RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 2: 338-344 (February 2026)

The Effect of the Chinese Herbal Additive Shikuqin on Rumen Microorganisms in Calves

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10.18805/IJAR.BF-2052

ABSTRACT

Background: Traditional Chinese medicine ShiKuQin, composed of *pomegranate rind*, *sophora flavescens* and *cortex fraxini*, has been proven effective in treating animal diarrhea. In order to understand the mechanism of SKQ in treating diarrhea, the aim is to explore its potential as an alternative to antibiotics.

Methods: The study was conducted in twelve calves of similar age, divided into a treatment group receiving SKQ as an additive and a control group. Rumen microbiota were analyzed using metagenomics sequencing.

Result: The analysis revealed that the number of microbial abundance and expressed genes in the treatment group was lower compared to the control group. Metastats and LEfSe variation analyses indicated significant reductions in the treatment group for *Methanoperedenaceae, Angelakisella, Actinophytocola, Klebsiella, Pseudoxanthomonas* and *Alistipes*. The treatment group also showed significantly higher levels of enzyme families GT64, GH24, GH13, CBM20 and GH31, whereas CE1 and GH29 levels were higher in the control group.

Key words: Calf, Chinese herbal medicine, Metagenomics, Microbiota, Rumen.

INTRODUCTION

Calf diarrhea is considered one of the most serious diseases of livestock from an economic standpoint (Cho and Yoon 2014; Qu *et al.*, 2023). Calf diarrhea can be caused by various factors. For example, environmental pollution and improper feeding management can lead to infections with bacteria, viruses and parasites in calves (Ngeleka *et al.,* 2019; Pansri *et al.,* 2022). To a certain extent, antibiotics have emerged as the predominant pharmaceuticals (Shukla *et al.*, 2022) and feed additives employed for the prevention of diarrhea in calves. Nonetheless, it is crucial to consider the potential implications associated with this practice, including the emergence of antibiotic resistance and the persistence of medicine residues over an extended period, which generated a serious concern for the safety of livestock products (Ali *et al.,* 2021). Previous research demonstrated that Chinese herbal medicines can provide beneficial effects for the prevention and treatment of animal diseases (Chen *et al.*, 2024) and have the potential to be used as effective substitutes for antibiotics (Millar *et al.,* 2021; Guo *et al.,* 2022).

Our previous studies have shown that SKQ exhibits remarkable antidiarrheal, anti-inflammatory and analgesic effects against gastrointestinal tract disorders (Yu *et al.,* 2019) and was not toxic to the central nervous system, cardiovascular system, or respiratory system (Yu *et al.,* 2018). The Chinese medicine SKQ is made of *Pomegranate rind, sophora flavescens* and *cortex fraxini* by hot water extraction. Chinese medicine SKQ, the three main active ingredients of traditional Chinese medicine are ellagic acid, matrine, oxymatrine, aesculin and aesculetin. *Pomegranate rind* is featured with beneficial properties such as carcino preventive effects, hemostasis activity, anti-inflammatory effects and antibacterial activities (Yassin *et al*., 2021; Rajamani *et al*., 2026). *Sophora flavescens,* rich in alkaloids and flavonoids, has multiple pharmacological activities, including anti-bacterial, anti-inflammatory, analgesic and antineoplastic effects (He *et al.,* 2015). *Cortex Fraxini* has been therapeutically used in clinical applications owing to its functions of antiperspiratory, antirheumatic, astringency-inducing and anti-dysenteric effects (Guo *et al.,* 2017). In this study, SKQ was applied as a therapeutic intervention for diarrhea,exploring its potential as an alternative to antibiotics.

Rumen bacteria function as a pivotal organ regulating host digestion, nutrient absorption and metabolism by generating bacterial metabolites and microbial-associated molecular patterns to maintain calf health and to prevent various diseases. However, the variation of rumen bacteria

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How to cite this article: Jiang, L., Zhao, Y., Ran, J., Luo, W., Zhou, J., Liu, J. and Yu, B. (2026). The Effect of the Chinese Herbal Additive Shikuqin on Rumen Microorganisms in Calves. *Indian Journal of Animal Research.* **60(2):** 338-344. doi: 10.18805/IJAR.BF-2052.

