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Effects of Combined Organic Selenium and Zinc Supplementation on *In Vitro* Ruminal Enzyme Activities and Relative Populations of Several Bacterial Species

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ABSTRACT

Selenium (Se) and zinc (Zn) are essential animal microminerals. Combining Se and Zn (Se-Zn) as a feed additive in its influence on rumen fermentation patterns is still very limited, so further investigation is needed. The present study explored the supplementation impact of combined Se-Zn from organic sources on rumen enzyme activity and relative abundance of several bacterial species through an *in vitro* method. Five treatments, each with six replicates were used in the study. The first group treated without Se and Zn supplementation (T0, control), the second group treated with 0.3 ppm Se + 60 ppm Zn (T1), the third group treated with 0.45 ppm Se + 60 ppm Zn (T2), the fourth group treated with 0.3 ppm Se + 90 ppm Zn (T3), and the fifth group treated with 0.45 ppm Se + 90 ppm Zn (T4). The parameters observed included rumen microbial enzyme activities (carboxyl methyl cellulase, amylase, protease) and the relative abundance of rumen microbes (*Ruminococcus* sp., *Ruminococcus flavefaciens, Ruminococcus albus, Streptococcus* sp., *Prevotella ruminicola,* and *Eubacterium ruminantium*). Results indicated that carboxyl methyl cellulase (CMC-ase) and amylase activities raised in T2, T3, and T4 in comparison to T1 and T0 treatments. Protease activity and protein enzyme content increased in T2 compared to all treatments. The relative abundance of *Ruminococcus* sp. and *Ruminococcus albus* was higher in T2 and T3 compared to T0 treatment. Furthermore, an elevated *Ruminococcus flavefaciens* was indicated in T2 compared to other treatments. The T2, T3, and T4 led to higher abundances of *Eubacterium ruminantium, Prevotella ruminicola,* and *Ruminococcus albus* compared to T0 and T1. It is concluded that organic Se and Zn enhanced the relative abundance of several bacterial species and the activity of enzymes in the rumen; optimal results are recommended when combining 0.45 ppm Se + 60 ppm Zn.

Keywords: Bacterial Species, Enzyme Activity, Rumen, Selenium, Zinc

INTRODUCTION

Multiple microorganisms contribute to the rumen fermentation process in ruminant animals, and the feed supply significantly impacts these microbial communities in the rumen. Within the rumen, the flora is commonly comprised of diverse bacteria that undertake specific digestive roles, for instance, amylolytic bacteria break down starches, cellulolytic microbes degrade cellulose, and protein-degrading variants metabolize nitrogenous compounds (Wei et al., 2022). Bacteria alter the feed substrate, the primary source of nutrition for the host animal, by secreting several specialized enzymes (Moon et al., 2021). Due to their immediate relationship to the feed offered, rumen microbes are essential to ruminant nutrition (Puniya et al., 2015; Takizawa et al., 2020). However, the primary source of ruminant feed comes from agricultural crop wastes and forages, which have diverse nutrient contents, including minerals. In addition, many things, such as climate, plant species, soil, and farming practices, affect the mineral content of plants (Spears et al., 2022). In various regions across several countries, animal feed derived from agricultural plant residues often exhibits deficiencies in essential micronutrients, such as selenium (Se) and zinc (Zn) (Kumar et al., 2013). Nevertheless, these microminerals are essential for animal metabolism, including microorganisms present in the rumen (Zheng et al., 2022).

As a glutathione peroxidase (GSH-Px) component, Se shields cellular membranes against peroxide damage. It has been demonstrated that feeding supplements increase the antioxidant status of ruminal microbes. These circumstances improve fermentation and rumen microorganism development, which benefits animal growth and productivity (Li et al., 2023). Protease, amylase, and carboxyl methyl cellulase (CMC-ase) enzyme activities were elevated by dietary Se at concentrations of 0.3 and 0.45 ppm (Anam et al., 2023). Rumen bacterial abundance rose when Se was added to feed (Du et al., 2019; Cui et al., 2021). *Ruminococcus-1*, *Prevotella*, and *Prevotellaceae-UCG-003* might be found in higher relative abundances when hydroxy-selenomethionine (0.6 ppm) was added to dairy cattle feed (Zheng et al., 2022). Furthermore, rumen fermentation was significantly impacted by Zn in the cattle diet (Chen et al., 2019; Vigh et al., 2023). An increase in nutrient-degrading bacteria was positively connected with increased rumen microbial enzyme activity, which could be achieved with Zn supplementation. The rumen microbial protein also increased with the addition

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of 60 ppm of Zn (Chen et al., 2020). Moreover, Zn supplementation increased *Ruminococcus albus* and *Streptococcus bovis* populations (Petrič et al., 2021). Dietary supplementation of 80 ppm Zn could provide a balanced gut microbiota that promotes better growth for calves (Hou et al., 2023).

