Veterinary & Life Science Innovations

Veterinary & Life Science Innovations 2025; 3(1):01-10

Review Article

Bromelain as a Functional Feed Additive to Enhance Protein Utilization, Gut Health, and Performance in Broiler Chickens

Henri Eko PRASETYO [1]

[1] Animal Nutritionist & Business Development, PT. Dinamika Megatama Citra (DMC) Indonesia Company
Jl. Raya Mojosari Ngoro Km. 3, Pungging, Dusun Pungging, Pungging, Kec. Pungging, Kabupaten Mojokerto, Jawa Timur 61384

(*) Corresponding author:
Henri Eko PRASETYO
E-mail: henriekoprasetyo@gmail.com

Received: 15-05-2025 | Accepted: 30-05-2025 | Published: 19-06-2025

Bromelain, a proteolytic enzyme complex derived from pineapple (*Ananas comosus*) waste, has attracted growing interest as a natural feed additive in broiler nutrition. Its primary mechanism of action involves the enzymatic hydrolysis of dietary proteins and antinutritional factors, leading to the conversion of complex protein molecules into absorbable peptides and amino acids. This process enhances nutrient digestibility, improves nitrogen retention, and ultimately supports more efficient growth performance. In addition to its digestive function, bromelain contributes to improved intestinal morphology by preserving villus height and structural integrity, thereby facilitating optimal nutrient absorption. These effects are particularly beneficial during the starter phase, when endogenous enzyme activity in young broilers remains underdeveloped. Beyond its gastrointestinal effects, bromelain also exhibits postmortem activity on muscle tissue by hydrolyzing key structural proteins such as actomyosin and titin. This enzymatic action enhances meat tenderness, increases water-holding capacity, and reduces cooking loss. Furthermore, by decreasing meat pH, bromelain may contribute to prolonged shelf life and improved overall meat quality. Despite these beneficial effects, several challenges persist, particularly regarding enzyme stability during feed processing, dosage optimization, and product standardization. Further molecular-level studies are warranted to elucidate bromelain's underlying mechanisms and to advance its application in sustainable poultry production systems.

Keywords: Bromelain, Egg Production, Gut Health, Poultry Nutrition, Proteolytic Enzyme

INTRODUCTION

The growing demand for sustainable and efficient poultry production has intensified the search for functional feed additives that not only enhance nutrient utilization but also support gastrointestinal health and overall performance. In recent years, naturally derived additives have gained increasing attention as viable alternatives to antibiotic growth promoters (AGPs), largely in response to escalating concerns over antimicrobial resistance and evolving consumer preferences for residue-free animal products (Wang et al., 2024). Despite global efforts to limit antibiotic use in livestock production, antimicrobial consumption in food-producing animals is projected to increase by 11.5% between 2017 and 2030 (Tiseo et al., 2020). This trend underscores the urgent need for safe, effective, and environmentally sustainable alternatives to conventional AGPs.

One promising candidate is bromelain, a proteolytic enzyme complex primarily extracted from the stem and fruit of the pineapple (*Ananas comosus*). With Indonesia's pineapple production reaching approximately 1.8 million tons in 2018 (Nanda et al., 2020), the valorization of pineapple by-products as a source of functional feed ingredients has become increasingly feasible. Notably, nearly 30% of the total weight of fresh pineapple consists of peel waste, which remains largely underutilized (Campos et al., 2020). This waste material contains significant amounts of bromelain, which can be extracted and incorporated into poultry feed formulations as a natural protease.

Due to its potent proteolytic activity, bromelain facilitates the hydrolysis of complex dietary proteins into smaller peptides and free amino acids, thereby improving protein digestibility and nitrogen retention within the poultry gastrointestinal tract (Nanda et al., 2020). In addition to its enzymatic role, bromelain exhibits a range of biological activities, including anti-inflammatory, immunomodulatory, and antimicrobial effects. These properties may collectively support gut health by modulating intestinal microbiota, enhancing mucosal structure, and reinforcing immune responses (Collins et al., 2023; Yenice et al., 2023; Gharib-Naseri et al., 2024).

Against this background, the present review aims to critically examine the role of bromelain as a functional feed additive in broiler nutrition. The discussion focuses on its potential to improve growth performance and meat quality, while also exploring its physicochemical characteristics, stability, nutritional effects, and pharmacokinetic profile. In addition, this review highlights existing research gaps and proposes future directions to optimize the integration of bromelain into sustainable poultry production systems.

