Research Journal of Agriculture
Volume 14, Number 6; June-2023;
ISSN: 2836-6050 | Impact Factor: 6.58
https://zapjournals.com/Journals/index.php/rja/index
Published By: Zendo Academic Publishing

INVERTEBRATE-DERIVED ENZYMES IN FOCUS: ARAZYME'S JOURNEY INTO ANIMAL NUTRITION

[1]Lee JW, [2]Yeom EK and [3]Choi YJ

Article Info

Keywords: Arazyme, invertebrate-derived enzymes, gut symbionts, bioactive molecules, and animal husbandry

Abstract

Enzymes derived from the gut symbionts of invertebrates have emerged as a promising and relatively unexplored resource in the realm of biologically active molecules. These enzymes play a pivotal role in facilitating the efficient utilization of a diverse array of nutrient resources by their invertebrate hosts (Jing et al., 2020). Consequently, the field of animal husbandry has exhibited substantial interest in harnessing these invertebrate-derived bioactive enzymes to enhance various aspects of animal nutrition and health (Kannan et al., 2019). One such intriguing enzyme is Arazyme, an alkaline metalloprotease secreted by Serratia proteamaculans, a gram-negative aerobic bacterium belonging to the genus Serratia. Isolated from the intestinal ecosystem of Nephila clavate (Fig. 1), a remarkable arachnid known as "棒络新妇" in Chinese and "무당거미" in Korean (Bersanetti et al., 2005; Kwak et al., 2007), Arazyme plays a crucial role in facilitating the digestion process within Nephila clavata. This enzyme, with a molecular size of 51.5 kDa, has even gained approval as a food additive (registration No. 2007-SA-0007) from the Ministry of Food and Drug Safety in the Republic of Korea. Significantly, Arazyme exhibits exceptional enzymatic activity across a wide pH and temperature range and displays impressive resistance to detergents, enhancing its potential for industrial applications (Bersanetti et al., 2005). As an innovative invertebrate-derived bioactive enzyme, Arazyme holds immense promise in the domain of animal husbandry. This manuscript presents a comprehensive review of the current understanding and knowledge concerning Arazyme, with the primary aim of providing valuable insights and references for its prospective applications. The versatile enzymatic properties of Arazyme open doors to a myriad of potential applications, ranging from animal feed supplementation to biotechnological advancements. This review explores the characteristics, mechanisms, and potential applications of Arazyme, shedding light on its multifaceted utility in various industries. Through a comprehensive synthesis of existing research, this review paves the

[1]College of Animal Science and Veterinary Medicine, Jinzhou Medical University, Jinzhou 121001, China
[2]Liaoning Kaiwei Biotechnology Co., Ltd., Jinzhou 121000, China
[3]Zhongke Gene Detection Service Co., Ltd., Jinzhou 121219, China

way for future investigations and innovations, encouraging further exploration of the diverse functionalities and applications of this remarkable invertebrate-derived enzyme.

Introduction

Biologically active enzymes derived from invertebrate-associated gut symbionts represent a promising and underexplored resource. These enzymes play a crucial role in the efficient utilization of diverse nutrient resources by invertebrates (Jing et al., 2020). Consequently, animal husbandry has shown considerable interest in harnessing these invertebrate-derived bioactive enzymes (Kannan et al., 2019).

Arazyme, an alkaline metalloprotease, is secreted by *Serratia proteamaculans*, a gram-negative aerobic bacterium belonging to the genus Serratia. It is a symbiotic bacterium isolated from the intestinal ecosystem of *Nephila clavate* (Fig. 1), a species of arachnid known as "棒络新妇" in Chinese and "무당거미" in Korean (Bersanetti et al., 2005; Kwak et al., 2007). Arazyme plays a pivotal role in facilitating the digestion process in *Nephila clavata*. With a size of 51.5 kDa, arazyme has been approved as a food additive (registration No. 2007-SA-0007) by the Ministry of Food and Drug Safety in the Republic of Korea. Notably, it exhibits remarkable enzymatic activity over a wide range of pH and temperature and displays resistance to detergents (Bersanetti et al., 2005).

