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DOI: 10.37722/AOVSAR.2026103

Use of Proteolytic Enzymes in Cattle Nutrition- A Review

Research Article **Archives on Veterinary Science and Research (ISSN: 2641-6867)** **AVSAR-172**

Ivannikov Zakhar*, Sarvilina Marina

University of Szeged, H-6800, Hódmezővásárhely, Andrássy út 15, Hungary

Received Date: May 24, 2026; **Accepted Date:** June 15, 2026; **Published Date:** June 25, 2026

***Corresponding author**: Sarvilina Marina, University of Szeged, H-6800, Hódmezővásárhely, Andrássy út 15, Hungary; Email: ivannikovzakhar2004@gmail.com

Abstract

The use of proteolytic enzymes in cattle feeding represents a promising direction for enhancing feed digestibility and potentially the Feed Convertion Ratio (FCR). Proteases mainly act as catalysts in the hydrolysis of peptide bonds producing easily digestible amino acids, which are important for adult animals and even more so in young stock, who require a sufficient amount of amino acids due to their intensive growth period. Optimizing amino acid assimilation with the help of proteases contributes to increased feed efficiency, higher average daily weight gains, and improved overall animal health. Furthermore, it allows for a reduction in the need for expensive protein supplements, thereby increasing production profitability. This article analyzes scientific studies aimed at investigating the effects of various protease dosages in cattle diets and also notes the effects of combining proteolytic enzymes with other feed additives, mainly exogenous enzymes. The effects of each experimental group were compared with both the control group and among themselves to evaluate optimal enzyme application strategies.

Keywords: feed additive, protease, assimilation, dairy cows, bulls

Introduction

It is estimated that by 2050 the population could grow to more than 9 billion people, this and the current socio-economic and global increase in urbanization indicates that the demand for food and particularly protein rich foods will continue to rise **[1].** Animal-based products account for 31% of dietary protein and 15% of global food energy supply. In the current growing global situation pressure on animal production has driven researchers to search for new sustainable feed resources **[2].** Cattle breeding is the leading branch of animal husbandry, accounting for more than half of the world's production of animal products **[3].** However, there are a number of pressing problems in dairy and beef cattle farming related to a decrease in overall productivity and insufficient feed conversion efficiency **[4].** One of the ways which people can use to increase the individual productivity of an animal is the usage of feed additives. One of the more promissing feed additives are the exogenous enzymes **[5].** Enzymes are biological molecules that act as catalysts, speeding up chemical responses in living organisms. They work by lowering the activation energy needed for a response to do, therefore adding the rate of the response without being consumed themselves **[6].** Enzymes play pivotal places in natural processes, similar as metabolism, digestion, and cell signaling **[7].**

Particularly interesting are the proteolytic enzymes which are known for increasing digestion and absorption of protein and its subsequent utilization **[8].** The efficient conversion of dietary protein into absorbable amino acids is paramount for optimizing the productivity of cattle. Proteolytic enzymes, also called “proteases,” divide the peptide bonds between two amino acids. Proteolytic enzymes are obtained through eukaryotic and prokaryotic organisms. Proteases can be classified into two main groups: exopeptidases or endopeptidases depending on the site of their action.**[9]** Exopeptidases detach the amino acids near the termini or “the ends" of the polypeptide chains. After this process there is the main chain and amino acid that was ripped form it, or di- or triptides also known as aminopeptidases. **[9]** Endopeptidases detach amino acids from the termini. Over several decades, the role of proteolytic enzymes in this process has been a subject of intensive scientific inquiry. Initial research primarily focused on the extensive enzymatic activity of the rumen microbiome **[10].** However, a growing body of work in recent years has shifted towards understanding and harnessing the potential of exogenous proteolytic enzymes as direct dietary supplements **[11].**

In recent years the research related to the use of exogenous proteolytic enzymes has moved from the simple efficacy in animal nutrition toward the molecular level of interaction between the enzymes and the microbiom of the rumen **[29].** There have been advances in the genomic understanding of the microorganisms which allows to create more stable versions enzymes **[29].** In addition, researchers have incorporated the use of microencapsulation which can further protect the enzymes, insuring an improved delivery system of the enzymes to the targeted organ **[30].** These developments are gradually shifting enzyme supplementation towards precision feeding strategies based on diet composition and animal physiology.

