Revolution. New Delhi: Springer,India; 81, 1375–1386.
83. Richon, V. M., Sandhoff, T. W., Rifkind, R. A., & Marks, P. A. (2000). Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proceedings of the National Academy of Sciences, *97*(18), 10014-10019.
84. Rogers, J. A., Davis, C. L., & Clark, J. H. (1982). Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral salts in dairy cows. *Journal of dairy science*, *65*(4), 577-586.
85. Roumpeka, D. D., Wallace, R. J., Escalettes, F., Fotheringham, I., & Watson, M. (2017). A review of bioinformatics tools for bio-prospecting from metagenomic sequence data. *Frontiers in genetics*, *8*, 23.
86. Russell JB, RE Muck, PJ Weimer. (2009). Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. FEMS Microbiology Economy 67, 183-197.
87. Russell JB, WM Sharp, RL Baldwin. (1979). The effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. Journal of Animal Science 48, 251-255
88. Sales-Duval M, F Lucas, G Blanchart. (2002). Effects of exogenous ammonia or free amino acids on proteolytic activity and protein breakdown products in Streptococcus bovis, Prevotella albensis, and Butyrivibrio fibrisolvens. Current Microbiology 44, 435-443.
89. Scala, G., Corona, M., &Maruccio, L. (2011). Structural, histochemical and immunocytochemical study of the forestomach mucosa in domestic ruminants. *Anatomia, histologia, embryologia*, *40*(1), 47-54.
90. Schrauzer, G. N. (2000). Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *The Journal of nutrition*, *130*(7), 1653-1656.
91. Serra, A. B., Nakamura, K., Matsui, T., Harumoto, T., & Fujihara, T. (1994). Inorganic selenium for sheep I. Selenium balance and selenium levels in the different ruminal fluid fractions. *Asian-Australasian Journal of Animal Sciences*, *7*(1), 83-
92. Shabi, Z., Bruckental, I., Zamwell, S., Tagari, H., & Arieli, A. (1999). Effects of extrusion of grain and feeding frequency on rumen fermentation, nutrient digestibility, and milk yield and composition in dairy cows. Journal of dairy science, 82(6), 1252-1260.
93. Shi, L., Xun, W., Yue, W., Zhang, C., Ren, Y., Liu, Q., et al. (2011). Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. Animal Feeding Science Technology. 163, 136–142.
94. Sirohi SK, Choudhury PK, Dagar SS et al (2012) Isolation, characterization and fi bre degradation potential of anaerobic rumen fungi from cattle. Animal Microbiology 63:1187–1194
95. Soto-Navarro, S. A., Krehbiel, C. R., Duff, G. C., Galyean, M. L., Brown, M. S., & Steiner, R. L. (2000). Influence of feed intake fluctuation and frequency of feeding on nutrient digestion, digesta kinetics, and ruminal fermentation profiles in limit-fed steers. *Journal of animal science*, *78*(8), 2215-2222.
96. Sutton, J. D., Hart, I. C., Brosters, W. H., Elliott, R. J., & Schuller, E. (1986). Feeding frequency for lactating cows: effects on rumen fermentation and blood metabolites and hormones. *British journal of Nutrition*, *56*(1), 181-192.
97. Swanson, K. C., Matthews, J. C., Matthews, A. D., Howell, J. A., Richards, C. J., & Harmon, D. L. (2000). Dietary carbohydrate source and energy intake influence the expression of pancreatic α-amylase in lambs. *The Journal of nutrition*, *130*(9), 2157-2165.
98. Trinci, A. P., Davies, D. R., Gull, K., Lawrence, M. I., Nielsen, B. B., Rickers, A., &Theodorou, M. K. (1994). Anaerobic fungi in herbivorous animals. *Mycological Research*, *98*(2),129-152.
99. Udén, P. (2011). Using a novel macro in vitro technique to estimate differences in absorption rates of volatile fatty acids in the rumen. *Journal of animal physiology and animal nutrition*, *95*(1), 27-33.
100. van Nevel CJ, DI Demeyer. (1996). Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Reproduction and Nutrition Dev 36, 53-63.
101. van Zwieten JT, AM van Vuuren, J Dijkstra. (2008). Effect of nylon bag and protozoa on in vitro corn starch disappearance. Journal Dairy Science 91, 1133-1139.
102. Wang, C., Liu, Q., Yang, W. Z., Dong, Q., Yang, X. M., He, D. C., ... & Huang, Y. X. (2009). Effects of selenium yeast on rumen fermentation, lactation performance and feed digestibilities in lactating dairy cows. *Livestock Science*, *126*(1-3), 239-244.
103. Wang, Y., & McAllister, T. A. (2002). Rumen microbes, enzymes and feed digestion-a review. *Asian-Australasian Journal of Animal Sciences*, *15*(11), 1659-1676.
104. Warner, R. G., Flatt, W. P., & Loosli, J. K. (1956). Ruminant nutrition, dietary factors influencing development of ruminant stomach. Journal of Agricultural and Food Chemistry, *4*(9), 788-792.
105. Waters, D. J., Shen, S., Cooley, D. M., Bostwick, D. G., Qian, J., Combs Jr, G. F., ... & Morris, J.S. (2003). Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. Journal of the National Cancer Institute, 95(3), 237-241.
106. Wedekind KJ, HR Mansfield, L Montgomery. (1988). Enumeration and isolation of cellulolytic and hemicellulolytic bacteria from human feces. Applied Enviromental Microbiology 54, 1530-1535.
107. Wubah, D. A., Akin, D. E., &Bomeman, W. S. (1993). Biology, fiber-degradation, and enzymology of anaerobic zoosporic fungi. Critical reviews in microbiology, 19(2), 99-115.

108. Yáñez-Ruiz DR, A Moumen, AI Martín García, E Molina Alcaide. (2004). Ruminal fermentation and degradation patterns, protozoa population, and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: Effect of PEG supply. Journal of Animal Science; 82, 2023-2032.
109. Yáñez-Ruiz, D. R., Abecia, L., and Newbold, C. J. (2015). Manipulating rumen microbiome and fermentation through interventions during early life: a review. Frontal Microbiology; 6:1133.
110. YANG C.-M. d. and G. A. VARGA (1989). Effect of Three Concentrate Feeding Frequencies on Rumen Protozoa, Rumen Digesta Kinetics, and Milk Yield in Dairy Cows. Journal of Dairy Science 72:950-957
111. Yang, W., Z. Shen and H. Martens. (2012). An energy-rich diet enhances expression of Na+/H+ exchanger isoform 1 and 3 messenger RNA in rumen epithelium of goat. Journal of Animal Science 90(1): 307-317
112. Ye, H., Liu, J., Feng, P., Zhu, W., & Mao, S. (2016). Grain-rich diets altered the colonic fermentation and mucosa-associated bacterial communities and induced mucosal injuries in goats. Scientific reports, 6(1), 1-13.
113. Zachara, B. A. (1992). Mammalian selenoproteins. Journal of trace elements and electrolytes Zhang, T. T., Zhao, G. Y., Zheng, W. S., Niu, W. J., and Lin, S. X. (2015). Effects of rare earth element lanthanum on rumen methane and volatile fatty acid production and microbial flora in vitro. Journal of Animal Physiology and Animal Nutrition. 99, 442–448.in health and disease, 6(3), 137-151.
114. Zhang, T. T., Zhao, G. Y., Zheng, W. S., Niu, W. J., and Lin, S. X. (2015). Effects of rare earth element lanthanum on rumen methane and volatile fatty acid production and microbial flora in vitro. Journal of Animal Physiology and Animal Nutrition. 99, 442–448.