

Chitin and Chitosan in Ruminant Diets

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The slow progress in the development of the subsector, particularly of alternative feed sources such as agro-industrial byproducts and unconventional feed resources, has deepened the gap in the availability of and accessibility to animal feed. Production of animal feed is highly resource demanding. It has been shown that increasing climate change, land degradation, and the recurrence of droughts have worsened the feed gap. In the backdrop of these challenges, there has been attention to food-not-feed components, which have great potential to substitute human-edible components in livestock feeding. Chitosan, a non-toxic polyglucosamine, is widely distributed in nature and used as a feed additive. Chitosan is obtained from the de-acetylation process of the chitin and is mostly present in shrimp, crabs, and insect exoskeletons, and has antimicrobial and anti-inflammatory, anti-oxidative, antitumor, and immune-stimulatory hypocholesterolemic properties.

Keywords: chitosan ; chitin ; ruminants ; microbiomes ; rumen enhancer

1. Introduction

In ruminant nutrition, many types of feed additives are used to improve production performance and to maintain the good health and metabolic condition of farm animals. The generally used feed additives are organic acids, feed enzymes pro and prebiotics, and herb extracts on the other hand, chitosan is a new and relatively less used in the diet of the animals [1][2]. Chitosan is a nontoxic polyglycosamine and is rarely present in nature (mushrooms) containing β -(1-4)-2-acetamido-D-glucose and β -(1-4)-2-amino-D-glucose units. It is a deacetylated to varying degrees form of chitin, widespread in nature component of the exoskeleton of shrimps, crabs, and insects [3][4]. Different from chitin, chitosan is soluble in acidic solutions [5][6], and it is moderately digested in the gastrointestinal tract of mono-gastric animals [7][8]. Chitosan is commercially obtained from chitin through the deacetylation process, in this process chitin is treated with a strong solution of sodium hydroxide at a higher temperature [4]. Chito-oligosaccharides are produced by chitosan depolymerization using acid hydrolysis, hydrolysis by physical methods, and enzymatic degradation [9]. Chitosan and its oligosaccharide derivatives have reactive functional groups, that is, amino acids and hydroxyl groups, unlike chitin they have antimicrobial [10][11], anti-inflammatory [12][13], anti-oxidative [14], antitumor [15], immunostimulatory [16][17], and hypocholesterolemic [18] properties.

In the environment, particularly in the agriculture sector, the emission of enteric methane contributes significantly, and the production of methane by ruminants also characterizes a substantial feed energy loss [19]. Proper provision of forage and choice of feed supplements can improve the total mixed ration quality and nutrient digestibility and alternatively improve the production performance of the animals and decrease the methane emission in an animal farming system [20].

2. Chemical Structures of Chitin and Chitosan

2.1. Chitin

In the world, chitin is the second most vital natural polymer and its *N*-deacetylated derivative chitosan has been known as a useful biopolymer that is used in the food industry, medicine, and agriculture. As already discussed, chitin is the second most plentiful natural polymer having structural polysaccharide [21][22]. A chief constituent of the carapaces, crusts, and crustacean shells such as crabs, shrimps, and lobsters, it is also a constituent of the cell walls in yeast and fungi [23].

The yearly production of chitin is about 10^{10} – 10^{12} tons [21]. Chitin (chemical formula $(C_8H_{13}O_5N)_n$) can only be soluble in concentrated mineral acids [24]. The structure of the chitin is a linear polymer containing mostly β (1→4)- linked 2-acetamido-2-deoxy- β -D-glucopyranose units and partially β -(1→4)-linked 2-amino-2-deoxy- β -D-glucopyranose. When the *N*-acetylation degree is lower than 50%, chitin can be soluble in an acidic solution having a pH less than 6 and later is called chitosan [25]. Therefore, chitosan is a combined name of the partially and fully de-acetylated chitin, however, a firm nomenclature concerning the degree of *N*-deacetylation between chitin and chitosan has not been defined [26].

In the nomenclature of the European chitin society (EUCHIS), chitosan and chitin should be divided by the solubility and insolubility in 0.1 M acetic acid [27]. Chitosan is a soluble material, whereas chitin is insoluble. The molecular weight of the chitin and chitosan is up to numerous million g/mol. The average molecular weight of commercially obtainable chitosan ranges between 3800–500,000 g/mol, and it has 2%–40% degree of N-acetylation [28].