From an economic standpoint, the inclusion of proteolytic enzymes in cattle diets can improve profitability through several mechanisms. Protease supplementation increases average daily weight gain by 4–8% in beef cattle and reduces feed conversion ratio by approximately 5%, leading to lower total feed costs per kilogram of gain **[29].** When it comes to dairy cattle, although there may be no significant increase in milk yield the improved utilization of nitrogen allows us to reduce expenses on supplements of rumen-undegradable proteins, saving up to 10–15% on protein feed costs **[31].** It should be pointed out that farmers will get the most out of proteolytic enzymes if used on high-productivity animals.

The goal of this review is to systematize and analyze current research on the addition of a proteolytic enzyme to the diet of dairy and beef cattle, as well as to evaluate the effectiveness of using a proteolytic supplement in feeding.

Mechanism of action of proteolytic enzymes

As stated previously proteases assist the body in digestion and convert proteins into their constituent amino acids. Beyond direct hydrolysis of dietary proteins, exogenous proteolytic enzymes modulate ruminal fermentation and protein utilization through several indirect but important pathways. First, proteases release small peptides and free amino acids more rapidly than rumen microbes alone, which stimulates the growth of proteolytic and cellulolytic bacteria (e.g., Ruminococcus albus, Butyrivibrio fibrisolvens), thereby enhancing fibre digestion **[32].** Second, an improved amino acid supply increases microbial protein synthesis in the rumen, as shown by higher concentrations of purine derivatives in urine – an indicator of microbial nitrogen flow to the duodenum. Third, exogenous proteases can reduce the formation of ammonia by minimizing excessive deamination; this is evidenced by consistently lower rumen ammonia- N and plasma urea nitrogen levels in supplemented animals **[33].** Finally, in diets containing anti- nutritional factors such as trypsin inhibitors (e.g., raw soybean meal), proteases help degrade these compounds, further improving protein digestibility.

Collectively, these mechanisms enhance the efficiency of nitrogen retention and reduce nitrogen excretion into the environment. There are several origins of proteolytic enzymes. Animal proteases are secreted in animals; the most popular example of this class is pepsin. Then there are plant-based proteases, for example papain (extracted from Carica papaya), bromelain (Ananas comosus), ficin (Ficus genus). The third group are proteolytic enzymes isolated from microorganisms These are typically fungi or bacteria such as Aspergillus niger and Bacillus spp. Examples include chymosin, pepsin A, subtilisin, trypsin **[12].** These are most commonly used as feed additives for farm animals. The mechanism of proteolytic enzymes isn't as well-known compared to other types of enzymes. This is mainly due to the very small scale of the reactions **[13].**

Mechanisms of Aspartic Proteases

Most researches were conducted using pepsin, as it is one of the most known and was one of the first proteases ever discovered, apart for tripsin. Because of this their mechanism is the most researched **[14].**

Essentially three major steps take place, including formation of the Michaelis complex.

1) Nucleophilic attack by an activated water molecule with transition state 1 (TS1) and formation of a tetrahedral intermediate (INT).
2) Nitrogen conversion (TS2).
3) Fission of the scissile bond and release of the products with new C- and N-termini (EP). Residue numbering corresponds to pepsin. (**B**) Free energy profile for the pepsin-like protease renin, with an additional reaction step including INT2 and TS3. (**C**) Active site of pepsin with a phosphonate inhibitor, mimicking TS2 (PDB 1QRP). White areas are polar, green areas are hydrophobic. Right panel: The dimeric HIV protease has one catalytic Asp25 per monomer (PDB 4HVP). A peptidic inhibitor is bound to the active site as ES analog **[15].**

Mechanisms of Cysteine Proteases

Cysteine, serine, and threonine proteases are characterized by a more complex reaction pathway involving multiple transition states and stable acyl intermediates. These acyl intermediates are pivotal for transpeptidation, a process particularly exploited by cysteine proteases through reactions with peptide α-amino groups, enabling applications like peptide ligation **[16].** Similar to other protease classes, cysteine proteases feature diverse specificity pockets. As a representative example, papain from *Carica papaya* demonstrates a moderate trypsin-like preference for P1-Arg residues and a defined specificity profile (Leu/Val-Arg/Lys↓Gln-Gln-Xaa-Asp at P2-P4′) **[17].** The functional oxyanion hole in papain is comprised of the catalytic Cys25 backbone NH and the Gln19 side-chain CONH2. Importantly, while His159 is essential for deacylation, the initial acylation steps in papain do not rely on general base or acid catalysis **[18].**