Submitted: 29-07-2025 **Accepted:** 12-01-2026 **Online:** 29-01-2026

remains largely unknown when feeding calves supplemented with the Chinese medicine SKQ. The present study focused on the impact of SKQ on the distribution of the rumen flora in calves and the possible outcomes of feeding calves SKQ as a supplement. We investigated the effects of SKQ on the rumen bacteria by metagenomics sequencing, by which the species distribution was comprehensively analyzed and functional annotation was performed to identify the physiological changes in calves.

MATERIALS AND METHODS

Preparation of Chinese medicine SKQ

The Chinese medicine SKQ was developed by the Veterinary Research and Animal Husbandry Institute, Guizhou Academy of Agricultural Sciences. The compound was extracted and concentrated by water at a ratio of 3 : 3 : 2 from pomegranate peel, *Sophora flavescens* and *Fraxini Cortex*. The concentrate was mixed with lactose at a ratio of 1:1 and dried by spray drying to make pellet feed. Concentrated feedstuff was purchased from Huirui Animal Husbandry Technology Ltd. in Linyi, Shandong Province. Corn, stems and silage were obtained from local cattle farms.

Analysis by HPLC

The effective components in the peel of the pomegranate, namely ellagic acid, the effective components in *Sophora flavescens* the, namely matrine and oxymatrine and the contents of aesculetin and aesculin in the cortex fraxini were detected according to the Chinese Pharmacopoeia (2020 Edition) Volume II. The detection was carried out using high-performance liquid chromatography (HPLC).

Animal care and experimental design

Twelve healthy calves approximately 5 monthes of age were chosen from a cattle farm in Tongren City (China,Guizhou yellow cattle). Their weights were around 80-100 kg. Animals (6 per group) were divided into treatment (Group A) and control (Group B) groups and housed separately. The calves were vaccinated and dewormed before the experiment. All calves were fed with same amount of feed using a single tank. The pre-trial period lasted for 10 days and the trial period lasted for 60 days. The calves were fed at 8:00 a.m. and 4:00 p.m. each day with free access to water. The feed formula of experimental animals is shown in Table 1. Calves in the control group were fed with forage only and calves in the experimental group were fed with forage containing 0.3% SKQ.

Sample collection

After the trial period, samples of rumen fluid from three calves randomly selected from each group were collected by using an orally administered stomach tube, for a total of six samples. The extracted rumen fluid was filtered through six layers of gauze. Approximately 50 mL of ruminal fluid from each sample was then placed in liquid nitrogen for later analysis.

Metagenomic sequencing

The rumen fluid samples were used to measure the variation in rumen microorganisms by metagenomic sequencing. The process of database building was divided into seven stages: DNA extraction of the sample, DNA tests, library construction, library detection, sequencing, quality

Table 1: Formula and nutrient composition of experimental animal feed (DM%)

Items	Mass fraction (%)	Nutrient level	Content
Corn straw	61.7	Synthetical net energy (MJ·kg^{-1})	5.4
Corn	17.6	Crude protein(CP/%)	13
Wheat bran	16.9	Neutral detergent fiber (NDF/%)	52
Baking soda	0.5	Crude ash(Ash/%)	10
Calcium carbonate	1.1	Calcium (Ca/%)	0.75
Common salt	0.6	Phosphorus(P/%)	0.4
Urea	1.6		
Total	100		

Table 2: Results of component analysis by HPLC.

	Name of ingredient	Appearance time (min)	Peak area	Concentration (ug/mL)	Content (mg/g)
Standard	Ellagic acid	6.545	6360584	50	/
	Matrine	5.041	238171	40	/
	Oxymatrine	7.215	668283	100	/
	Aesculin	4.628	2653585	100	/
	Aesculetin	8.774	4168860	100	/
Sample	Ellagic acid	6.585	10499909	82.539	1.17
	Matrine + oxymatrine	5.038, 7.216	140125, 434074	23.534, 64.954	4.21
	Aesculin + aesculetin	4.599, 8.713	1262716, 1725991	47.585, 41.402	2.19

control and information analysis. The sequencing was performed by Novogene Co., Ltd. In addition, the databases used to analyze genetic data were the Kyoto Encyclopedia of Genes and Genomes (KEGG), Version: 2018.01, evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG), Version: 4.5 and the Carbohydrate-Active enzymes Database (CAZy), Version: 2018.01.

RESULTS AND DISCUSSION

Composition of SKQ

HPLC analysis indicated that the SKQ contained matrine and oxymatrine at 4.21 mg/g, aesculin and aesculetin at 2.19 mg/g and ellagic acid at 1.17 mg/g (Table 2). The content of active ingredients in the traditional Chinese medicine compound meets the requirements of the Chinese Pharmacopoeia (2020 Edition) Volume II.