The current study focused on using organic rather than inorganic minerals. According to recent research, organic forms of minerals may prove to be more readily absorbed by the body than inorganic types due to higher bioavailability levels (Bakhshizadeh et al., 2019; Zheng et al., 2022). In addition, organic mineral supplementation can help livestock reduce environmental damage by increasing retention and absorption (Shaeffer et al., 2017). As described above, several studies have examined the effect of administering organic Se or Zn separately in ruminant feed on ruminal fermentation profiles (Chen et al., 2020; Anam et al., 2023). On the other hand, little is known regarding the organic Se-Zn combinations modifying the composition of bacteria and enzyme activity in the rumen. The present study explored the supplementation impact of combined Se and Zn from organic sources on rumen enzyme activity and relative abundance of several bacterial species through *in vitro* methods.

MATERIALS AND METHODS

Ethical approval

The Universitas Gadjah Mada, Indonesia, Animal Ethics Committee (025/EC-FKH/Eks./2023) approved all animal-handled protocols used in the current study.

Experimental design

Se and Zn were supplied as chelated-methionine containing Se and Zn at 0.4% and 15%, respectively. Five combinations of Se-Zn were evaluated, control (no Se and Zn supplementation, T0), 0.3 ppm + 60 ppm (T1), 0.45 ppm + 60 ppm (T2), 0.3 mg ppm + 90 mg ppm (T3), 0.45 ppm + 90 ppm (T4). The feeding of trace minerals into the feed was based on the dry matter (DM) of the feed used. In this case, Se and Zn were mixed with the basal premix (0.5% DM) and added to the substrate. The basal premix (per kg) contained Vitamin A 200,000 IU, Vitamin D 80,000 IU, Vitamin E 200 IU, Ca 243.4 g, P 3.2 g, K 277.9 g, Mg 1.8 g, Na 24.3 g, S 130.4 mg, Fe 12.5 mg, Mn 1.2 mg, Cu 179.4 mg, Co 5.4 mg, and I 1.2 mg. The basal diet consisted of forage to concentrate ratio of 60:40 (DM basis, percentage) based on elephant grass, wheat bran, ground corn, rice bran, dried palm kernel, and local soybean meal (Table 1).

Two Bali cattle (male and female) fitted with permanent rumen cannulas were used as rumen inoculum donors. The farm research facility at Universitas Gadjah Mada provided the cattle utilized in the present study. Healthy cattle were approximately five years old and weighed 320 ± 5 kg. During adaptation and experimentation, the animals received meals twice daily, administered at 6 a.m. and 3 p.m., alongside unrestricted access to water. Before feeding, rumen fluid was collected and filtered to eliminate any leftover feed before being transferred into a thermos flask. The rumen liquid from two cattle, each totaling 1,200 ml, was combined. Carbon dioxide (CO_2) flowed into the bottle for approximately 1 minute to remove the oxygen. Furthermore, 500mg of grounded feed substrate was transferred to a fermentation bottle, and artificial saliva and rumen inoculum were heated to 39°C and put into the fermentation bottle immediately. Each bottle was filled with 50ml of rumen fluid and artificial saliva, made in a 1:4 ratio. The artificial saliva was made anaerobically, as described by Tilley and Terry (1963). Incubation was carried out at 39°C for 48 hours with six replicates (for each treatment) and six blanks. The blanks contained only artificial saliva and rumen fluid without feed substrate. After 48 hours of incubation, the samples were centrifuged at 3,000 g for 15 minutes to separate the incubated liquid from the feed residue. The supernatant, which constitutes rumen liquid, was sampled for further analysis, including rumen enzyme activity and microbiota abundance.