CHARACTERISTICS AND STABILITY OF BROMELAIN ENZYME

Bromelain is a cysteine protease derived from the pineapple (*Ananas comosus*) plant, widely recognized for its broad spectrum of applications in both therapeutic and industrial contexts. This enzyme exhibits broad substrate specificity, effectively hydrolyzing low molecular weight compounds such as synthetic amides and dipeptides, as well as high molecular weight proteins including fibrin, albumin, casein, angiotensin II, and bradykinin (Pavan et al., 2012). Bromelain demonstrates selective cleavage of peptide bonds, particularly those involving glycine, alanine, and leucine residues (Rajan et al., 2022).

From a practical standpoint, enzyme stability is a critical determinant of its effectiveness. Bromelain generally exhibits optimal stability within a pH range of 5.0–6.0 and at temperatures below 60°C (Mohamad and Sedrah, 2023). However, variations in reported stability parameters are evident across studies, likely due to differences in enzyme sources and extraction techniques. For instance, Sree et al. (2012) reported peak enzymatic activity at pH 6.5 and 50°C, while Mohapatra et al. (2013) noted stability across a broader pH range of 3.0–7.0 and temperatures between 40°C and 60°C. In contrast, Manzoor et al. (2016) observed sustained enzymatic activity across pH 5.5–8.0 and temperatures ranging from 35.5°C to 71°C.

Bromelain activity is also influenced by the presence of metal ions. Inhibitory effects have been reported in the presence of Fe^{3+} and Cu^{2+}, which significantly diminish enzymatic activity (Liang et al., 2012; Zhou et al., 2021). Conversely, the incorporation of stabilizing agents such as polyethylene glycol has been shown to improve shelf-life and preserve enzymatic functionality during storage (Zhou et al., 2021).

Recent efforts to enhance bromelain stability have focused on immobilization technologies. Techniques such as adsorption onto *Bacillus* spores, conjugation with gold nanoparticles, and cross-linking with glutaraldehyde have demonstrated significant improvements in both thermal and storage stability (Tacias-Pascacio et al., 2024; Banerjee et al., 2020). These immobilization strategies not only enhance resistance to elevated temperatures and denaturing agents but also broaden bromelain's utility in industrial applications that involve harsh processing conditions.

In addition, recombinant forms of bromelain have been developed and exhibit superior thermal stability compared to conventional commercial preparations (Banerjee et al., 2020). These advancements hold promise for expanding the use of bromelain in a variety of demanding environments, including pharmaceutical formulations, food processing systems, and enzyme-based therapeutic interventions.

NUTRITIONAL EFFECTS AND PHARMACOKINETICS OF BROMELAIN

Bromelain, although a high-molecular-weight enzyme, has been shown in several studies to be partially absorbed through the intestinal mucosa. Seifert et al. (1979) reported that up to 40% of radioactively labeled bromelain (^125I-bromelain) could be absorbed from the gastrointestinal tract in its intact form. This observation has been supported by additional studies employing diverse analytical techniques, which further confirm the potential for systemic absorption (Castell, 1976; Kolac et al., 1996).

Despite these findings, the extent to which bromelain remains enzymatically active in systemic circulation at physiologically relevant concentrations remains a subject of debate. Once absorbed, bromelain is known to rapidly bind to plasma antiproteinases, particularly α2-macroglobulin (AMG) and α1-antitrypsin, which serve as endogenous regulators of proteolytic activity. While these complexes limit uncontrolled proteolysis, they also complicate the precise quantification of free, unbound bromelain in circulation. Notably, bromelain bound to AMG has been shown to retain partial enzymatic activity (Shiraishi et al., 2004).

Pharmacokinetic evaluations have provided further insight into its systemic profile. Castell (1976) reported a plasma half-life of approximately 6 to 9 hours, with a mean area under the curve (AUC) concentration ranging from 2.5 to 4 ng/mL following oral administration of 8.6 grams of bromelain over a period of three consecutive days. These findings indicate that, beyond its local effects within the gastrointestinal tract, bromelain may exert systemic bioactivity after oral administration, although further studies are needed to elucidate its precise pharmacodynamic relevance.

EFFECT OF BROMELAIN ON BROILER PERFORMANCE

The incorporation of bromelain into broiler diets has shown promising potential in promoting growth performance, enhancing nutrient utilization, and preserving gastrointestinal health and functionality. Multiple studies have demonstrated that dietary inclusion of bromelain at 30 g/kg significantly improves final body weight (BW), average daily gain (ADG), and feed conversion ratio (FCR) in Ross 308 broilers over a six-week period (Yenice et al., 2023). These improvements are largely attributed to bromelain's proteolytic activity, which facilitates the breakdown of complex dietary proteins into simpler peptides and free amino acids, thereby increasing nutrient availability and absorption.