Chitosan and chitin have commercial importance because in these two compounds the nitrogen content is high (6.89%), and these compounds have greater abilities in biocompatibility, biodegradability, non-toxicity, and adsorption [29]. In addition, chitin and chitosan have lower toxicity [30]. Chitosan is highly insoluble and has a lesser chemical reactivity. The difference between chitin and chitosan depends on the degree of deacetylation. If the threshold of deacetylation is above 50%, it is referred to as chitosan. The deacetylation ranges from 44.1% to 98.0%, depending on the species. Grasshoppers, honeybee beetles, shrimp shells, and blowfly larvae all are examples of the highest deacetylation. In addition, commercial chitin is obtained from crustaceans and aquatic invertebrates and has similar products and futures that are in insects produced chitin with like qualities [31].

2.2. Chitosan

Chitosan is composed of two repeated units of D-glucosamine and N-acetyl-D-glucosamine and these units are linked together by $\beta(1 \rightarrow 4)$ linkage, while chitin, a linear polysaccharide composed of two repeated units, D-glucosamine and N-acetyl-D-glucosamine, linked by $\beta(1 \rightarrow 4)$ linkages. Chitosan is considered a natural compound, biocompatible and non-toxic, biodegradable and bioactive mucoadhesive compound, and is commonly used as a food product in Japan in 1983 and Korea in 1995 and in 2012 food and in addition drug administration United States recommended chitosan as for food production. The chitosan is fully or partially de-acetylated biopolymer chitin. Chitosan is the second most polysaccharide in nature having a greater molecular weight and a polycationic polymer. Normally chitosan is found in the exoskeleton of insects, mollusks, crustaceans, and some algae, however, a large quantity of chitosan is obtained from marine crustaceans [32]. In a year, shells of crustacean are generated through the extraction of chitin (106–107 tons), and from this extraction different protein and chitosan from this waste has added value [33]. Numerous studies proposed that chitosan has antimicrobial properties and worked against the killing of the bacteria and different fungi like filamentous fungi and yeast, it also studied that chitosan also has antiviral and anti-inflammatory, analgesic, anticholesteromic and homeostatic effects [34]. Chitosan can be used directly, or it can be mixed with other polymers such as inoculants of silage, for food processing and preservation, biotechnology, water treatment, tissue engineering, the cosmetic industry, and the pharmaceuticals textile [24]. Nowadays, in ruminants particularly in beef and dairy cattle chitosan is also used in animal feeding and it improved rumen fermentation and digestibility [35]. The extraction of chitosan can be done either by biological or chemical methods. The industrial and chemical process starts with the removal of different minerals like calcium chloride and known as demineralization followed by deproteinization and decolorization like carotene and astaxanthin. Lastly, the deacetylation process is carried out through potassium or sodium hydroxide [36]. While the biological method is considered environment friendly. In this method demineralization is done by using lactic acid and protease is used for deproteinization, discoloration acetone or organic solvents are used, and lastly, bacteria are used for deacetylation. Recently A new method of extraction, also used known as the microwave irradiation method, has been recently developed [37]. The raw material (crustaceans species), which is used for chitosan, method of extraction, and seasonal variation play a crucial role in the quality of the final product [38].

3. Supplementation of Chitin and Chitosan and Its Impact on Performance of Ruminants

In dairy cows, higher milk production depends on the energy requirement of dairy cows and proper energy requirement is among the greatest challenge [39]. To overcome this challenge most of the studies suggested using dietary additives supplementation, to improve the rumen digestion process. The most abundant used feed additives in animal diets are primary substances with antimicrobial activity, mainly ionophore, which has been successful in surging protein efficiency and energy utilization [40]. The use of antibiotics in the diet of the animal, however, is facing decreased social acceptance due to possible residues in animal products and the development of resistant strains of bacteria [41]. Goiri et al. [42] stated that chitosan can be used as a rumen fermentation modulator and digestion process. In addition, chitosan is mostly used in medicine and for the preservation of food due to its antimicrobial activity and is also a non-toxic and biodegradable biopolymer. Chitosan is obtained by the deacetylation of chitin, the most plentiful biopolymer in the world after cellulose, and it is a key component of the exoskeleton of crustaceans and insects [43]. Goiri et al. [44] stated that the inclusion of chitosan prevents in vitro bio-hydrogenation, and upsurges unsaturated fatty acids levels in Rusitec® assay though in their other study Goiri et al. [44] showed that the concentration of propionate in rumen increased, the concentration of ammonium reduced and no impact of chitosan inclusion was observed on feed intake and total tract digestibility in sheep. An alternative approach to overcome the energy requirements of the animals producing high milk is to use feeds with high

energy density, such as those rich in lipids. Jenkins et al. [45] stated that in animals feed lipids have higher energy density and may influence the rumen fermentation, changing the fatty acid profile in milk.