Table 1. Ingredient and nutritional composition of the experimental diet

Ingredient	Value (%)	Nutrient level	Value (%)
Elephant grass	60.00	Dry matter	89.78
Wheat bran	10.00	Crude protein	14.78
Ground corn	5.00	Extract ether	3.44
Rice bran	5.00	Ash	8.21
Dried palm kernel	13.00	Acid detergent fiber	34.56
Local soybean meal	7.00	Neutral detergent fiber	52.11

Analyses

The AOAC (2005) procedure was used to determine the nutrient content of the feed substrate. Casein, amylum, and CMC substrates were used to measure protease, amylase, and CMC-ase enzyme activities, respectively (Anam et al., 2023). Ruminal genomic DNA was extracted utilizing the FavorPrep™ DNA Kit following the manufacturer's instructions. Microbial 16S rRNA was amplified, focusing on the hypervariable V3-V4 region, using primer sets V338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and V806R (5′- GGACTACHVGGGTWTCTAAT-3′; Gui et al., 2021). Bioinformatics analysis followed the methodology explained by Fregulia et al. (2022), where sequencing data were identified using QIIME2 v9.2023. The relative abundance of bacterial species observed included *Ruminococcus* sp., *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Streptococcus* sp., *Prevotella ruminicola*, and *Eubacterium ruminantium*.

Statistical analysis

The data were assessed using a one-way ANOVA with a completely randomized design, conducted with IBM SPSS Statistical software (version 26). The Duncan multiple range test was employed to test for differences in means. Differences in mean data were considered significant at $p < 0.05$. The association between ruminal bacterial species populations and enzyme activities was assessed using a Spearman-rank correlation analysis.

RESULTS

Table 2 presents the results of *in vitro* rumen enzyme activities. CMC-ase levels for T2, T3, and T4 were higher than T1 and T0 ($p < 0.05$). Amylase levels for T2, T3, and T4 were also higher than T1 and T0 ($p < 0.05$). In addition, enzyme protein levels for T2 were higher across all treatments ($p < 0.05$). Figure 1 depicts the bacterial species abundance across the different dietary treatments. According to the data, there was a significant improvement of *Ruminococcus* sp. in T2 and T3 compared to T0 and an equivalent rise in T4 compared to T0 ($p < 0.05$). *Ruminococcus flavefaciens* showed higher in T2 than other groups ($p < 0.05$). *Ruminococcus albus* increased in T2, T3, and T4 than in T0 and T1 ($p < 0.05$). Compared to T0, *Streptococcus* sp. increased in T2 and T4 ($p < 0.05$). Furthermore, in T2, T3, and T4, compared to other treatments, there were greater levels of *Eubacterium ruminantium* and *Prevotella ruminicola* ($p < 0.05$). As shown in Figure 2, the correlation between rumen enzyme activities and populations of bacterial species was determined using Spearman's correlation analysis under different combinations of supplemental Se-Zn. In general, it has been shown that *Ruminococcus* sp., *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Streptococcus* sp., *Prevotella ruminicola*, and *Eubacterium ruminantium* had positive relationships with CMC-ase, amylase, and protease activities.

Table 2. Ruminal enzyme activities as affected by various combined organic Se and Zn supplementations in the diet of Bali cattle

Item \ Treatment	T0	T1	T2	T3	T4	SEM	*p*-value
CMC-ase (U/g)	3.40^{b}	3.85^{b}	5.01^{a}	4.79^{a}	4.46^{a}	0.18	< 0.001
Amylase (U/g)	16.90^{c}	18.11^{b}	20.42^{a}	20.42^{a}	19.89^{a}	0.31	< 0.001
Protease (U/g)	141.91^{d}	149.26^{c}	165.38^{a}	161.36^{ab}	153.64^{bc}	1.99	< 0.001
Enzyme protein (mg/ml)	0.78^{c}	0.92^{bc}	1.08^{a}	1.04^{ab}	0.89^{bc}	0.03	0.002

T0: Basal diet, no additive, T1: T0 + 0.3 ppm Se + 60 ppm Zn, T2: T0 + 0.45 ppm Se + 0.60 ppm Zn, T3: T0 + 0.30 ppm Se + 90 ppm Zn, T4: T0 + 0.45 ppm Se + 90 ppm Zn. SEM: Standard Error of the Mean. Means with different superscript letters in the same row are significantly different at $p < 0.05$.

