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Review

Role of Chitin and Chitosan in Ruminant Diets and Their Impact on Digestibility, Microbiota and Performance of Ruminants

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Abstract: 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. Recently, 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 hypo-cholesterolemic properties. This review article discusses the results of recent studies focusing on the effects of chitosan and chitin on the performance of dairy cows, beef steers, sheep, and goats. In addition, the effects of chitosan and chitin on feed intake, feed digestibility, rumen fermentation, and microbiota are also discussed. Available evidence suggests that chitosan and chitin used as a feed additive for ruminants including dairy cows, beef steers, sheep, goats, and yaks have useful biological effects, including immune-modulatory, antimicrobial, and other important properties. These properties of chitosan and chitin are different from the other feed additives and have a positive impact on production performance, feed digestibility, rumen fermentation, and bacterial population in dairy cows, beef steers, sheep, goats, and yaks. There is promising evidence that chitosan and chitin can be used as additives in livestock feed and that well-designed feeding interventions focusing on these compounds in ruminants are highly encouraged.

Keywords: chitosan; chitin; ruminants; microbiomes; rumen enhancer; methane; fermentation efficiency

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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].

Therefore, the objectives of the current review paper are to review and discuss the results of different studies which were conducted regarding supplementation of the chitin and chitosan in ruminants such as dairy and beef cattle, sheep, and goats and to understand the effects of chitosan and chitin on production yield, growth performance, wool yield, feed intake and digestibility, rumen fermentation and bacterial community and usage are presented.

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. In this structure, the chitin is insoluble in water and only soluble in common specific organic solvents such as N, N-dimethylacetamide (DMAc)-LiCl [21], hexafluoroacetone or hexafluoro-2-propanol and the chemical structure of the chitin is presented in Figure 1 [25]. 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 [26]. 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 [27].

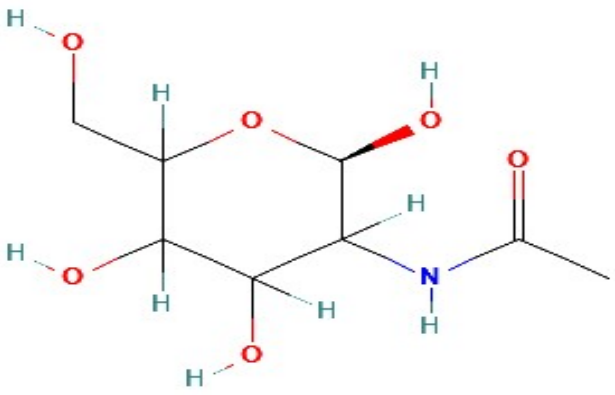

Figure 1. Chemical structure of the chitin; Source Pubchem (https://pubchem.ncbi.nlm.nih.gov/; (accessed on 10 September 2022)).

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 [28]. 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 [29].

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 [30]. In addition, chitin and chitosan have lower toxicity [31]. 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 [32].

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 β(1→4) linkage, while chitosan, a linear polysaccharide composed of two repeated units, D-glucosamine and N-acetyl-D-glucosamine, linked by β-(1→4) linkages. Chitosan is categorized in terms of intrinsic properties such as molecular weight, viscosity, and degree of deacetylation, and the chemical structure of the chitosan is presented in Figure 2 [33]. 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 Stated 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 [34]. 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 [35]. 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 [36]. 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 [37]. 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 [38]. 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 [39]. 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 [40].