Bromelain also contributes to better intestinal health by reducing digesta viscosity and enhancing villus height and integrity. Increased villus height reflects a larger absorptive surface area, which supports more efficient nutrient uptake (Kostiuchenko et al., 2022). Furthermore, bromelain supplementation has been associated with reduced nitrogen excretion in feces, suggesting enhanced protein utilization and contributing to environmentally sustainable poultry production through lower nitrogen emissions (Rahman and Yang, 2018; Akit et al., 2019).

Notably, a significant reduction in abdominal fat has been observed in Cobb CP 707 broilers fed diets supplemented with up to 20% pineapple waste fermented using a consortium of microorganisms—including *Candida parapsilosis*, *Candida melinii*, *Hansenula subpelliculosa*, *Hansenula malanga*, *Aspergillus niger*, *Aspergillus oryzae*, and *Saccharomyces cerevisiae*—over a 42-day period (Mandey et al., 2017). This reduction may indicate more efficient energy metabolism and a shift toward lean tissue accretion. However, contrasting findings were reported by Heryandi et al. (2018), who observed no significant changes in abdominal fat in Arbor Acres broilers fed diets containing 12% pineapple waste fermented with a local microbial solution derived from bamboo shoots over five weeks.

Bromelain's efficacy appears to be more pronounced during the starter phase, likely due to the limited production of endogenous digestive enzymes in young chicks. In Cobb 500 broilers, bromelain supplementation at 0.1% during this early phase significantly enhanced protein and fat digestibility (Akit et al., 2019). As broilers mature, increased endogenous enzyme activity may necessitate higher doses to maintain comparable effects (Castro et al., 2020).

Beyond its digestive benefits, bromelain also offers protective effects against gastrointestinal disturbances caused by antinutritional factors such as allergenic proteins in soybean meal, which can impair microvilli structure and compromise nutrient absorption. Bromelain facilitates the hydrolysis of these proteins, thereby supporting mucosal health and maintaining nutrient uptake efficiency (Kostiuchenko et al., 2022). This function is further supported by its anti-inflammatory properties, which contribute to the recovery of intestinal tissue and preservation of mucosal architecture (Nobre et al., 2025).

In addition, bromelain appears to influence gut microbial composition. Supplementation with 0.05% and 0.1% bromelain in Cobb 500 broilers over 21 days has been linked to a decrease in *Escherichia coli* populations and an increase in beneficial *Lactobacillus* spp., which are crucial for maintaining microbial homeostasis in the gut (Akit et al., 2019). These microbial shifts contribute to a healthier intestinal environment, reduce the likelihood of infection and inflammation, and ultimately support improved nutrient utilization (Collins et al., 2023). A comprehensive summary of bromelain's effects on broiler and layer performance is presented in Table 1.

PROTEOLYTIC EFFECTS OF BROMELAIN ON MEAT TEXTURE AND QUALITY

Bromelain has been widely applied as a natural tenderizer across various types of meat, including beef, chicken, squid (Jun-Hui et al., 2020; Woinue et al., 2021), goat (Razali et al., 2023), pork, and assorted poultry cuts (Nanda et al., 2020). This proteolytic enzyme effectively hydrolyzes several key myofibrillar proteins within muscle tissue—such as actomyosin, titin, and nebulin—as evidenced by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses (Hage et al., 2012).

Chaurasiya et al. (2015) further demonstrated bromelain's tenderizing efficacy by comparing different enzyme preparations. Meat treated with reverse micellar extraction-purified bromelain (RMEB) showed a 52.1% reduction in Warner–Bratzler shear force (WBSF), significantly outperforming the 26.7% reduction observed with commercial stem bromelain (CSB).