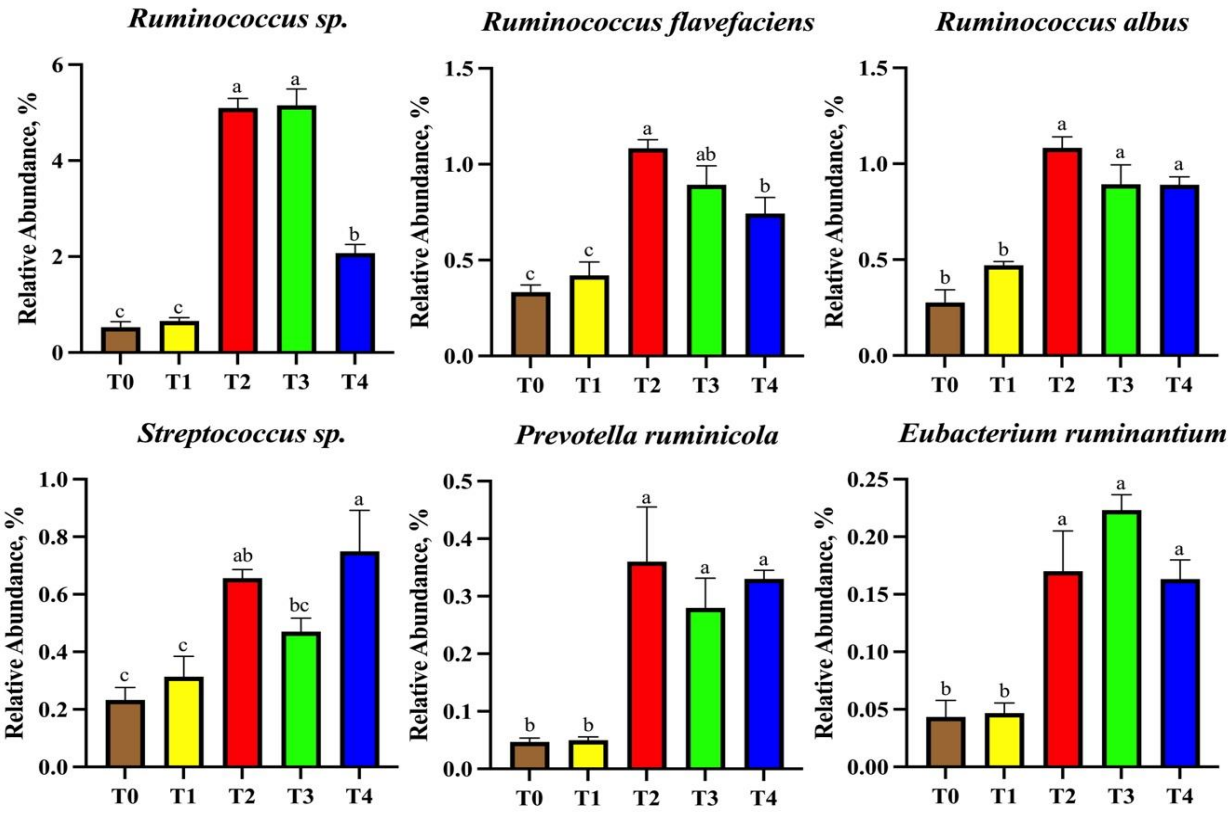

Figure 1. Ruminal bacterial abundance with different levels of combined organic selenium and zinc supplementations. T0: Basal diet, no additive, T1: T0 + 0.3 ppm Se + 60 ppm Zn, T2: T0 + 0.45 ppm Se + 0.60 ppm Zn, T3: T0 + 0.30 ppm Se + 90 ppm Zn, T4: T0 + 0.45 ppm Se + 90 ppm Zn. Means with different letters in the same figure are significantly different at $p < 0.05$.