As a novel invertebrate-derived bioactive enzyme, arazyme holds broad application prospects in animal husbandry. This manuscript provided a review of the current knowledge and understanding of arazyme, aiming to offer valuable references for its potential applications.

Characteristic of arazyme

Arazyme, an enzyme with zinc-containing metalloprotease activity, relies on the presence of zinc for its function (Kwak et al., 2007). Its deduced amino acid sequence exhibits significant homology to serralysins found in other enteric bacteria (Kwak et al., 2007). Arazyme acts as a proteolytic enzyme, capable of hydrolyzing substances such as albumin, casein, elastin, hemoglobin, keratin, and collagen (Piao et al., 2009). Notably, its proteolytic activity remains unaffected by inhibitors of aspartate, cysteine, and serine proteases, as well as pepsin, trypsin, and chymotrypsin. However, its activity is strongly inhibited by compounds like 1,10phenanthroline and ethylenediaminetetraacetic acid (Bersanetti et al., 2005; Kwak et al., 2007). Furthermore, arazyme demonstrates optimal enzymatic activity at pH levels of 6.0 or higher and can function efficiently at temperatures up to 50°C (Bersanetti et al., 2005).

Immunoregulatory property

Arazyme has been extensively studied for its immunoregulatory properties. Pereira et al. (2016) demonstrated that *in vivo* injection of both active and heat-inactivated arazyme exhibited significant effects in reducing tumor development, including primary and metastatic tumors. These effects were attributed to the activation of toll-like receptor 4, leading to increased levels of interferon gamma and elevated counts of activated $CD8^+$ T lymphocytes. In a separate study, Pereira et al. (2014) found that intraperitoneal administration of arazyme exerted a dose-dependent cytostatic effect on human and murine tumor cells by reducing the expression of CD44 molecules on the tumor cell surface, thereby interfering with cell adhesion. Additionally, arazyme induced the production of protease-specific immunoglobulin G, which exhibited cross-reactivity with tumor matrix metalloproteinase-8. Ghadaksaz et al. (2022) developed a fusion protein, Arazyme-linker-TGFαL3, with high affinity for the epidermal growth factor receptor. In silico immune simulation demonstrated that this chimera protein has the potential to prevent cancer development by inducing an immune response and inhibiting cell proliferation. Similarly, Rahmani et al. (2023) designed a fusion protein, Arazyme-linker-herceptinHER2, which showed promising results as a

candidate for breast cancer treatment. In silico immune simulation demonstrated that the predicted B cell and T cell epitopes of the fusion protein were capable of eliciting an immune response.

Overall, the mechanism of arazyme's immunoregulatory properties involves toll-like receptor 4 activation, increased interferon gamma levels, activation of $CD8^+$ T lymphocytes, reduction of CD44 molecules, and production of specific immunoglobulins. These studies collectively highlight the immunomodulatory potential of arazyme and its ability to impact immune responses, offering promising prospects for its application in immunotherapy.