The mechanism underlying bromelain's tenderizing effect centers on its ability to degrade structural muscle proteins into smaller peptides and low-molecular-weight fragments

Table 1. Effect of Bromelain on broiler and layer performance

Breed	Bromelain dosage	Primary Outcomes	Refrences
Cobb 500	0–0.5% in feed	• Enhances protein and fat digestibility, intestinal villus height, FCR, body weight gain, and optimizes serum enzyme profiles • Reduces *Escherichia coli* populations	Akit *et al*. 2019
Ross 308	0.4 mL/kg (via gavage) 0.8 mL/kg (via gavage or drinking water)	• Improves body weight gain and FCR • Reduces intestinal lesion scores, particularly in the jejunum • Decreases fecal oocyst counts • Maintains intestinal integrity comparable to non-challenged birds	Gharib-Naseri *et al*. 2024
-	• 20% or 100% fish meal replaced with bromelain-treated feather meal • 20% or 40% soybean meal replaced	• Improves body weight gain and FCR • Enhances crude protein digestibility • Reduces fecal ammonia and hydrogen sulfide gas emissions • Lowers feed cost per unit weight gain • Maintains nutrient digestibility (dry matter, ether extract, organic matter, phosphorus) across treatments	Kim and Ko 2005
-	30%, 50%, and 100% concentration of pineapple extract (Josephine variety) in feed	• Reduces meat hardness by up to 86% • Lowers meat pH from 5.87 to 4.99 • Enhances meat tenderness without compromising microbiological safety • Converts agricultural waste (pineapple core) into value-added natural tenderizing ingredient	Hussain *et al*. 2022
Arbor Acres	0.05% bromelain added to the fermentation solution, together with 34% probiotic fermentation solution, was used for soybean meal fermentation.	• Enhances fermented soybean meal quality when combined with probiotics and bromelain • Improves BWG and feed intake compared to unfermented soybean meal group • Increases relative spleen weight (suggestive of improved immune status) • Mitigates the negative effects of fish meal replacement on growth performance • Offers potential for reducing feed cost without compromising broiler performance	Li *et al*. 2014
-	Pineapple peel extract 0%, 25%, 50%, 75%, and 100% w/v	• Increases protein content of ex-layer chicken breast meat • Reduces water content and pH value • Improves meat tenderness and quality using natural bromelain from pineapple skin • Converts agricultural waste (pineapple skin) into a functional meat tenderizer	Purnamasari *et al*. 2014
Cobb	Fermented Pineapple Peel Meal 0%, 7.5%, 15%, or 22.5% in feed	• Improves nutrient consumption in broiler chickens	Ibrahim *et al*. 2016

-	Fermented Pineapple Peel Meal 0%, 5%, 10%, or 15% in feed	• Decreases feed consumption • No significant effect on final body weight • No significant changes in relative weight of liver, pancreas, ventriculus, and small intestine	Handoko *et al*. 2013
Cobb 500	Pineapple Leaf Powder 1%, 2%, and 3% PLP in feed	• Increases body weight • Decreases FCR • Improves red blood count, hemoglobin, and hematocrit • Reduces serum creatinine and blood urea nitrogen • Decreases caecal coliforms and E. coli, while increasing lactobacillus population	Rahman *et al*. 2018
Lohman MB 202 P	Bromelain 0, 600, 1200, 1800, 2400, or 3000 GDU/kg in feed	• No significant effect on BWG or FCR	Mahfudhoh *et al*. 2023
Ross 308	0–0.45g/kg in feed	• Enhances FCR, body weight gain, villus height and crypt depth, high-density lipoprotein (HDL) concentrations • Reduces serum cholesterol and low-density lipoprotein (LDL) levels	Yenice *et al*. 2023
Broiler	Bromelain crude extract 2–8% in feed	• Enhances feed intake and body weight gain, and FCR	Fitasari and Soenardi 2012
White Leghorn	0.14/0.28g/kg in feed	• Enhances eggshell thickness, liver and serum cholesterol, protein, and immunoglobulin G	Lien *et al*. 2012
Isa Brown	0.025–0.075% in feed	• Enhances blood protein, albumin, cholesterol, immunoglobulin Y	Rafis *et al*. 2024

(Ketnawa and Rawdkuen, 2011; Tacias-Pascacio et al., 2023). Supporting this, Nadzirah et al. (2016) reported a strong correlation between the extent of proteolysis and improvements in meat tenderness. Bromelain's action disrupts collagen structures, alters myosin complexes, and cleaves specific protein chains—modifications that collectively enhance meat texture (Feng et al., 2018).

This hydrolytic activity is reflected in increased levels of trichloroacetic acid (TCA)-soluble proteins, indicative of protein degradation. Chaurasiya et al. (2015) noted that CSB-treated meat contained 10.2 mg/g of TCA-soluble proteins, slightly higher than the 9.1 mg/g observed in RMEB-treated samples. Similarly, Ketnawa and Rawdkuen (2011) reported a 27% increase in TCA-soluble peptides when bromelain—applied at 20% of meat weight—was used to tenderize chicken, beef, and squid. Furthermore, bromelain-derived hydrolysates not only improve protein content but also impart a pronounced umami flavor to chicken meat, primarily due to elevated levels of free glutamic acid (Puspitasari et al., 2022).