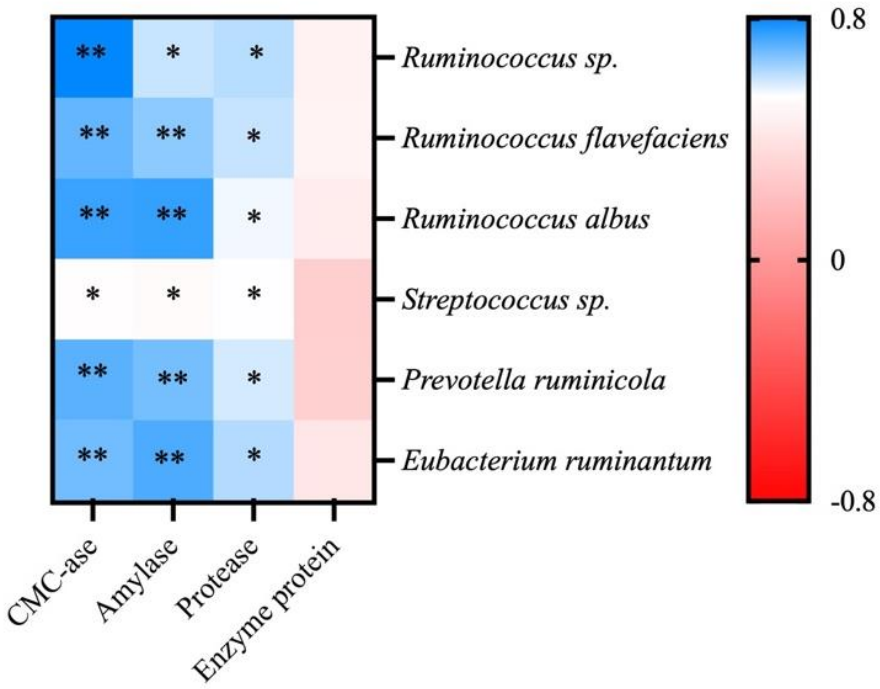

Figure 2. The heat map displays the correlation of rumen ruminal enzyme activities with bacterial abundance. Spearman's correlation coefficients were calculated and the values between -0.8 and 0.80 in the color key indicate negative (red) and positive (blue) correlations. CMC-ase: carboxymethyl cellulase. *: $p < 0.05$ and **: $p < 0.01$.

DISCUSSION

Bacteria comprise about 95% of the entire microbiota and are the most abundant microorganisms. These bacteria secreted enzymes that play an essential function in the feed degradation process (Hao et al., 2021). In the current research, supplementing some variations of Se-Zn additive in ration boosted the activity of the CMC-ase enzyme linear with the increase in fiber-degrading bacteria abundance. Se snares free radicals and guards against oxidative damage to cell membranes (Surai et al., 2019). Čobanova et al. (2016) found that after incorporating 0.4 ppm of Se into sheep diets, rumen microbes and protozoa exhibited elevated GSH-Px activity. Anam et al. (2023) reported that adding 0.45 ppm organic Se revealed CMC-ase activity. In line with Liu et al. (2019), the inclusion of inorganic Se in the diet of Holstein dairy cattle led to an increase in the numbers of *Ruminococcus albus* and *Ruminococcus flavefaciens* compared to the unsupplemented group. Additionally, Zn has antioxidant properties that might boost some enzyme activities. When Zn supplementation was applied to dairy cows, Wang et al. (2021) observed a linear rise in CMC-ase activity as well as in the number of fiber-degrading bacteria, *Ruminococcus albus* and *Ruminococcus flavefaciens*. These two bacteria belong to the two main bacteria in the *Ruminococcus* phylum category, which can produce cellulase and hemicellulase. *Ruminococcus* is known for breaking down cellulose and hemicellulose (Kim et al., 2018; Takizawa et al., 2020). The enzyme mentioned in this study, CMC-ase, breaks down β-1,4-glucan and generates free chain ends by targeting low-crystalline regions in cellulose fibers (Sun and Cheng, 2002).