Chitosan is obtained from chitin (a by-product of the fishing industry, especially from shrimp, lobster, krill, and crab) through deacetylation, the second most plentiful biopolymer in nature. Furthermore, Dias et al. [46] reported that chitosan is non-toxic, biodegradable, and has been recognized by US Food and Drug Administration (2012) to use in food. Numerous chitosan applications were reviewed by Senel and McClure [47]; lately, chitosan can be used in silage preparation as an inoculant due to its antimicrobial property [35] and provided as a rumen modulator to confined beef cattle [48] and dairy cows [49] with promising results. Though the antimicrobial mechanism of chitosan is not fully explicated, the intracellular leakage mechanism is the most accepted theory by the scientific community [43]. Dias et al. 2017 [46] reported that positively charged chitosan attaches to the negatively charged bacterial surface, changing the permeability of the membrane (hydrolysis of peptidoglycans), resulting in intercellular component leakage and thus cell death. Henry et al. [50] investigated that chitosan supplementation to beef cattle with a most-forage diet improved the digestibility of neutral detergent fiber, acid detergent fiber, and DM. These authors evaluated the influence of chitosan on in vitro batch cultures and also defined higher production of total volatile fatty acid for batches with chitosan compared to those with monensin. Furthermore, Belanche et al. [51] stated that in a rumen that chitosan transferred the fermentation pattern from the production of acetate towards propionate. Therefore, it is predicted that the supplementation of chitosan to grazing cattle should be beneficial for the digestibility of fiber, pasture intake, and rumen fermentation. Furthermore, the speed of concentrate intake of grazing cattle may change rumen fermentation by suddenly reducing the pH of the rumen and therefore impairing rumen fermentation. Goiri et al. [42] investigated upsurging different concentrations of chitosan in vitro trials and observed that chitosan up surged pH in fermentation batches having concentrate to forage mixture of 20:80. This was conducted to study the effect of higher doses of chitosan feed intake, rumen fermentation, total apparent digestibility microbial protein synthesis, nitrogen utilization. and urea and creatine metabolism of grazing beef cattle.

4. Advantages of Supplementing Chitin and Chitosan in Ruminant Diets

Different research studies revealed that supplementation of both chitin and chitosan enhanced the production performance of dairy cows, and also improved the feed intake and digestibility, rumen fermentation as well as a bacterial community [39][52]. In addition, Abd- Elkader et al. [53] reported that chitosan supplementation in the diet of goats did not affect the total ruminal protozoa count and motility, and no significant effect of chitosan was found on the volatile fatty acid production in the rumen of goat. On the contrary, Wencelova et al. [54] reported that the inclusion of chitosan in the diet of sheep decreased the total ruminal protozoa and improved the rumen fermentation. Zhang et al. [55] concluded that the inclusion of seleno-chitosan increased the growth rate, and wool production and improved the blood parameters of the Chinese Marino sheep. Chitosan supplementation to lambs improved the feed intake, digestibility of neutral detergent fiber, dry matter, and crude protein and upsurged nitrogen balance and production of microbial protein. However, no significant effect was observed on the production performance of the feedlot lambs [56]. In vitro study conducted by Jayanegara et al. [57] showed that the use of chitin from black soldier flies decreased methane emission. Nevertheless, different results obtained could be attributed to the feeding systems, physiological conditions, as well as ruminant species.

5. Future Applications of Chitin and Chitosan in Ruminant Feeding

Earlier studies showed that chitin and chitosan were mainly used in the diet of the different ruminants to improve the dry matter intake, feed digestibility, rumen fermentation, bacterial community, production performance, body weight gain, and other production parameters. However, there is still ample information required to study the effects of chitosan and chitin, particularly on the starved yaks, and to investigate the influence of these additives on blood biochemistry hormones feed intake, feed digestibility, rumen fermentation, microbiota, milk, meat production and growth rate and meat quality of the yaks. In addition, different doses of diets may be used in different ruminants to study the influence of chitin and chitosan on carbohydrate, lipid, and protein metabolism. Even with full findings, furthermore, to the best of researchers' knowledge, no study is conducted to study the supplementation of chitin or chitosan on metabolomics in dairy cows, beef steers, sheep, goats, and yaks.

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