Anti-inflammatory property

Bersanetti et al. (2005) observed that arazyme exhibited high hydrolytic activity on substance P, which is secreted by inflammatory cells, as well as peptides related to bradykinin, during *in vitro* co-culture. Additionally, Kim et al. (2015) conducted studies demonstrating that oral treatment of arazyme attenuated the development of atopic dermatitis-like lesions in mice induced by 2,4-Dinitrochlorobenzene. This attenuation was achieved through the reduction of epidermal thickening, infiltration of inflammatory cells into the dermis, and suppression of interleukin 4, interleukin 13, and immunoglobulin E levels. In another study by Kim et al. (2013), THP-1 human monocytic and EoL-1 human eosinophilic cells were treated *in vitro* with *Dermatophagoides pteronyssinus* extract and administered with arazyme. The researchers observed that arazyme alleviated the severity of atopic dermatitis by regulating the expression of thymus and activation-regulated chemokine and skin barrier proteins in keratinocytes. They found that arazyme inhibited the production of monocyte chemoattractant protein 1, interleukin 6, and interleukin 8 in THP-1 and EoL-1 cells. Furthermore, arazyme suppressed the secretion of interleukin 6 and interleukin 8 in HMC-1 cells, reduced thymus and activation-regulated chemokine, monocyte chemoattractant protein 1, interleukin 6, and interleukin 8 levels in HaCaT cells, and upregulated the production of filaggrin, involucrin, and loricrin in HaCaT cells. Additionally, Kim et al. (2014) observed that arazyme administration inhibited lipopolysaccharide-induced apoptosis in human umbilical vein endothelial cells *in vitro*. This inhibition was attributed to the suppression of monocyte chemoattractant protein 1 and interleukin 6 secretion, as well as the downregulation of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 expression, and reduction in reactive oxygen species production. Kim and Lee (2014) investigated the inhibitory effects of arazyme on neutrophil apoptosis in allergic diseases, specifically allergic rhinitis and asthma, *in vitro*. They discovered that arazyme effectively inhibits neutrophil apoptosis through the PI3K/Akt/ERK/NF-κB pathway and the caspase 9/3 pathway. In the context of animal health, providing arazymecontaining diet has shown potential in reducing the occurrence of subclinical mastitis in dairy cows (Liu et al., 2007). This reduction is manifested by a decrease in somatic cell count in milk.

In summary, the mechanism underlying arazyme's anti-inflammatory property involves multiple potential pathways. Arazyme may interact with protease-activated receptors and exhibit high hydrolytic activity on specific peptides. Additionally, it regulates the production of pro-inflammatory cytokines and chemokines and protects against apoptosis and oxidative stress. Although more research is needed to fully understand the precise molecular mechanisms, these findings collectively suggest that arazyme acts as an anti-inflammatory agent through various mechanisms, making it a promising candidate for inflammation regulation.

Anti-bacterial property

In a study conducted by Kim et al. (2021), it was found that arazyme at a concentration of 250 mg/mL exhibited remarkable anti-bacterial properties *in vitro*. The researchers observed that arazyme inhibited the growth of oral opportunistic pathogens such as *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, and *Streptococcus mutans*. Notably, arazyme demonstrated over 80% inhibition against *C. albicans*, which is a major

contributor to denture stomatitis. These findings highlight the potential of arazyme as an effective agent for controlling harmful bacterial growth and preventing oral infections associated with denture use.

However, it is important to note that while this study demonstrated the anti-bacterial properties of arazyme, further research is necessary to elucidate the underlying mechanisms by which arazyme exerts its effects on bacterial growth. Future experiments can help unravel the specific molecular interactions and pathways involved in the antibacterial activity of arazyme, providing a deeper understanding of its potential applications in controlling bacterial infections.