Amylolytic bacteria, including *Prevotella ruminicola* and *Streptococcus bovis* can degrade nutrients, such as xylan, pectin, and starch (Wei et al., 2022). In addition, *Prevotella ruminicola* is responsible for the metabolism of peptides and proteins in the rumen (Wallace et al., 1997). The enzyme activity tests performed in the present research revealed that amylase and protease levels within the T2 group samples were markedly higher than those of the T0 or other analyzed groups. This finding may indicate that amylolytic and proteolytic flora may have better reactions in T2 treatment. Rumen microorganisms incorporate Se into proteins and their components. Similarly, the level of amylase activity was significantly increased when dairy cattle feed was supplemented with Se up to 0.5 ppm (Liu et al., 2019). In addition, Zn can help rumen fermentation patterns by directly altering microbial enzyme functions (Hilal et al., 2016). Dietary 70 ppm organic Zn caused an improvement in the relative population of *Streptococcus bovis* (Petrič et al., 2021). *Prevotella ruminicola* also developed with proteolytic bacteria as a result of the addition of inorganic Zn at 30 ppm (Wang et al., 2021). According to similar findings, rumen protease enzyme activity was raised by organic Se inclusion at a level of 0.45 ppm (Anam et al., 2023). The Se interacts closely with Zn, and both play crucial roles at the cellular level, depending on their form and dose (Yildiz et al., 2019). Giving 0.5 ppm Se plus 50 ppm Zn boosted daily body weight

gain in Awassi sheep by 31.03% as compared to Se alone (Al-Taie and Almahdawi, 2021). The improvement in sheep performance was associated with the increase in rumen enzyme activity, which degraded feed more efficiently.

CONCLUSION

In summary, the inclusion of organic Se and Zn enhanced the relative abundance of several bacterial species and the activity of enzymes in the rumen, optimal results are recommended when combining 0.45 ppm Se + 60 ppm Zn. Nevertheless, more investigation is required to find whether this result may be directly applied to live ruminant animals.

DECLARATIONS

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Authors' contributions

Moh Sofi'ul Anam and Ali Agus: conceptualization, data collection, and writing. Budi Prasetyo Widyobroto and Andriyani Astuti: data curation, validation, and editing. Gunawan: methodology, writing, and review. All authors checked and approved the analyzed data and the final edition of the manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

All authors have reviewed the manuscripts for ethical issues such as plagiarism, misconduct, data fabrication, and double submission.

Availability of data and materials

The datasets produced in the present study can be obtained from the corresponding author upon a reasonable inquiry.

REFERENCES

Al-Taie YAM and Almahdawi MKK (2021). Effect of selenium and zinc doses and interaction between them on production performance and some carcass traits of Awassi lambs. Annals of the Romanian Society for Cell Biology, 25(4): 2506-2520. Available at: https://annalsofrscb.ro/index.php/journal/article/view/2782

Anam MS, Astuti A, Widyobroto BP, Gunawan, and Agus A (2023). *In vitro* ruminal cumulative gas and methane production, enzyme activity, fermentation profile and nutrient digestibility on feed supplemented with organic selenium. American Journal of Animal and Veterinary Sciences, 18(4): 261-272. DOI: https://www.doi.org/10.3844/ajavsp.2023.261.272

Association of official analytical chemists (AOAC) (2005). Official methods of analysis, 18th Edition. Arlington., Washington, DC, USA.

Bakhshizadeh S, Aghjehgheshlagh FM, Taghizadeh A, Seifdavati J, and Navidshad B (2019). Effect of zinc sources on milk yield, milk composition and plasma concentration of metabolites in dairy cows. South African Journal of Animal Science, 49(5): 884-891. DOI: https://www.doi.org/10.4314/SAJAS.V49I5.11

Chen F, Li Y, Shen Y, Guo Y, Zhao X, Li Q, Cao Y, Zhang X, Li Y, Wang Z et al. (2020). Effects of prepartum zinc-methionine supplementation on feed digestibility, rumen fermentation patterns, immunity status, and passive transfer of immunity in dairy cows. Journal of Dairy Science, 103(10): 8976-8985. DOI: https://www.doi.org/10.3168/jds.2019-17991

Chen M, Xi Y, Zhang L, Zeng H, Li Y, and Han Z (2019). Effects of zinc-bearing palygorskite on rumen fermentation *in vitro*. Asian-Australasian Journal of Animal Sciences, 32(1): 63-71. DOI: https://www.doi.org/https://doi.org/10.5713/ajas.17.0920

Čobanova K, Faix Š, Plachá I, Mihaliková K, Váradyová Z, Kišidayová S, and Grešáková L (2016). Effects of different dietary selenium sources on antioxidant status and blood phagocytic activity in sheep. Biological Trace Element Research, 175(2): 339-346. DOI: https://www.doi.org/10.1007/s12011-016-0794-0