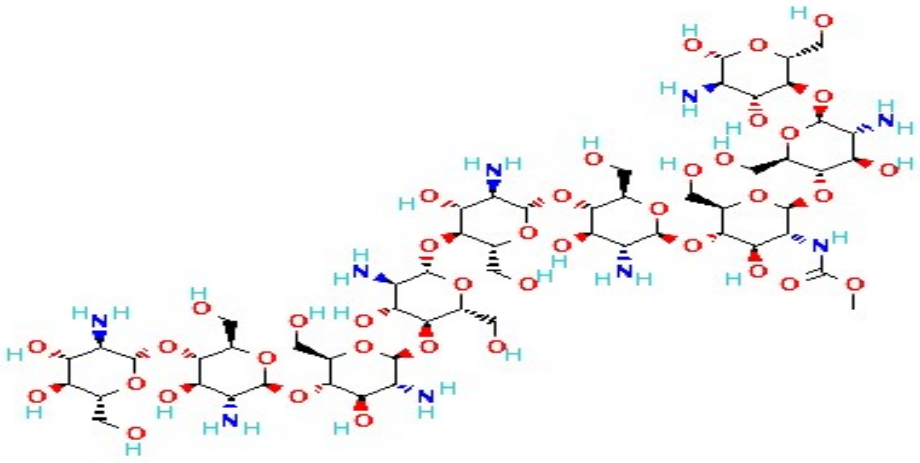

Figure 2. Chemical structure of the chitosan: Source Pubchem (https://pubchem.ncbi.nlm.nih.gov/; (accessed on 10 September 2022)).

3. Chitin and Chitosan Sources

3.1. Chitin

The main source of Chitin is the exoskeleton of mollusks, insects, fungi, and crustaceans. However, the chief chitin source is the shells of the shrimps and crabs [41]. The chitin is normally present in two forms (allomorphs) contingent on the source, these two forms are α and β-forms furthermore in γ-chitin, this form is commonly available in a combined form with either α or β forms than a different form [21]. From these forms, α-Chitin is the most plentiful form which is available and is obtained from the crustacean's exoskeleton mainly crabs and shrimps. While the β-Chitin and γ-chitin can be received from the squid pens and yeast respectively [42]. The α-chitin can be obtained from β-Chitin through an alkaline process followed by passing through water [43]. Numerous methods and procedures are used to obtain the chitin from different sources and earlier research published [44]. The composition of the crustacean shells is proteins (30%–40%), calcium carbonate (30%–50%), chitin (20%–30%), and pigments. The above-mentioned composition of crustacean shells is varying depends on the different species and also from season to season [45]. There are various steps like washing, grinding, and sieving followed by the removal of different minerals like calcium carbonate in dilute acidic acid and known as demineralization then deproteinization (NaOH or KOH) is used to obtain the chitin from the crustacean shells chemically and deproteinization process, enzymatic hydrolysis is used [21]. In addition, it is also reported that different microorganisms are also used for demineralization and deproteinization [21].

3.2. Chitosan

The chitin is radially converted into chitosan through partial or complete de-acetylation of the chitin in solid and dissolved under enzymatic hydrolysis (chitin deacetylase) or alkaline condition in industries. Chitosan is produced from the natural source of chitin, and it affects the production parameters and preparation. Different studies showed that the β-chitin form is higher reactive in N-deacetylation compared to α-chitin [46]. The change in chitin morphology semi-crystalline is due to the chitosan received in a solid-state reaction having a heterogeneous distribution of N-acetyl groups along the molecular chains [37]. The change in the process of chitosan production like temperature, alkali concentration, and alkali ratio to shell also means that the production of chitosan contains different chitosan in molecular weight and N-acetylation degree. Different types of impurities are added in the preparation of the chitosan from the chitin like heavy metals, protein, acid, and alkaline residues. Different studies proposed that the impurities content and different factors like weight-average molecular weight, polydispersity, degree of N-acetylation (DA), and

pattern of acetylation (PA) in commercially available chitosan are unknown [47]. Chitosan microstructure knowledge is vital for understanding the structural properties and activity relationships in them, and distinct importance in this respect is cited in the biomedical use of chitosan [47]. In addition, the biological, synthesized, active, and water-soluble derivatives of chitin and chitosan are also essential.

4. Modes of Actions in the Rumen on Substrates, Microbiomes, Fermentation, Volatile Fatty Acids, pH, Microbial Protein Synthesis, and Methane Mitigation

Chitin and chitosan use as feed additives in ruminants have been well-illustrated and are presented as follows.