Bromelain also positively influences water-holding capacity (WHC), an important quality attribute in meat. Chaurasiya et al. (2015) observed a substantial increase in WHC in RMEB-treated samples (91.1%) compared to CSB-treated (55.6%) and control (56.6%) groups. This enhancement is linked to bromelain-induced denaturation of myofibrillar proteins (Jun-Hui et al., 2020). According to Hughes et al. (2014), improved WHC contributes to greater tenderness and reduces cooking loss during heat processing.

In addition to textural modifications, bromelain treatment has been shown to lower the pH of meat. Chaurasiya et al. (2015) reported a decrease in pH from 6.1 to 5.8 following CSB treatment, and to 5.7 in RMEB-treated meat. Similar pH reductions have been documented by Ketnawa and Rawdkuen (2011) across various meat types. These changes are likely due to the release of amino acids resulting from proteolytic cleavage, as well as the naturally acidic pH of pineapple juice (approximately 3.36), a common source of bromelain.

Notably, Tørngren et al. (2018) emphasized that pH values below 5.8 are beneficial for extending the shelf life of vacuum-packaged meat, as such acidic conditions inhibit the proliferation of spoilage microorganisms responsible for greening and off-odor formation. Thus, in addition to its role in improving sensory attributes, bromelain's acidifying effect may also enhance the microbiological stability and shelf life of meat products.

FUTURE PERSPECTIVES AND RESEARCH GAPS

While bromelain holds considerable promise as a functional feed additive in poultry nutrition, several critical areas warrant further investigation. Its positive effects on protein digestibility, intestinal morphology, and overall performance are well documented; however, the molecular mechanisms underpinning these benefits—particularly those related to intestinal barrier integrity, immune modulation, and the regulation of nutrient transporter expression—remain insufficiently characterized. In this regard, omics-based approaches may provide valuable insights into the complex biological pathways involved.

To date, most studies have focused predominantly on broilers, with limited data available for laying hens—especially during the late production phase—despite notable physiological and nutritional differences across production stages that may influence bromelain's efficacy. Additionally, the potential synergistic or antagonistic interactions between bromelain and other feed additives, such as probiotics or exogenous enzymes, remain largely unexplored, even though such combinations could optimize feed efficiency.

Another important challenge lies in the variability of bromelain activity, which can be influenced by its botanical source, extraction methods, and stability during feed processing. These inconsistencies underscore the need for standardized production and formulation protocols to ensure consistent bioactivity. Furthermore, the long-term safety of bromelain use in poultry diets, as well as the potential for residue accumulation in animal-derived products, has not yet been comprehensively assessed and requires further evaluation to ensure consumer safety.

CONCLUSION

Bromelain exerts its primary effects in poultry nutrition through its proteolytic activity, which facilitates the hydrolysis of dietary and antinutritional proteins into simpler peptides and amino acids. This mechanism enhances nutrient digestibility, promotes intestinal absorption, and improves nitrogen retention, particularly in young broilers with underdeveloped endogenous enzyme systems. Bromelain also supports gut morphology by preserving villus structure and reducing mucosal irritation, contributing to improved feed efficiency. In postmortem muscle, its targeted action on myofibrillar proteins improves meat tenderness, water-holding capacity, and pH stability. Despite these advantages, further research is needed to elucidate molecular pathways especially its influence on intestinal transporters, enzyme expression, and barrier integrity to optimize its use as a functional additive in poultry feed.

REFRENCES

1. Akit H, Mohd Noor Zainudin NN, Abdul Wahid NA, Zakaria SN, Foo HL, Loh TC. Dietary bromelain improves nutrient digestibility, digesta viscosity and intestinal villus height as well as reduces intestinal *E. coli* population of broiler chickens. *Mal J Anim Sci*. 2019;22(1):1-16.
2. Banerjee S, Arora A, Vijayaraghavan R, Patti AF. Extraction and crosslinking of bromelain aggregates for improved stability and reusability from pineapple processing waste. *Int J Biol Macromol*. 2020;158:318-326. doi: 10.1016/j.ijbiomac.2020.04.220