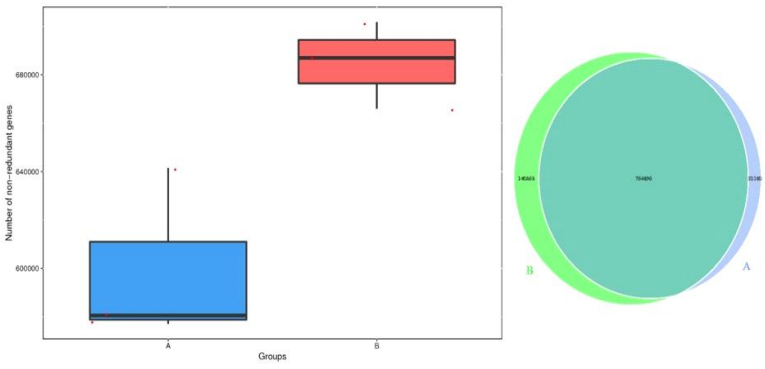
Metagenomics sequencing

Analysis of gene number differences

To investigate the differences in the number of genes among the various groups, the sequencing company created a bar chart showing the differences in the number of genes between the groups based on the data results. To analyze the common and specific information of genes between different samples (groups), the Venn diagram was drawn (Fig 1) It showed that the total number of genes identified in the study was 764,896, with 50,180 genes exclusive to group A and 140,868 genes exclusive to group B. The results showed that the experimental group was lower than the control group.

Species annotation

Analysis of the relative abundance of species in each group. The species based on annotation and abundance were compared between group A and group B. To be specific, the top ten most abundant species at the genus level were *Prevotella*, *Bacteroides*, *Acetobacter*, *Fibrobacter*, *Paraprevotella*, *Clostridium*, *Succiniclasticum*, *Alistipes*, *Ruminococcus* and *Klebsiella* in group A and *Prevotella*, *Bacteroides*, *Klebsiella*, *Succiniclasticum*, *Clostridium*, *Alistipes*, *Paraprevotella*, *Ruminococcus*, *Fibrobacter* and *Acetobacter* in group B. But the results clearly show that the content of *Prevotella*, *Klebsiella, Alistipes* in group A was significantly lower than that in group B (Fig 2). The literature indicates that infected frogs that were exposed to both *Klebsiella pneumoniae* and *Elizabethkingia miricola* exhibited faster and higher mortality rates compared to



Group A was the experimental group of traditional Chinese medicine and group B was the control group.

Fig 1: Distribution of gene numbers in each group.

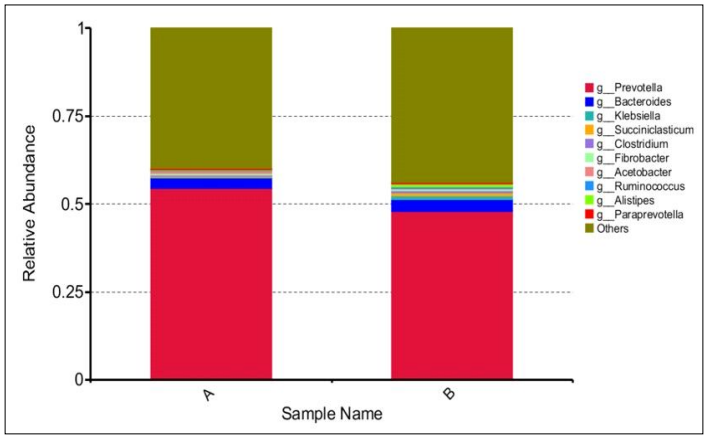


Fig 2: Relative abundance of species.

frogs infected with each bacterium separately (Li *et al.,* 2023), revealing the pathogenicity of *Elizabethkingia* miricola. Studies have shown that the microbial composition of the SKQ-fed group is better because there is less *Elizabethkingia*, reducing the risk of negative effects. Studies have shown that *Klebsiella* is involved in several human infections (Wyres and Holt, 2016) and has multidrug-resistant ability (Rodríguez-Medina *et al.,* 2019). Studies have shown that *Klebsiella* is involved in several human infections (Wyres and Holt, 2016) and has multidrug-resistant ability (Rodríguez-Medina *et al.,* 2019). The genus *Alistipes* are a kind of anaerobic bacteria reported to be involved in inflammation, cancer and other diseases (Parker *et al.,* 2020). In this study, the SKQ group contained fewer harmful bacteria.