4.1. Feed Efficiency, Rumen Fermentation, Volatile Fatty Acid, and Milk Composition and Production

Currently, chitosan has attained great attention in the preparation of different medicine and the preservation of various food items because of its biodegradability, antimicrobial, and nontoxic qualities [48,49]. As already mentioned, the deacetylation of the chitin process is used to obtain the chitosan and it is an N-acetyl-D-glucosamine polymer (natural biopolymer). After cellulose, chitin is the second most plentiful copious organic compound in the world and originated from the exoskeleton of some arthropods and crustaceans. In addition, it is also present in the cell wall of plants [50].

Different research studies on dairy cows investigated the impact of chitosan on rumen fermentation [51,52]. Goiri et al. [53] and Araújo et al. [54] conducted research in sheep, steers, and dairy cows and concluded that chitosan reduces the acetate-to-propionate ratio in rumen fluid respectively. In addition, volatile fatty acid production also reduces biohydrogenation in the rumen [52]. Lately, Del Valle et al. [52] stated that chitosan increased the unsaturated fatty acid levels in milk and also improved the feed efficiency of cows fed a soybean-oil-free diet. Though, when chitosan was supplemented with soybean oil, these researchers stated a negative impact on the milk production of cows. The motives for the latter impact of the chitosan are not clear due to any change in feed intake and feed digestibility, and volatile fatty acid levels in the rumen were noticed between cows provided feed with soybean oil and the diet with soybean oil along with chitosan.

Besides, Zanferari et al. [51] stated that chitosan supplemented with whole raw soybean alters the rumen fermentation and bacterial population in dairy cows and also increased the concentration of the unsaturated fatty acid in milk but reduces the feed intake and feed digestibility, synthesis of microbial protein and milk production. Nevertheless, results indicate that supplementation of chitosan in a diet with no lipid supplementation in dairy cows improves feed efficiency, also increasing the level of the unsaturated fatty acid in milk and *cis*-9,*trans*-11 CLA.

In ruminants, the diet containing chitosan changes rumen fermentation with the chitosan altering pattern of fermentation towards an efficient pathway of energy when added to in vitro trials [55,56]. Goiri et al. [53] and Dias et al. [57] reported that rumen ammonia levels were reduced with the addition of chitosan in the diet of sheep and beef steers diet. In addition, supplementation of chitosan in the diet of dairy cows and beef steers alters the production of volatile fatty acids of less acetic acid to more propionic acid, thus reducing the ratio of acetic to propionic acid [58]. Furthermore, few researchers reported that chitosan inclusion in the diet alters rumen fermentation and upsurges the apparent digestibility. Furthermore, regarding alters in rumen fermentation, some researchers observed that chitosan increased dry matter apparent digestibility, crude protein, and neutral detergent fiber, whereas no impact on feed intake [54,58]. Nonetheless, Mingoti et al. [59] and Dias et al. [57] reported that alike findings were obtained in dry matter apparent digestibility and crude protein, though concurrently detecting a decrease in dry matter, crude protein, and NDF intake. So far, the influence of supplementation of chitosan on rumen fermentation has focused chiefly on a total mixed ration containing corn silage [60]. Jiménez-Ocampo et al. [61] conducted research on chitosan and naringin supplementation

to cross-bred heifers and concluded that both feed additives had no impacts on ruminal fermentation and methane production.

In addition, Kirwan et al. [50] reported that there is no effect of chitosan inclusion on dry matter intake in beef heifers, however, chitosan reduced the total tract digestibility, dry matter, and crude protein, and no effect was noted in the apparent total tract digestibility of the NDF. Chitosan inclusion in the diet of the beef heifers increased the rumen pH and ruminal ammonia concentrations. Supplementation of chitosan had no impact on the concentration of butyric acid in the rumen. Whereas the addition of chitosan in the low protein total mixed ration tended to reduce the acetic acid to propionic acid ratio. Supplementation of chitosan and concentration of crude did not affect the concentrations of the valeric acid, isovaleric acid, and iso-butyric acid.