Cui X, Wang Z, Tan Y, Chang S, Zheng H, Wang H, Yan T, Guru T, and Hou F (2021). Selenium yeast dietary supplement affects rumen bacterial population dynamics and fermentation parameters of Tibetan sheep (*Ovis aries*) in Alpine Meadow. Frontiers in Microbiology, 12: 663945. DOI: https://www.doi.org/10.3389/fmicb.2021.663945

Du HS, Wang C, Wu ZZ, Zhang GW, Liu Q, Guo G, Huo WJ, Zhang YL, Pei CX, and Zhang SL (2019). Effects of rumen-protected folic acid and rumen-protected sodium selenite supplementation on lactation performance, nutrient digestion, ruminal fermentation and blood metabolites in dairy cows. Journal of the Science of Food and Agriculture, 99(13): 5826-5833. DOI: https://www.doi.org/10.1002/jsfa.9853

Fregulia P, Campos MM, Dias RJP, Liu J, Guo W, Pereira LGR, Machado MA, Faza DR de LR, Guan LL, Garnsworthy PC et al. (2022). Taxonomic and predicted functional signatures reveal linkages between the rumen microbiota and feed efficiency in dairy cattle raised in tropical areas. Frontiers in Microbiology, 13: 1025173. DOI: https://www.doi.org/10.3389/fmicb.2022.1025173

Gui LS, Raza SHA, Ahmed AFAE, Zhou L, Hou S, Khan I, Kakar IU, Abd El-Aziz AH, Jia J, Sun Y et al. (2021). Altered milk yield and rumen microbial abundance in response to concentrate supplementation during the cold season in Tibetan sheep. Electronic Journal of Biotechnology, 53: 80-86. DOI: https://www.doi.org/10.1016/j.ejbt.2021.07.001

Hao Y, Guo C, Gong Y, Sun X, Wang W, Wang Y, Yang H, Cao Z, and Li S (2021). Rumen fermentation, digestive enzyme activity, and bacteria composition between pre-weaning and post-weaning dairy calves. Animals, 11(9): 2527. DOI: https://www.doi.org/10.3390/ani11092527

Hilal EY, Elkhairey MAE, and Osman AAA (2016). The role of zinc, manganese and copper in rumen metabolism and immune function: A review article. Open Journal of Animal Sciences, 6(4): 71654. DOI: https://www.doi.org/10.4236/ojas.2016.64035

Hou P, Li B, Wang Y, Li D, Huang X, Sun W, Liang X, and Zhang E (2023). The effect of dietary supplementation with zinc amino acids on immunity, antioxidant capacity, and gut microbiota composition in calves. Animals, 13(9): 1570. DOI: https://www.doi.org/10.3390/ani13091570

Kim YH, Nagata R, Ohkubo A, Ohtani N, Kushibiki S, Ichijo T, and Sato S (2018). Changes in ruminal and reticular pH and bacterial communities in Holstein cattle fed a high-grain diet. BMC Veterinary Research, 14(1): 310. DOI: https://www.doi.org/10.1186/s12917-018-1637-3

Kumar P, Yadav B, and Yadav S (2013). Effect of zinc and selenium supplementation on antioxidative status of seminal plasma and testosterone, T 4 and T 3 level in goat blood serum. Journal of Applied Animal Research, 41(4): 382-386. DOI: https://www.doi.org/10.1080/09712119.2013.783482

Li L, Qu L, and Li T (2023). The effects of selenohomolanthionine supplementation on the rumen eukaryotic diversity of Shaanbei white cashmere wether goats. Scientific Reports, 13(1): 13134. DOI: https://www.doi.org/10.1038/s41598-023-39953-2

Liu YJ, Wang C, Liu Q, Guo G, Huo WJ, Zhang YL, Pei CX, Zhang SL, and Zhang J (2019). Effects of sodium selenite addition on ruminal fermentation, microflora and urinary excretion of purine derivatives in Holstein dairy bulls. Journal of Animal Physiology and Animal Nutrition, 103(6): 1719-1726. DOI: https://www.doi.org/10.1111/jpn.13193

Moon CD, Carvalho L, Kirk MR, McCulloch AF, Kittelmann S, Young W, Janssen PH, and Leathwick DM (2021). Effects of long-acting, broad spectra anthelmintic treatments on the rumen microbial community compositions of grazing sheep. Scientific Reports, 11(1): 3836. DOI: https://www.doi.org/10.1038/s41598-021-82815-y