Metastats analysis of different species between groups

Metastats (White *et al.,* 2009) was used to analyze species abundance data. As shown in Fig 3, the genus *Angelakisella* from family *Ruminococcaceae* and genus *Actinophytocola from Pseudonocardiaceae* in group A were significantly lower than that in group B ($p<0.05$). Additionally, the family Candidatus, genus *Methanoperedenaceae* in Group A was extremely lower than that of Group B ($p<0.01$). (Fig 3). The study demonstrated that the relative abundance of the genus *Angelakisella* was negatively correlated to the levels of gastrointestinal active peptides (Chai *et al.,* 2021). Although notable alterations have been found in the present study, bacteria such as *Methanoperedenaceae* and *Actinophytocola* were not discussed here due to the lack of literature reports.

LEfSe analysis of different species between groups

LEfSe analysis of different species between groups: LEfSe analysis was used to screen for significant differences between groups. The rank sum test was used to detect the differences in species among groups and LDA (linear discriminant analysis) was used to achieve dimension reduction and evaluate the impact of the differential species (Segata *et al.,* 2011). The LEfSe analysis included three parts, an LDA value distribution histogram, an evolutionary branching diagram (phylogenetic distribution) and a

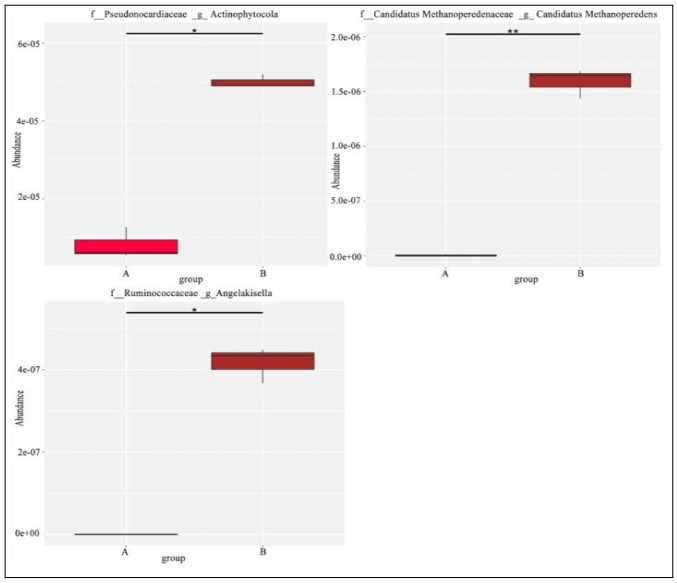


Fig 3: Different species in each group by metastats analysis.

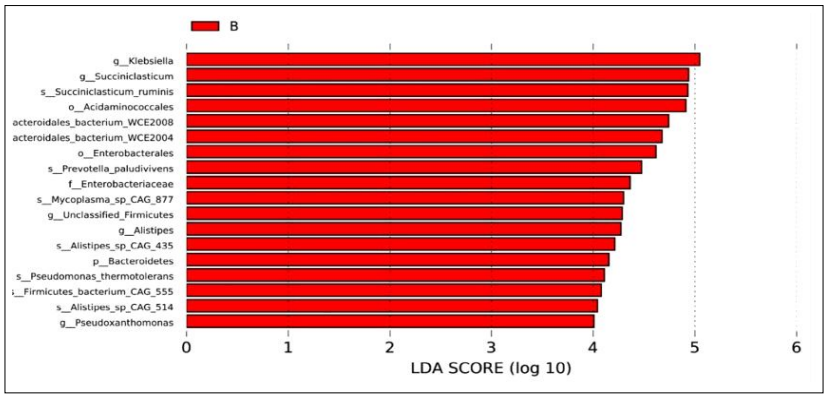


Fig 4: Different species in each group according to the LDA score.