The chitosan inclusion in the diet decreased the digestibility of DM, OM, and CP, but this did not alter the dry matter intake. This decrease in the digestibility of nutrients was likely because of the antimicrobial quality of chitosan against rumen microbiota [62]. In ruminants, protozoa play a vital role in protein degradation [63], with defaunation typically ensuing in decreased degradation of protein [64]. Regarding chitosan, numerous hypotheses have been planned as the mode of action. The chitosan is polycationic and this is extensively accepted by a different theory, this polycationic nature is because of these positive charges of the protonated amino groups (NH_3^+), which permits it to interconnect with the negative charge of the outer cell membrane of diverse microbiota, producing wide changes to the surface of the cell, leading to leakage of intracellular substances, resulting in the death of cell [50]. The outer peptidoglycan layer is greater reachable in gram-positive compared to gram-negative bacteria, to which the utmost importance of the fibrolytic bacteria belongs. Goiri et al. [53] stated the digestibility of OM reduced in vitro experiment treated with chitosan, indicating activity towards cellulolytic bacteria, though Belanche et al. [56] reported that chitosan inclusion reduced the activity of protozoa and cellulolytic bacteria in the rumen [56], accountable for the reduction in degradation of feed and rumen fermentation rate. Decreasing the solubility of chitosan (<85% deacetylated) and its addition to the diet, can weaken the negative impact on the digestibility of feed [50,56].

The environmental pH in the rumen plays a key role in the antibacterial mode of action of chitosan. Kong et al. [50] reported that when pH is less than molecules pKa (6.3–6.5), chitosan becomes polycationic, which causes electrostatic interaction between the chitosan and the anionic components of the microorganism's surface, though, on the contrary, hydrophobic and chelating effects are accountable for the antibacterial action of chitosan when the environment is above the pKa. The pH in the rumen plays a key role in better performance and stability of the rumen due to its impact on the bacterial population and rumen fermentation products as well as on the normal physiological function of the rumen. The change in the pH of the rumen was comparatively less and would likely not be biologically significant. In the rumen, cellulolytic bacteria are accountable for the digestion of fiber rumen, and the pH of the rumen starts to go down below 6.2 their action starts to reduce [65]. In the rumen, the negative correlation between the total volatile fatty acid level and ruminal pH highlights the pH-decreasing potential of volatile fatty acid accumulation [66]. Chitosan inclusion in the diet did not significantly affect the individual concentration of volatile fatty acid or total volatile fatty acid levels. Though, the digestibility of nutrients was reduced with the addition of chitosan, thus decreasing the production potential of volatile fatty acids. The ammonia has a greater pKa value (9.21) and as significance, almost all ammonia is present in the form of NH_4^+ in the rumen. In rumen production of ammonia can assist the pH regulation by the NH_4^+ disposal. Consequently, excessive ammonia supply from the degradation of the amine terminals in chitosan may elucidate the upsurge in pH of the rumen related to the chitosan addition. In rumen greater levels of ammonia related to feeding the high protein diet were expected due to increased dietary percentages of RDP [67]. As CP increases the diet, there is higher deamination of amino acids released from protein degradation, which increases ammonia. The upsurge in iso-valeric acid related to the low protein diets is a significance of the deamination process

and decarboxylation of the branch-chained amino acids [68]. The inclusion of chitosan in high protein diet upsurged the concentration of ammonia, the similar results were also reported by Araújo et al. [54], where an upsurge in the concentration of ammonia was noted in steers supplemented with chitosan. The upsurge in the ammonia concentration in the rumen with a high protein diet suggests that this was probably due to an extra addition of ammonia from the amine group degradation in chitosan and a lesser uptake of ammonia by the microbes of the rumen, rather than upsurge proteolysis [56]. Kang-Meznarich and Broderick [69] stated that 1.94 to 5 mmol L^{-1} is the optimum level of ruminal ammonia level is satisfactory for the synthesis of microbiota and digestion of fiber, portentous the concentration of ammonia generated in the low protein diets were below the optimum, which might clarify why no changes were found in a concentration of ruminal ammonia between the two low protein diets.