3. Campos DA, Ribeiro TB, Teixeira JA, Pastrana L, Pintado MM. Integral valorization of pineapple (*Ananas comosus* L.) by products through a green chemistry approach towards added value ingredients. *Food*. 2020;9(60):1-22. doi: 10.3390/foods9010060
4. Castro SF, Bertechini AG, Lima EMC, Clemente AHS, Ferreira VGG, Carvalho JCC. Effect of different levels of supplementary alpha-amylase in finishing broilers. *Acta Sci Anim Sci*. 2020;42:e47546. doi: 10.4025/actascianimsci.v42i1.47546
5. Castell JV. Intestinal absorption of undegraded brome lain in humans. In: Gardner MLG, Steffens KJ. *Absorption of orally administered enzymes*. Springer, Berlin; 1995:47–60.
6. Chaurasiya RS, Sakhare PZ, Bhaskar N, Hebbar HU. Efficacy of reverse micellar extracted fruit bromelain in meat tenderization. *Food Sci Technol*. 2015;52(6):3870-3880. doi: 10.1007/s13197-014-1454-z
7. Collins A, Bowring B. Pre-treatment with bromelain prevents intestinal dysbiosis in pigs with post-weaning diarrhea, without increasing antimicrobial resistance in *Escherichia coli*. *Animals*. 2023;13:3229. doi: 10.3390/ ani13203229
8. Feng X, Hang S, Zhou Y, Liu Q, Yang H. Bromelain kinetics and mechanism on myofibril from golden pomfret (*Trachinotus blochii*). *J Food Sci*. 2018;83(8):2148-2158. doi: 10.1111/1750-3841.14212
9. Fitasari E, Soenardi. Effek penambahan ekstrak kasar enzim bromelin dalam pakan terhadap penampilan produksi. *Buana Sains*. 2012;1:17-24.
10. Gharib-Naseri K, Kheravii SK, Nguyen HT, Wu SB. Bromelain can reduce the negative effects of a subclinical necrotic enteritis in broiler chickens. *Poult Sci*. 2024;103(4):103560. doi: 10.1016/j.psj.2024.103560
11. Hage DS, Anguizola JA, Bi C, Li R, Matsuda R, Papastavros E, Pfaunmiller E, Vargas J, Zheng X. Pharmaceutical and biomedical applications of affinity chromatography: recent trends and developments. *J Pharm Biomed Anal*. 2012;1-13. doi: 10.1016/j.jpba.2012.01.004
12. Handoko H, Nurhayati, Nelwida. The use of pineapple peel flour in the diet on the relative weight of digestive organs and small intestine of broiler chickens supplemented with yogurt. *J Res Univ Jambi Ser Sci.* 2013;15(1):53–95.
13. Heryandi Y, Adrizal, Ningsih N, Mahata ME. Carcass characteristics and organ development of broilers fed fermented pineapple peel [*Ananas comosus* (L.) Merr] waste using a local microorganism solution derived from bamboo sprouts. *Int J Poult Sci*. 2018;17: 229-233. doi: 10.3923/ijps.2018.229.233
14. Hughes JM, Oiseth SK, Purslow PP, Warner RD. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Sci*. 2014;98(3):520-32. doi: 10.1016/j.meatsci.2014.05.022
15. Hussain N, Weng CH, Munawar N. Effects of different concentrations of pineapple core extract and maceration process on free-range chicken meat quality. *Ital J Food Sci.* 2022;34(1):124–31. doi:10.15586/ijfs.v34i1.2086.
16. Ibrahim W, Mutia R, Nurhayati, Nelwida, Berliana. Fermented pineapple peel supplementation with addition of medicinal weeds on nutrient intake consumption of broiler chicken. *Agripet.* 2016;16(2):76–82.
17. Jun-Hui X, Hui-Juan C, Bin Z, Hui Y. The mechanistic effect of bromelain and papain on tenderization in jumbo squid (*Dosidicus gigas*) muscle. *Food Res Int*. 2020;131:108991. doi: 10.1016/j.foodres.2020.108991
18. Ketnawa S, Chaiwut P, Rawdkuen S. Aqueous two-phase extraction of bromelain from pineapple peels ('PhuLae' cultv.) and its biochemical properties. *Food Sci Biotechnol*. 2011; 20(5):1219-1226. doi: 10.1007/s10068-011-0168-5
19. Kim JH, Ko YD. Effect of dietary protease (bromelain) treated feather meal on the performance and nutrient utilization in broilers. *J Anim Sci Technol.* 2005;47(2):221–32. doi:10.5187/jast.2005.47.2.221
20. Kolac C, Streichhan P, Lehr CM. Oral bioavailability of proteolytic enzymes. *Eur J Pharm Biopharm*. 1996;42:222–232.
21. Li CY, Lu JJ, Wu CP, Lien TF. Effects of probiotics and bromelain fermented soybean meal replacing fish meal on growth performance, nutrient retention and carcass traits of broilers. *Livest Sci.* 2014;163:94–101. doi:10.1016/j.livsci.2014.02.005
22. Shiraishi I, Melendez J, Ahn Y, Skavdahl M, Murphy E, Welch S, Schaefer E, Walsh K, Rosenzweig A, Torella D, Nurzynska D, Kajstura J, Leri A, Sussman P. Nuclear targeting of Akt enhances kinase activity and survival of cardiomyocytes. Circ Res.2004;94(7): 884 891.
23. Kostiuchenko O, Kravchenko N, Markus J, Burleigh S, Fedkiv O, Cao L, Letasiova S, Skibo G, Fåk Hållenius F, Prykhodko O. Effects of proteases from pineapple and papaya on protein digestive capacity and gut microbiota in healthy C57BL/6 mice and dose-manner response on