Organ protection property

In the study conducted by Park et al. (2008), the effects of oral arazyme on acute hepatic injury induced by CCl4 in mice were investigated. The findings revealed that arazyme plays a protective role by upregulating the expression of antioxidative proteins, inhibiting the TGF-β/Smad3 signaling pathway through downregulation of Smad3 and p-Smad3 expression levels, and preventing the decrease of SMP30 expression. These results suggest that arazyme has the potential to protect hepatocytes from chemokineinduced hepatic damage. In a separate study by Li et al. (2019), the hepatoprotective effects of oral arazyme in high-fat diet-induced non-alcoholic fatty liver disease-like mice were examined. The administration of arazyme demonstrated protective effects against hepatic steatosis and hepatitis, and inhibited the progression of non-alcoholic fatty liver disease. Arazyme exerted its hepatoprotective effects by inhibiting hepatic fatty acid and triglyceride synthesis, suppressing SREBP-1-mediated lipid accumulation and macrophage-mediated inflammation, and reducing palmitic acid-induced lipogenesis in HepG2 hepatocytes. Moreover, arazyme reduced macrophage recruitment by inhibiting the expression of inflammatory cytokines in the liver. Li et al. (2016) further investigated the hepatoprotective effects of oral arazyme in high-fat diet-induced non-alcoholic fatty liver diseaselike mice. The supplementation of arazyme in the diet exhibited hepatoprotective effects by decreasing hepatic lipid accumulation and improving insulin resistance. Arazyme supplementation resulted in reduced plasma levels of alanine aminotransferase, thyroglobulin, nonesterified fatty acids, glucose, and hemoglobin A1C. Additionally, arazyme improved hepatic steatosis and fibrosis, decreased hepatic triglyceride and total cholesterol contents, improved glucose tolerance, and reduced pancreatic insulin contents and islet size. In cell studies, arazyme increased AKT phosphorylation in palmitic acid-induced HepG2 cells, improved insulin secretion and synthesis in pancreatic MIN6 β-cells, and reduced hepatic lipid accumulation while reversing insulin resistance.

Overall, oral arazyme administration is able to upregulate antioxidative proteins, inhibit the TGF-β/Smad3 signaling pathway, prevent the decrease of SMP30 expression, inhibit hepatic fatty acid and triglyceride synthesis, enhance AKT phosphorylation, and improve insulin resistance. These mechanisms collectively contribute to the hepatoprotective effects of arazyme, ultimately improving liver function in various liver injury and non-alcoholic fatty liver disease models.

Application of arazyme in feedstuff industry

The feed industry can extract several benefits from the use of arazyme. Firstly, arazyme, as a proteolytic enzyme, has the ability to hydrolyze proteins in feedstuff materials (Piao et al., 2009). This enzymatic hydrolysis results in increased levels of digestible small molecular substances, particularly amino acids and peptides, present in the ingredient. Zi et al. (2011) conducted an enzymatic hydrolysis study on soybean meal using a combination of 0.5% phytase and 0.02% arazyme, which resulted in increased levels of free cysteine, aspartic acid, threonine, methionine, and leucine in the hydrolyzed soybean meal. Kim (2009) reported that when arazyme was applied to hydrolyze corn gluten meal, it led to elevated levels of free leucine. Similarly, hydrolysis of soybean meal with arazyme resulted in increased level of free phenylalanine, valine, leucine, and isoleucine (Kim, 2009). Furthermore, the hydrolysis of distiller's dried grains with solubles using arazyme increased the contents of free

cysteine, valine, and isoleucine (Kim, 2009). Fish meal hydrolyzed with arazyme also exhibited increased level of free cysteine (Kim, 2009). Piao et al. (2009) note that when arazyme was applied to hydrolyze meat meal, fish meal, soybean meal, cottonseed meal, rapeseed meal, corn flour, and corn gluten meal, there were notable increases in the contents of both essential and non-essential free amino acids, particularly lysine, threonine, and tryptophan. Additionally, Liang and Piao (2010) found that arazyme hydrolysis of black rice bran resulted in increased free peptide content. By enhancing the free amino acid profiles of feedstuff materials, arazyme can improve the nutritional value of animal diets.

Additionally, the utilization of arazyme can help optimize feed formulations by providing a cost-effective alternative to expensive protein sources, such as plasma protein powder. Guan and Sun (2010) demonstrated that replacing 4% plasma protein powder in the diet with 0.1% arazyme and 3% fish meal effectively reduced feed costs without compromising growth performance.

Overall, the use of arazyme in the feed industry offers benefits such as improved feedstuffs free amino acid profiles and cost savings in feed formulation. These advantages contribute to the production of high-quality and cost-effective animal feeds, ultimately benefiting both feed manufacturers and livestock producers.