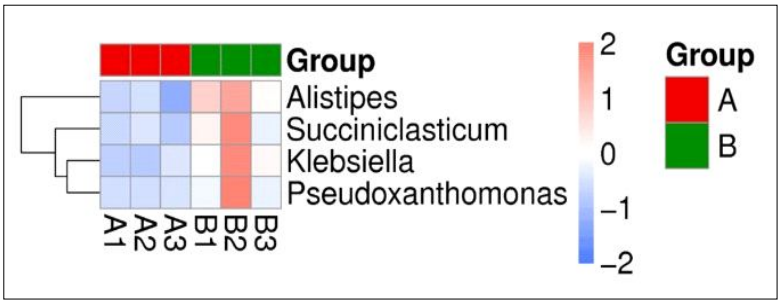


Fig 5: Different species in each group by LEfSe analysis shown as a cluster heat map.

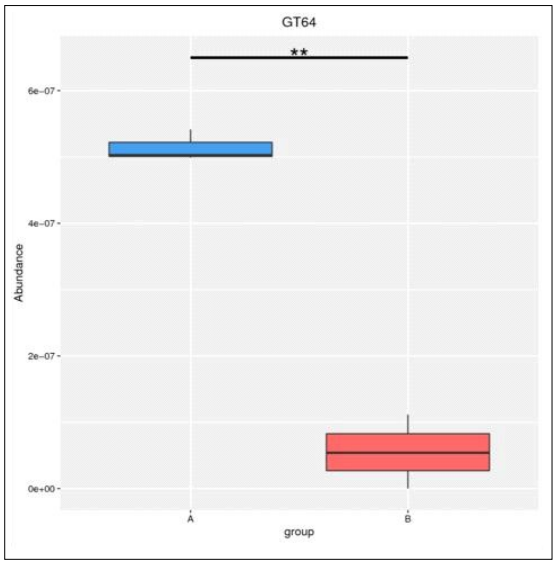


Fig 6: Different functions between groups by metastats analysis.

comparison diagram of abundance with biomarkers having significant differences between groups (Fig 4).

The histograms show that the genera *Klebsiella*, *Pseudoxanthomonas*, *Alistipes* and *Succiniclasticum* had significant reductions in group A (Fig 5). The analysis results are consistent with species annotation.

Annotation of common functions

Sketch of annotated gene numbers

The microbial gene function annotation based on KEGG indicated that the genes associated with metabolism were the highest abundant. those with the number of genes above 10000 being carbohydrate, amino acid, cofactors and vitamins, nucleotide metabolism and energy and glycan biosynthesis metabolism.

The annotation based on eggNOG showed that the functions with the number of genes above 20000 were cell wall/membrane/envelope biogenesis, carbohydrate transport and metabolism, replication-recombination-repair, amino acid transport and metabolism, translation-ribosomal structure and biogenesis, inorganic ion transport and metabolism and signal transduction mechanisms.

The annotation based on CAZy illustrated that the main enzymes were glycoside hydrolases involved with 27384 genes, glycosyl transferases with 9228 genes, carbohydrate-binding modules with 4384 genes, carbohydrate esterases with 3590 genes and polysaccharide lyases with 1280 genes.

Metastats analysis of different functions between groups

Metastats analysis was used to research the functions similar to the above analysis for the species between groups. The results showed that GT64 belonging to the class of glycosyltransferases, was highly abundant in group A compared with group B ($p<0.01$) (Fig 6). At present, the GT64 glycosyltransferase family has been studied in animals, including humans and mice and the human GT64 members are also known as the Exostosio family, consisting of five members, respectively exostosin 1 (EXT1), exostosin 2 (EXT2) and exostosin-like 1-3 (EXTL1-3), which play an important role in the synthesis of heparan sulfate (Wilson *et al.,* 2022). Research has demonstrated that heparan sulfate is widely distributed and structurally similar in animal tissues. Additionally, heparan sulfate has a variety of biological activities and functions, including cell adhesion, regulation of cell growth and proliferation, developmental processes, blood coagulation and tumor metastasis (Rabenstein, 2002). Whether this has any impact on the growth and reproduction of the calves still needs further study.

LEfSe analysis of different function between groups

LEfSe analyses revealed that the LDA score of Amino acid transport and metabolism from the eggNOG database at level 1 was significantly different in Group B. The LDA scores of CE1 and GH29 from CAZy families at level 2 were much higher in Group B, while GH24, GH13, CBM20 and GH31 had higher scores in Group A (Fig 7).