mucosal permeability in human reconstructed intestinal 3D tissue model. *Metabolites*. 2022;12:1027. doi: 10.3390/ metabo12111027
24. Liang H, Li M, Shi M, Liao A, Wu R. Study on the stability of fruit bromelain. *Adv Mater*. 2012;421:19-22. doi: 10.4028/www.scientific.net/AMR.421.19
25. Lien TF, Cheng YH, Wu YP. Effects of supplemental bromelain on egg production and quality, serum and liver traits of laying hens. *J Anim Sci Adv*. 2012;2(4):386-391.
26. Mahfudhoh NLN, Sajidan, Budiharjo A. The effect of dietary bromelain enzyme on broiler chicken (*Gallus gallus*) growth performance. In: *Proceedings of the 4th International Conference on Life Sciences and Biotechnology (ICOLIB 2021);* 2021. doi:10.2991/978-94-6463-062-6_24.
27. Mandey JS, Tulung B, Leke JR, Sondakh BFJ. Performance and carcass quality of broiler chickens fed diet containing pineapple waste meal fermented by "ragi tape". *IOP Conf Ser: Earth Environ Sci*. 2017;1213042:012042. doi: 10.1088/1755-1315/102/1/012042
28. Manzoor Z, Nawaz A, Mukhtar H, Haq I. Bromelain: methods of extraction, purification and therapeutic applications. *Braz Arch Biol Technol*. 2016;59:e16150010. doi: 10.1590/1678-4324-2016150010
29. Mohamad SA, Sedrah ZT. The use of bromelain enzyme extracted from pineapple crown as anti-browning in apple juice and anti-bacterial. *Iraqi J Mark Res Consum Prot*. 2023;15(2): 94-104 doi: 10.28936/jmracpc15.2.2023.(10)
30. Mohapatra VM, Rao MV, Ranjan M. Comparative study of the increased production and characterization of bromelain from peel, pulp and stem pineapple (*Anannus commas*). *Int J Adv Res Technol*. 2013;2:249-279.
31. Nadzirah KZ, Zainal S, Noriham A, Normah I. Application of bromelain powder produced from pineapple crowns in tenderising beef round cuts. Inter Food Res J. 2016;23(4):1590-1599.
32. Nanda RF, Rini B, Syukri D, Thu NNA, Kasim A. A review: application of bromelain enzymes in animal food products. *And Int J Agric Nat Sci*. 2020;1(1):18–24. doi: 10.25077/aijans.v1.i01.33-44.2020
33. Nobre TA, de Sousa AA, Pereira IC, Carvalho Pedrosa-Santos ÁM, Lopes LO, Debia N, El-Nashar HAS, El-Shazly M, Islam MT, Castro E Sousa JM, Torres-Leal FL. Bromelain as a natural anti-inflammatory drug: a systematic review. *Nat Prod Res*. 2025;39(5):1258-1271. doi: 10.1080/14786419.2024.2342553
34. Pavan R, Jain S, Shraddha, Kumar A. Properties and therapeutic application of bromelain: a review. *Biotechnol Res Int*. 2012;2012:976203. doi: 10.1155/2012/976203
35. Purnamasari E, Eltha A, Febrina D, Irawati E. Utilization of skin extract pineapple (*Ananas comosus* L. Merr) to improve the quality of ex-layer chicken. *Sagu*. 2014;13(2):1–6.
36. Puspitasari E, Rosida DF, Putra AYT, Priyanto AD. Physicochemical properties of apple snail protein hydrolysate (*Pila ampullacea*) and its potential as flavor enhancer. *IJ-FANRes*. 2022; 3(1):27-32.
37. Rafis, Noor H, Sudarman, Asep, Mutia, Rita. Suplementasi enzim bromelain dalam pakan terhadap performa, kualitas telur dan profil darah ayam akhir periode bertelur. 2024. http://repository.ipb.ac.id/handle/123456789/137661
38. Rahman MM, Yang DK. Effects of *Ananas comosus* leaf powder on broiler performance, haematology, biochemistry, and gut microbial population. *Rev Bras Zootec*. 2018;47. doi: 10.1590/rbz4720170064
39. Rajan PK, Dunna NR, Venkatabalasubramanian S. A comprehensive overview on the anti-inflammatory, antitumor, and ferroptosis functions of bromelain: an emerging cysteine protease. *Expert Opin Biol Ther*. 2022;22(5):615-625. doi: 10.1080/14712598.2022.2042250
40. Razali R, Kumar V, Budiman C. Tenderness and physicochemical characteristics of meat treated by recombinant bromelain of MD2 pineapple from a codon-optimized synthetic gene. *Emir J Food Agric*. 2023;35(10):878-889. doi: 10.9755/ejfa.2023.v35.i10.3164
41. Seifert J, Ganser R, Brendel W. Die resorption eines proteolytischesproteins pflanzlichen ursprunge aus dem magen-darmtraktin das blut und in die lymphe von erwachsenen ratten. *Z Gastroenterol*. 1979;17:1–8. doi: 10.1007/978-3-642-68415-9_17
42. Sree PPS, Jayakumar K, Mathai V, Chintu S, Sarath BK. Immobilization and kinetic studies of bromelain: a plant cysteine protease from pineapple (*Ananas comusus*) plant parts. *Int J Med Heal Sci*. 2012;1:10-16.
43. Tacias-Pascacio VG, Castañeda-Valbuena D, Tavano O, Murcia ÁB, Torrestina-Sánchez B, Fernandez-Lafuente R. Peptides with biological and technofunctional properties produced by bromelain hydrolysis of proteins from different sources: A review. *Int J Biol Macromol*. 2023;253(Pt 5):127244. doi: 10.1016/j.ijbiomac.2023.127244