Factors affecting enzyme effectiveness

The efficacy of exogenous proteases in cattle is not uniform and varies significantly with feeding system characteristics. Key modulatory factors include:

Diet composition: High forage diets or rations which include low quality roughages typically show more improvements in digestibility rather than high concentrate diets, where there is more rumen degradable protein **[29].**

Enzyme origin: Fungal proteases (Aspergillus niger) are more active at acidic pH (optimal 3–5) and thus work better post- ruminally, while bacterial proteases (Bacillus spp.) often have neutral to alkaline optima and may act primarily in the rumen.

How the enzyme was introduced into the diet: Soaking the feed has proven to be a more effective method, rather than mixing or spraying the enzymes on top of the feed.

Physiological state of the animal: Animals with a lower protease activity (e.g. young calves and old cows, animals in the early stages of lactation) benefit more from these additives rather than cows in the drying off period or beef cattle close to their slaughter weight.

Understanding these factors allows for more effective usage of these enzymes, depending on the situation.

Proteases in feeding dairy cows

First, it is important to understand the effects of proteolytic enzymes on milk yields and milk content.

The precise mode of action of PE in ruminant diets had not yet been clearly delineated. However, evidence suggests improved nutrient digestibility when a variety of feeds were treated with proteolytic enzymes **[19].**

Feeding of healthy cows

There is a number of experiments, where healthy dairy cows were fed proteolytic enzymes and compared to a control group, which was fed a standard diet without any additives, absent in the experimental diets.

The digestibility of all feed elements (e.g. starch, protein, etc.) increases, while the total dry matter intake was reduced. This had an effect on the overall feed efficiency, supposedly because of the increased digestibility of nutrients thanks to the proteolytic enzymes. There has also been a significant decrease in somatic cell count.

There was no apparent change in milk yields, nor in the concentration of milk fat and protein.

Although there have been increases in milk lactose and decreases in milk urea nitrogen **[20, 21].**

But there are also effects depending on different types of diets (e.g. high and low forage diets). Increase in starch digestibility was only seen in high forage diets, while digestibility of neutral detergent fiber increased only in cows fed low forage diets **[20].**

The decrease of blood and milk urea nitrogen can be explained by proteases having a positive effect on nitrogen utilization by microorganisms in the rumen.

Feeding proteases with other exogenous enzymes

Exogenous enzymes are often used in combination with other additives, including other enzymes.

The most common combinations are made with exogenous amilolytic enzymes **[22].** This experiment was conducted using different diets, two of which used a combination of both amilases and proteases, only in different proportions.

Although there were similar results with the control group, there were still some changes in the digestion and utilization of nutrients by cows **[23].** Starch digestability was greater in animals fed with both enzymes than in groups fed only amylase. Feeding the blends of amylase and protease resulted in greater feed sorting index for long particles (>19 mm) and lower feed sorting index for small particles (<4 mm) than feeding amylase alone **[24].**

Neither ruminal pH nor concentrations of NH3-N were altered by treatments. Milk and lactose yields were greater in cows under diets mixed with enzymes compared with the control group. Fat and protein yields tended to be greater in enzyme fed cows than in the control group **[25].** Other treatment comparisons did not reveal differences in yields of milk or solids. Milk concentration of solids (fat, protein, and lactose) were similar between treatments. Milk urea nitrogen concentration was greater in cows fed enzymes, whereas cows fed treatments containing protease had higher milk urea nitrogen concentrations than those in the amylase group. Feed efficiency, which is found by diving the milk yield by the dry matter intake tended to be greater in cows fed exogenous enzymes than in the control group.

Proteases in beef cattle nutrition

Using exogenous proteolytic enzymes in feeding beef cattle and farm animals used for meat is a much more studied topic. Since the main function of proteases is the hydrolysis of peptide bonds it is natural to conclude that this feed additive would be the most effective in meat production animals, because they require a large amount of attention to the protein amount and protein utilisation. The feeding of beef cattle could be divided into two branches: feeding of adults and elderly bulls, and feeding of beef steers **[26].**

Feeding adult and elderly beef cattle

Elderly animals are known to experience age-related declines in digestive function, which can hinder the digestion of dietary proteins and thereby negatively impact overall health and production. This is why it could be desired to use proteases as feed additives.