GH24 is one of the glycoside hydrolase family which includes a lysozyme gene firstly found in chicken egg white (Blake *et al.,* 1967). SKQ may show a potential antimicrobial activity by upregulation of lysozyme. The GH13 family contains nearly 30 enzymes that are involved in starch degradation, hydrolyzing glycosidic bonds, polysaccharide degradation and glycosyl transfer. The GH31 family contains enzymes to hydrolyze oligosaccharides as well as to transfer mannose, which can cleave glucosidic bonds of glucose, galactose and mannose (Brun *et al.,* 2020). CBM20 contains starch-binding domains (SBD) that can

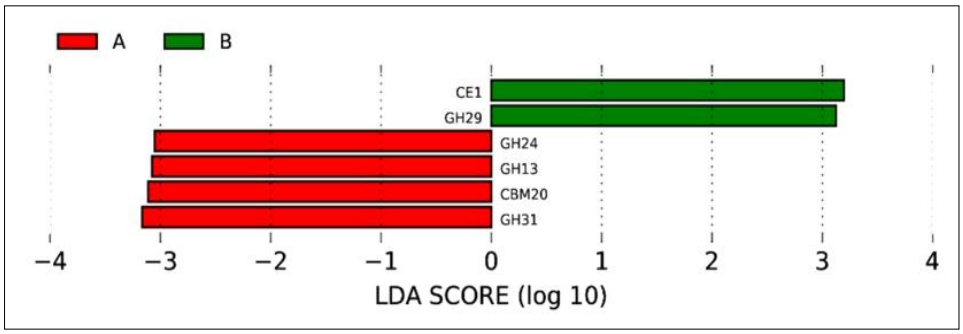


Fig 7: Different functions between groups by LEfSe analysis *via* LDA scores.

interact with cyclodextrins (Jia *et al.,* 2017). It has been reported that CBM20 can boost catalytic efficiency combined with cyclodextrin glycosyltransferase.

The GH29 family shows its ability to hydrolyze practically all fucose-containing compounds *via* hemicellulolytic and a-fucosidase activity (Curci *et al.,* 2021). The CE1 family contains acetyl xylan esterase, cinnamoyl esterase and feruloyl esterase, which are associated with cellulose and polysaccharide degradation and lipid metabolism (Lombard *et al.,* 2014).

CONCLUSION

From the analysis of metagenomic sequencing, the abundance of beneficial bacteria in the experimental group increased (such as *Prevotella* and *Bacteroides*) and the abundance of inflammatory bacteria decreased (such as genus *Alistipes* and *Angelakisella*), suggesting a potential shift toward an anti-inflammatory microbial profile. At the same time, drug resistance and pathogenic microorganisms increased in the control group, such as *Elizabethkingia, Klebsiella, Pseudoxanthomonas,* which in turn caused changes in the contents of various carbohydrate active enzymes, especially the contents of sugar-degrading enzymes. This suggests that the supplementation of Chinese herbal additive Shikuqin may enhance the degradation of dietary carbohydrates, which could promote animal protein synthesis and potentially improve immune function, indicating that the degradation of sugar after the addition of Chinese herbal additive Shikuqin may promote animal protein synthesis and improve host immunity.

ACKNOWLEDGEMENT

The present study was supported by Beef Cattle Modern Agricultural Technology System of Guizhou Province (No.GZRNCYJSTX-05), the Science and Technology Support Program of Guizhou Province (Research Project No. QKHZC-2023-021), Guizhou Provincial Key Laboratory of Livestock and Poultry Genetic Resources Innovation and Utilization (ZSYS[2025]034).

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Ali, A., Liaqat, S., Tariq, H., Abbas, S., Arshad, M., Li, W.J. and Ahmed, I. (2021). Neonatal calf diarrhea: A potent reservoir of multi-drug resistant bacteria, environmental contamination and public health hazard in Pakistan. *The Science of the Total Environment*. **799:** 149450. doi: 10.1016/j.scitotenv.2021.149450.

Blake, C.C., Johnson, L.N., Mair, G.A., North, A.C., Phillips, D.C. and Sarma, V.R. (1967). Crystallographic studies of the activity of hen egg-white lysozyme. *Proceedings of the Royal Society of London Series B, Biological Sciences*. **167 (1009):** 378-388. doi: 10.1098/rspb.1967.0035.

Brun, A., Mendez-Aranda, D., Magallanes, M.E., Karasov, W.H., Martínez, Del Rio, C., Baldwin, M.W. and Caviedes-Vidal, E. (2020). Duplications and functional convergence of intestinal carbohydrate-digesting enzymes. *Molecular Biology and Evolution*. **37(6):** 1657-1666. doi: 10.1093/molbev/msaa034.