Application of arazyme in animal husbandry

In this manuscript, we conducted a review of scientific studies investigating the effects of dietary arazyme supplementation in various animal species, including poultry (Table 1), swine (Table 2), ruminant (Table 3), and aquatic animals (Table 4). The results of these studies consistently demonstrate the positive effect of incorporating arazyme into the diet of farming animals, leading to improvements in their growth and production performance.

Specifically, in poultry husbandry, the supplementation of arazyme to the diet has been shown to improve performance and reduce excreta noxious gas emissions in both broilers and layers (Kim et al., 2009; Kim, 2009). Similarly, in swine husbandry, arazyme supplementation has been found to enhance the efficiency of nutrient utilization, leading to improved growth performance, carcass traits, meat quality, nutritional value of pork, and offspring qualit (Ju et al., 2010; Wen, 2005; Song et al., 2022; Qu et al., 2009; Wang et al., 2009; Xu et al., 2009). In ruminant husbandry, the inclusion of arazyme has shown promising results in improving rumen fermentation, ultimately translating into improved milk quality, milk production, and growth performance (Wang, 2019; Zhang and Sun, 2009). Furthermore, in the context of aquaculture, the addition of arazyme to fish and shrimp diets has demonstrated beneficial effects on nutrient digestibility, antioxidant capacity, and growth performance (Wu et al., 2007; Xie et al., 2009).

The consistent positive outcomes observed across these studies highlight the potential of arazyme as a valuable tool in animal nutrition. The ability of arazyme to enhance growth and production performance in various species underscores its importance as a supplement in animal feed formulations. Further research and exploration of optimal dosage levels and other delivery methods are warranted to fully harness the benefits of arazyme in animal husbandry.

Conclusion

In conclusion, arazyme, a biologically active enzyme derived from invertebrate-associated gut symbionts, holds significant potential for various applications. It exhibits remarkable enzymatic activity over a wide range of pH and temperature, making it suitable for use in different conditions. Arazyme has been extensively studied for its immunoregulatory, anti-inflammatory, antibacterial, and organ protection properties. In the feedstuff industry, arazyme has been proven effective in hydrolyzing proteins in feed materials, leading to enhanced free amino acid profiles and peptide contents. This offers opportunities to improve the nutritional value of feed formulations and reduce feed costs. In animal husbandry, arazyme has consistently shown positive effects on growth and production performance in poultry, swine, ruminants, and aquatic animals. It improves nutrient utilization, intestinal health,

and meat quality, while reducing noxious gas emission. These findings emphasize the potential of arazyme as a valuable tool in an imal nutrition.

Overall, the comprehensive review of arazyme presented in this manuscript provides valuable references for its potential applications. Further research is warranted to explore optimal dosage levels, delivery methods, and underlying mechanisms of action.

Animal Welfare Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adh ered to. No ethical approval was required as this is a review article with no original research data.

Author Contributions Statement

DX Dang, WS Meng, SH Li: Writing - original draft, Writing - review & editing. T Wang, D Li: Methodology, Supervision, Writing - review & editing.

Conflict of Interest

I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Data Availability Statement

No new data were generated or analyzed in support of this research.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Bersanetti PA, Park HY, Bae KS, Son KH, Shin DH, Hirata IY, Juliano MA, Carmona AK and Juliano L (2005)

Characterization of arazyme, an exocellular metalloprotease isolated from Serratia proteamaculans culture medium. *Enzyme and Microbial Technology* 37, 574-581.

Guan J and Sun G (2010) A preliminary investigation on the effects of spider enzyme substitution for plasma protein powder in creep feed. *Feed and Livestock* 5, 44-46.

Ghadaksaz A, Imani Fooladi AA, Mahmoodzadeh HH, Nejad ST and Amin M (2022) Targeting the EGFR in cancer cells by fusion protein consisting of arazyme and third loop of TGF-alpha: an in silico study. *Journal of Biomolecular Structure and Dynamics* 40, 11744-11757.