In the elderly model, protein digestibility was significantly impaired when contrasted with the adult model ($P < 0.05$). Specifically, gastric phase digestibility was 36.56% in the elderly versus 48.93% in adults, and small intestinal phase digestibility was 55.62% in the elderly compared to 69.60% in adults. These observations are consistent with those of [cite the mentioned study] in static in vitro digestion studies of diverse food matrices. The introduction of Protease-DS, however, significantly boosted protein digestibility in the elderly model, raising it to 44.62% in the gastric phase and 63.60% in the small intestinal phase. This marked increase in protein hydrolysis aligns with existing literature demonstrating the efficacy of protease supplementation in enhancing in vitro protein digestibility, such as the reported increase in pea protein digestibility from 54.4% to 81.6% with microbial protease. Therefore, Protease-DS supplementation substantially improved protein digestion in the elderly model, approaching adult levels **[27].**

Parallel to protein digestibility, amino acid absorption also exhibits greater impairment in aged livestock than in younger individuals. The inclusion of protease supplementation resulted in a greater release of crucial essential amino acids, such as arginine, lysine, and methionine, when compared to the control elderly model. These findings strongly suggest that Protease-DS significantly augmented the digestion of beef protein in the elderly model, leading to a greater liberation of essential amino acids. Consequently, this improved amino acid profile holds promise for enhancing the nutritional adequacy of protein for elderly individuals, potentially contributing to the maintenance of their physiological functions **[27].**

Feeding beef steers

During the intensive growth period of beef steers, it is crucial to feed them optimally, because in this period the animals will gain the most weight until maturity.

Exogenous protelytic enzymes have a positive effect on the digestibility and utilization of nutrients by stimulating rumen micro flora propagation and growth. The implementation of proteases in beef steer diets has increased the digestibility of dry matter (by 2.7%), proteins (by 6.8%), fibers (by 3.0%) and lipids (by 2.8%) **[28].**

However, it has been noted during the micronutrial analysis of the ration and the analysis of the fecal matter that when using exogenous proteolytic enzymes derived from fungi there has been a decrease in macro element absorption (Na, Mg, Na, P, Ca), while the absorption of a number of micro elements has increased (Zn, Li, I, etc.).

It is also important to notice that the addition of exogenous protelytic enzymes during the growth phase had no impact on the body weight of the steers. Only in the final phase the BW and final BW increased due to protease supplementation. Total tract N digestibility is also worthy of notice because it would mean that the usage of proteases has a positive impact on the environment **[28].**

Limitations

Although there are clear benefits from using proteolytic enzymes sertain nuances should be kept in mind when utilizing them. Firstly, the relatively high cost of preparation means that incorporating these additives in the diets of low productivity animals may not cover the expenses which will bring losses to the farm. Second, enzymes are sensitive to most types of feed processing (e.g. heating, extrusion, pelleting), also they spoil very quickly if the right conditions are not met **[32].** Third, there is a risk of over-supplementation. Excessive protease activity can lead to excessive ammonia production in the rumen, reduced feed intake, and increased urea excretion, which negates environmental benefits. Finally, many commercial enzyme products are “black boxes” – their exact composition and activity units are not transparent, making it difficult for nutritionists to compare products or formulate precisely. Finally, the composition and active ingredients are difficult to identify, which complicates things if nutritionists would want to compare different proteolytic enzymes from various manufacturers.

Conclusion

The paper was meant to review the use of exogenous proteolytic enzymes on dairy and beef cattle of different ages.

It was concluded that proteases in feeding dairy cows have a positive impact on the overall digestibility of nutrients and DMI, although there were no significant changes in the milk composition or yields.

Combining EPE with other exogenous enzymes also showed that they can complement each other.

Feeding beef cattle with proteases proved more effective than with dairy cows, because there was more impact on the body weight of the steers. Also, proteases increased the feed conversion and effectiveness in elderly cattle’s.

Future research could address:

Large-scale trials for a better estimation on the financial and environmental effect. Further development of the enzyme delivery system to protect them from rumen degradation. Research of enzyme activity and standardization, which will allow nutritionists to compare different products

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Citation: Zakhar I, Marina S *(2026); Use of Proteolytic Enzymes in Cattle Nutrition- A Review. Arch Vet Sci Res: AVSAR-172.*