Chai, M., Wang, L., Li, X., Zhao, J., Zhang, H., Wang, G. and Chen, W. (2021). Different *Bifidobacterium bifidum* strains change the intestinal flora composition of mice *via* different mechanisms to alleviate loperamide-induced constipation. *Food and function*. **12(13):** 6058-6069. doi: 10.1039/d1fo00559f.

Chen, P., Liu, J., Ma, B., Li, Z., Qin, Q. and Wu, H. (2024). Efficacy of chinese and western medicine on endometritis in dairy cows: A systematic review and meta-analysis. *Indian Journal of Animal Research.* **58(7):** 1081-1087. doi: 10.18805/IJAR.BF-1648.

Cho, Y.I. and Yoon K.J. (2014). An overview of calf diarrhea - infectious etiology, diagnosis and intervention. *Journal of Veterinary Science*. **15(1):** 1-17. doi: 10.4142/jvs.2014.15.1.1.

Curci, N., Strazzulli, A., Iacono, R., De Lise, F., Maurelli, L., Di Fenza, M., Cobucci-Ponzano, B. and Moracci, M. (2021). Xyloglucan oligosaccharides hydrolysis by Exo-acting glycoside hydrolases from hyperthermophilic microorganism *Saccharolobus solfataricus*. *International Journal of Molecular Sciences*. **22(7): 3325.** doi: 10.3390/ijms22073325.

Guo, B.B., Wu, J.P., Chen, J.W., Zhang, H. and Li, J.J. (2022). Effects of Chinese medicine herbal residues on antibiotic resistance genes and the bacterial community in chicken manure composting. *The Journal of Antibiotics*. **75(3):** 164-171. doi: 10.1038/s41429-022-00505-x.

Guo, S., Guo, T., Cheng, N., Liu, Q., Zhang, Y., Bai, L., Zhang, L., Cao, W., Ho, C.T. and Bai, N. (2017). Hepatoprotective standardized EtOH-water extract from the seeds of *Fraxinus rhynchophylla* Hance. *Journal of Traditional and Complementary Medicine*. **7(2):** 158-164. doi: 10.1016/j.jtcme.2016.05.001.

He, X., Fang, J., Huang, L., Wang, J. and Huang, X. (2015). *Sophora flavescens* Ait: Traditional usage, phytochemistry and pharmacology of an important traditional Chinese medicine. *Journal of Ethnopharmacology*. **172:** 10-29. doi: 10.1016/j.jep.2015.06.010.

Jia, X., Guo, Y., Lin, X., You, M., Lin, C., Chen, L. and Chen, J. (2017). Fusion of a family 20 carbohydrate-binding module (CBM20) with cyclodextrin glycosyltransferase of *Geobacillus* sp. CHB1 improves catalytic efficiency. *Journal of Basic Microbiology*. **57(6):** 471-480. doi: 10.1002/jobm.201600628.

Li, S., Wang, X., Lu, Y., Wang, J., Yu, D., Zhou, Z., Wei, J., Liu, L., Liu, J., Liu, F., Su, Y. and Xu, H. (2023). Co-infections of *Klebsiella pneumoniae* and *Elizabethkingia miricola* in black-spotted frogs (Pelophylax nigromaculatus). *Microbial Pathogenesis*. **180:** 106150. doi: 10.1016/j.micpath.2023.106150.

Lombard, V., Golaconda, R.H., Drula, E., Coutinho, P.M., Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic acids research*. 42(Database issue): D490-5. doi: 10.1093/nar/gkt1178.

Millar, B.C., Rao J.R., Moore J.E. (2021). Fighting antimicrobial resistance (AMR): Chinese herbal medicine as a source of novel antimicrobials - an update. *Letters in Applied Microbiology*. **73(4):** 400-407. doi: 10.1111/lam.13534.

Ngeleka, M., Godson, D., Vanier, G., Desmarais, G., Wojnarowicz, C., Sayi, S., Huang, Y., Movasseghi, R. and Fairbrother, J.M. (2019). Frequency of *Escherichia coli* virotypes in calf diarrhea and intestinal morphologic changes associated with these virotypes or other diarrheagenic pathogens. *Journal of Veterinary Diagnostic Investigation: Official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*. **31(4):** 611-615. doi: 10.1177/1040638719857783.