44. Tacias-Pascacio VG, Castañeda-Valbuena D, Tavano O, Abellanas-Perez P, de Andrades D, Santiz-Gómez JA, Berenguer-Murcia Á, Fernandez-Lafuente R. A review on the immobilization of bromelain. *Int J Biol Macromol*. 2024;273(2):133089. doi: 10.1016/j.ijbiomac.2024.133089
45. Tiseo K, Huber L, Gilbert M, Robinson TP, Van Boeckel TP. Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics*. 2020;9(12):918. doi: 10.3390/antibiotics9120918
46. Tørngren MA, Darré M, Gunvig A, Bardenshtein A. Case studies of packaging and processing solutions to improve meat quality and safety. *Meat Sci*. 2018;144:149-158. doi: 10.1016/j.meatsci.2018.06.018
47. Wang J, Deng L, Chen M, Che Y, Li L, Zhu L, Chen G, Feng T. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: a review of the literature of the last decade. *Anim Nutr*. 2024;17:244-264. doi: 10.1016/j.aninu.2024.01.012
48. Woinue Y, Chaurasiya RS, Sharma R. Meat tenderization using bromelain enzyme extracted from pineapple waste. *Food Res*. 2021;5(2):363-370. doi: 10.26656/fr.2017.5(2).525
49. Yenice G, Atasever M, Kara A, Özkanlar S, Gelen SU, İskender HA, Gür C, Gedikli S. Effects of bromelain on growth performance, biochemistry, antioxidant metabolism, meat quality, and intestinal morphology of broilers. *Braz Arch Biol Technol*. 2023;66:e23220852. doi: 10.1590/1678-4324-2023220852
50. Zhou W, Ye C, Geng L, Chen G, Wang X, Chen W, Sa R, Zhang J, Zhang X. Purification and characterization of bromelain from pineapple (*Ananas comosus* L.) peel waste. *J Food Sci*. 2021;86(2):385-393. doi: 10.1111/1750-3841.15563