Ju B, Shen D and Zhang Z (2010) The effects of spider enzyme on nutrient digestibility in pig diets and pork quality. *Feed and Livestock* 2, 48-51.

Jing TZ, Qi FH and Wang ZY (2020) Most dominant roles of insect gut bacteria: digestion, detoxification, or essential nutrient provision?. *Microbiome* 8, 1-20.

Kwak JY, Lee KE, Shin DH, Maeng JS, Park DS, Oh HW, Son KH, Bae KS and Park HY (2007) Biochemical and genetic characterization of arazyme, an extracellular metalloprotease produced from Serratia proteamaculans HY-3. *Journal of microbiology and biotechnology* 17, 761-768.

Kim JU, Kim JY, Kim JS, Lee BK, Lee SY, Lee WS, You SJ, Ahn BK, Kim EJ, Park HY, Son KH, Shin DH and Kang CW (2009) Effects of spider-derived protease (Arazyme®) supplementation of corn-soy diets on the performance in laying hens at the late production. *Korean Journal of Poultry Science* 36, 47-55.

Kim J (2009) Effects of Spider-Derived Protease (Arazyme®) Supplementation of Corn-Soy Diets on the Performance in Layer and Broiler Chickens. Dissertation, Graduate School of Konkuk University.

Kim IS, Kim MJ, Shin DH, Son KH, Park HY and Lee JS (2013) Arazyme inhibits cytokine expression and upregulates skin barrier protein expression. *Molecular Medicine Reports* 8, 551-556.

Kim IS, Yang EJ, Shin DH, Son KH, Park HY and Lee JS (2014) Effect of arazyme on the lipopolysaccharide-induced inflammatory response in human endothelial cells. *Molecular Medicine Reports* 10, 1025-1029.

Kim IS and Lee JS (2014) Suppressive effect of arazyme on neutrophil apoptosis in normal and allergic subjects. *Biomedical Science Letters* 20, 244-249.

Kim IS, Lee NR, Baek SY, Kim EJ, Kim JS, Jeong TS, Shin DH, Park HY and Lee JS (2015) Inhibitory effect of arazyme on the development of atopic dermatitis-like lesions in BALB/c and Nc/Nga mice. *Molecular Medicine Reports* 11, 3995-4001.

Kannan M, Mubarakali D, Thiyonila B, Krishnan M, Padmanaban B and Shantkriti S (2019) Insect gut as a bioresource for potential enzymes-an unexploited area for industrial biotechnology. *Biocatalysis and Agricultural Biotechnology* 18, 101010.

Kim JH, Lee HN, Bae SK, Shin DH, Ku BH, Park HY and Jeong TS (2021) Development of a novel denture care agent with highly active enzyme, arazyme. *BMC Oral Health* 21, 1-9.

Liu D, Cheng Y, Lu D and Jin X (2007) Effects of Highly Active Biological Protease (Power-cell) on Production Performance of Dairy Cows Suffering from Subclinical Mastitis. *Chinese journal of animal science* 34, 81-83.

Liang D and Piao M (2010) Response surface optimization of Arazyme protease for the enzymatic hydrolysis of black rice bran. *Cereals and oils processing* 9, 119-122.

Li H, Park HM, Bak J, Lim SY, Shin DH, Park HY and Jeong TS (2016) Arazyme Ameliorates Hepatic Steatosis and Insulin Resistance in Diet-induced Non-alcoholic Fatty Liver Disease Mouse Model. *Proceedings of the Annual Conference of the Korean Society of Food Science and Nutrition* 1, 420.

Li H, Yoo W, Park HM, Lim SY, Shin DH, Kim S, Park HY and Jeong TS (2019) Arazyme suppresses hepatic steatosis and steatohepatitis in diet-induced non-alcoholic fatty liver disease-like mouse model. *International Journal of Molecular Sciences* 20, 2325.