Pansri, P., Svensmark, B., Liu, G., Thamsborg, S.M., Kudirkiene, E., Nielsen, H.V., Goecke, N.B. and Olsen, J.E. (2022). Evaluation of a novel multiplex qPCR method for rapid detection and quantification of pathogens associated with calf diarrhoea. *Journal of Applied Microbiology*. **133(4):** 2516-2527. doi: 10.1111/jam.15722.

Qu, J.C., Meng, C., Li, L.Y., Zhu, H. and Yin, G.A. (2023). Epidemiology of diarrhea for Chinese dairy calves: A systematic review and meta-analysis. *Indian Journal of Animal Research*. **57(2):** 139-146. doi: 10.18805/IJAR.BF-1549.

Parker, B.J., Wearsch, P.A., Veloo, A.C.M. and Rodriguez-Palacios, A. (2020). The genus alistipes: Gut bacteria with emerging implications to inflammation, cancer and mental health. *Frontiers in Immunology*. **11:** 906. doi: 10.3389/fimmu.2020.00906.

Rabenstein, D.L. (2002). Heparin and heparan sulfate: Structure and function. *Natural Product Reports*. **19(3):** 312-331. doi: 10.1039/b100916h.

Rajamani, P., Bagthasingh, C., Panchavarnam, S., Rani, V., Shalini, R., Amirtharaj, Vijay, K.S. and David, P.E. (2026). Pomegranate rind-assisted green synthesis of ZnO nanoparticles: Characterization, antioxidant activity and antibacterial potential against bacterial fish disease. *Indian Journal of Animal Research*. **59:** 95-102. doi: 10.18805/IJAR.B-5684.

Rodríguez-Medina, N., Barrios-Camacho, H., Duran-Bedolla, J. and Garza-Ramos, U. (2019). *Klebsiella variicola*: An emerging pathogen in humans. *Emerging Microbes and Infections*. **8(1):** 973-988. doi: 10.1080/22221751.2019.1634981.

Segata, N., Izard J., Waldron L., Gevers D., Miropolsky L., Garrett W.S. and Huttenhower C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*. **12(6):** R60. doi: 10.1186/gb-2011-12-6-r60.

Shukla, S., Nayak, A., Sharma, R.K., Shukla, P.C. and Singh, R.V. (2022). Bacteriophages: A potential new therapeutic alternate to antibiotics to treat chronic septic wounds in large animals. *Indian Journal of Animal Research.* **56(10):** 1279-1282. doi: 10.18805/IJAR.B-4317.

White, J.R., Nagarajan, N. and Pop, M. (2009). Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Computational Biology*. **5(4):** e1000352. doi: 10.1371/journal.pcbi.1000352.

Wilson, L.F.L., Dendooven, T., Hardwick, S.W., Echevarría-Poza, A., Tryfona T., Krogh, K., Chirgadze, D.Y., Luisi, B.F., Logan, D.T., Mani, K. and Dupree, P. (2022). The structure of EXTL3 helps to explain the different roles of bi-domain exostosins in heparan sulfate synthesis. *Nature Communications*. **13(1):** 3314. doi: 10.1038/s41467-022-31048-2.

Wyres, K.L. and Holt K.E. (2016). Klebsiella pneumoniae Population Genomics and Antimicrobial-Resistant Clones. *Trends in Microbiology*. **24(12):** 944-956. doi: 10.1016/j.tim.2016.09.007.

Yassin, M.T., Mostafa A.A. and Al Askar A.A. (2021). *In vitro* evaluation of biological activities and phytochemical analysis of different solvent extracts of *Punica granatum* L. (Pomegranate) Peels. *Plants (Basel, Switzerland)*. **10(12).** doi: 10.3390/plants10122742.

Yu, B., Chen, X., Jiang, L., Shang, Y., Song, X., Zhou, S., Xu, J., Yu, J., Fu, Q., Peng, S., Liu, S., Yang, Y. and Yin, Z. (2018). Acute and subchronic toxicity and the evaluation of safety pharmacology of Chinese herbal compound preparation "Shikuqin". *Pakistan Journal of Pharmaceutical Sciences*. **31(6(Special)):** 2855-2862.

Yu, B., Yu, J., Jiang, L., Chen, X., Song, X., Zhou, S., Xu, J., Wu, J., Tu, Z., Song, Z., Yin, Z. and Shang, Y. (2019). Antibacterial, antidiarrheal, anti-inflammatory and analgesic activities of compound Shikuqin powder. *Pakistan Journal of Pharmaceutical Sciences*. **32(3Special):** 1333-1342.