Petrič D, Mravčáková D, Kucková K, Kišidayová S, Cieslak A, Szumacher-Strabel M, Huang H, Kolodziejski P, Lukomska A, Slusarczyk S et al. (2021). Impact of zinc and/or herbal mixture on ruminal fermentation, microbiota, and histopathology in lambs. Frontiers in Veterinary Science, 8: 630971. DOI: https://www.doi.org/10.3389/fvets.2021.630971

Puniya AK, Singh R, and Kamra DN (2015). Rumen microbiology: From evolution to revolution. Springer., New Delhi, India, pp. 357-379. Available at: https://vetbooks.ir/rumen-microbiology-from-evolution-to-revolution/

Shaeffer GL, Lloyd KE, and Spears JW (2017). Bioavailability of zinc hydroxychloride relative to zinc sulfate in growing cattle fed a corn-cottonseed hull-based diet. Animal Feed Science and Technology, 232: 1-5. DOI: https://www.doi.org/10.1016/j.anifeedsci.2017.07.013

Spears JW, Brandao VLN, and Heldt J (2022). Invited review: Assessing trace mineral status in ruminants, and factors that affect measurements of trace mineral status. Applied Animal Science, 38(3): 252-267. DOI: https://www.doi.org/10.15232/aas.2021-02232

Sun Y and Cheng J (2022). Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresource Technology, 83(1): 1-11. DOI: https://www.doi.org/10.1016/S0960-8524(01)00212-7

Surai PF, Kochish II, Fisinin VI, and Juniper DT (2019). Revisiting oxidative stress and the use of organic selenium in dairy cow nutrition. Animals, 9(7): 462. DOI: https://www.doi.org/10.3390/ani9070462

Takizawa S, Asano R, Fukuda Y, Feng M, Baba Y, Abe K, Tada C, and Nakai Y (2020). Change of endoglucanase activity and rumen microbial community during biodegradation of cellulose using rumen microbiota. Frontiers in Microbiology, 11: 1-12. DOI: https://www.doi.org/10.3389/fmicb.2020.603818

Tilley JMA and Terry RA (1963). A two-stage technique for the *in vitro* digestion of forage crops. Journal of the British Grassland Society, 18: 104-111. DOI: http://www.doi.org/10.1111/j.1365-2494.1963.tb00335.x

Vigh A, Criste A, Gragnic K, Moquet L, and Gerard C (2023). Ruminal solubility and bioavailability of inorganic trace mineral sources and effects on fermentation activity measured *in vitro*. Agriculture, 13(4): 879. DOI: https://www.doi.org/10.3390/agriculture13040879

Wallace RJ, McKain N, Broderick GA, Rode LM, Walker ND, Newbold CJ, and Kopecny J (1997). Peptidases of the rumen bacterium, *Prevotella ruminicola*. Anaerobe, 3(1): 35-42. DOI: https://www.doi.org/10.1006/anae.1996.0065

Wang C, Xu YZ, Han L, Liu Q, Guo G, Huo WJ, Zhang J, Chen L, Zhang YL, Pei CX et al. (2021). Effects of zinc sulfate and coated zinc sulfate on lactation performance, nutrient digestion and rumen fermentation in Holstein dairy cows. Livestock Science, 251: 104673. DOI: https://www.doi.org/10.1016/j.livsci.2021.104673

Wei W, Zhen Y, Wang Y, Shahzad K, and Wang M (2022). Advances of rumen functional bacteria and the application of micro-encapsulation fermentation technology in ruminants: A review. Fermentation, 8(10): 564. DOI: https://www.doi.org/10.3390/fermentation8100564

Yildiz A, Kaya Y, and Tanriverdi O (2019). Effect of the interaction between selenium and zinc on DNA repair in association with cancer prevention. Journal of Cancer Prevention, 24(3): 146-154. DOI: https://www.doi.org/10.15430/jcp.2019.24.3.146

Zheng Y, He T, Xie T, Wang J, Yang Z, Sun X, Wang W, and Li S (2022). Hydroxy-selenomethionine supplementation promotes the *in vitro* rumen fermentation of dairy cows by altering the relative abundance of rumen microorganisms. Journal of Applied Microbiology, 132(4): 2583-2593. DOI: https://www.doi.org/10.1111/jam.15392