Park JK, Jeong DH, Park HY, Son KH, Shin DH, Do SH, Yang HJ, Yuan DW, Hong IW, Goo MJ, Lee HR, Ki MR, Ishigami A and Jeong KS (2008) Hepatoprotective effect of Arazyme on CCl4-induced acute hepatic injury in SMP30 knock-out mice. *Toxicology* 246, 132-142.

Piao M, Shen D, Sun G, Piao G and Zhang Z (2009) Research on the proteolytic efficacy of spider protease on protein degradation in animal and plant feedstock materials. *Feed and Livestock* 10, 51-54.

Pereira FV, Ferreira-Guimaraes CA, Paschoalin T, Scutti JA, Melo FM, Silva LS, Melo ACL, Silva P, Tiago M, Matsuo AL, Juliano L, Juliano MA, Carmona AK, Travassos LR and Rodrigues EG (2014) A natural bacterial-derived product, the metalloprotease arazyme, inhibits metastatic murine melanoma by inducing MMP-8 cross-reactive antibodies. *PloS one* 9, e96141.

Pereira FV, Melo AC, de Melo FM, Mourão-Sá D, Silva P, Berzaghi R, Herbozo CCA, Coelho-dos-Reis J, Scutti JA, Origassa CST, Pereira RM, Juliano L, Juliano MA, Carmona AK, Câmara NOS, Tsuji M, Travassos LR and

Rodrigues EG (2016) TLR4-mediated immunomodulatory properties of the bacterial metalloprotease arazyme in preclinical tumor models. *Oncoimmunology* 5, e1178420.

Qu N, Li Y and Guan J (2009) The impact of spider enzyme on the production performance of sows and piglets. *Feed and Livestock* 9, 52-54.

Rahmani F, Imani FAA, Ajoudanifar H and Soleimani NA (2023) In silico and experimental methods for designing a potent anticancer arazyme-herceptin fusion protein in HER2-positive breast cancer. Journal of Molecular Modeling 29, 160.

Song C, Li C, Liu Y, Qi K, Dou Y and Zheng Z (2022) Effects of Arazyme on Reproductive Performance of Pregnant Sows and Growth Performance of Nursery Piglets. *Feed industry* 43, 36-39.

Wen Y (2005) Effect of metalloprotease on piglets productivity. Dissertation, Yanbian University.

Wu L, Xie J, Wang G, Yu D, Hu C and Niu J (2007) Effect of the metalloprotease on growth performance digestibility and nonspecific immune of hybrid tilapia Oreochromis niloticus × O. aureus. *South China Fisheries Science* 3, 8-13.

Wang H, Li H, Lin J and Chen J (2009) The effects of spider enzyme on the production performance of sows. *Feed and Livestock* 3, 45-47.

Wang M (2019) Application of spider enzymes in ruminants. Dissertation Henan Agricultural University.

Xu Y, Gong J, Zhang Z, Duan G and Fan Z (2009) The impact of spider enzyme on the production performance of piglets. *Feed and Livestock* 4, 56-58

Xie J, Yu D, Wang G, Wu L, Hu C and Guan S (2009) The effects of adding spider enzyme to feed on the growth performance, non-specific immune response, and aquaculture water environment of the Pacific white shrimp (Litopenaeus vannamei). *Feed and Livestock* 7, 52-54.

Zhang S and Sun G (2009) The effects of Kangnaibao (Power-cell) on the production performance of dairy cows. *Proceedings of the China Dairy Association 2009 Conference* 1, 148-149.

Zi PM, Kim ID, Shin DH, Lee JW, Yeom EK and Choi YJ (2011) Consecutive treatment with phytase and arazyme influence protein hydrolysis of soybean meal. *African Journal of Biotechnology* 10, 7868-7873