Earlier research investigated that chitosan supplementation in ruminant diets upsurges propionic acid levels in the rumen [58], whereas Araújo et al. [54] reported that chitosan inclusion in the diet reduces the acetic acid levels in the ruminal coupled with the upsurge of propionic acid levels as a result of increased feed intake and nutrient digestibility. The change in the products of the rumen fermentation within the rumen may be a result of the chitosan degradation in the rumen, with the remaining carbon skeleton used by certain bacteria [70]. While supplementation of chitosan did not affect volatile fatty acid profiles in the rumen, the negative impact on the digestibility of nutrients may have potentially pretentious production of volatile fatty acid due to the consequence of inefficient eating and chewing efficiency. On the other hand, the earlier research stated that the concentration of acetic acid: Propionic acid was considerably greater, showing the greater contribution of the NDF from the GS offered, affecting both feeding behavior and volatile fatty acid levels of the animals [71]. Though, the CP concentration affects the volatile fatty acid profile in the rumen. Providing higher concentrations of the CP in the diet resulted in lower acetic acid and higher propionic acid levels in the rumen. Supplementing higher protein diets would provide additional supply to a higher percentage of RDP [67], which has been shown to upsurge the concentration of propionic acid and reduces the level of acetic acid in the rumen [72].

Chitosan is a biopolymer (N-acetyl-d-glycosamide) derived from the deacetylation of chitin—the second most abundant polysaccharide in nature and the major component of exoskeleton from crustaceans and insects [73]. Chitosan has demonstrated antimicrobial activity against several bacteria, fungi, and yeasts [48] and is considered generally recognized as safe by the US Food and Drug Administration since 2012 (GRN#443; FDA, 2012). Chitosan interrelates with the outer membrane proteins instigating bacterial cell membrane disturbance and death of cells [74]. While researchers have investigated that chitosan can be used as a feed additive for silage preservation [37], or to treat metritis [75] and mastitis in dairy cattle [76], Numerous research studies have investigated the impact of Chitosan on rumen fermentation in vivo trials, particularly with dairy cows [52]. Lately, Del Valle et al. [52] demonstrated that chitosan improved the feed intake and upsurge unsaturated fatty acid concentrations in the milk of dairy cows fed a soybean-oil-free diet. Though, when chitosan was supplemented in diets with soybean oil, these researchers reported a negative relative impact on the performance of dairy cows. The motives for the latter relative impact are not clear due to no change in feed intake and digestibility, and volatile fatty acid concentration in the rumen was detected between cows supplemented diet with soybean oil and a diet with soybean oil along with chitosan.

Zanferari et al. [51] stated that chitosan decreased the feed intake (DM, OM, CP, NDF, and NFC) and digestibility of nutrients (DM, OM, NDF, and EE) in dairy cows. Chitosan also upsurges the ruminal pH and propionate molar proportion than cows fed no chitosan. Though, chitosan reduced acetate and valerate concentrations in the rumen and acetate to propionate ratio and acetate molar proportion in the rumen. No chitosan by WRS interaction effects was observed on ruminal bacterial populations assessed in this experiment. Chitosan decreased ($p = 0.001$) the relative population of the *Butyrivibrio* group in comparison with the other treatments. Diets containing WRS negatively affected the rumen bacterial

populations from the *Butyrivibrio* group and *F. succinogenes*; however, WRS increased the relative rumen bacterial population of *S. bovis*. The provision of chitosan in a diet with no WRS and relatively low EE has not altered nutrient intake in ruminants [53,54,58]. On the other hand, chitosan provision to cows fed a diet containing soybean oil decreases nutrient intake [52]. In agreement with the latter study, the combination of chitosan and WRS decreased the intake of nutrients except for EE. In this experiment, the reduced feed intake of cows fed chitosan + WRS is likely related to changes in ruminal fermentation and negative effects on nutrient digestibility. Although the exact mechanism of chitosan on the gastrointestinal tract of cows is not fully understood, changes in ruminal fermentation and nutrient utilization [54], are very similar to those observed when feeding ionophores [77]. Similar to ionophores and chitosan, unsaturated fatty acid affects ruminal metabolism by altering the ruminal microbial population [78]. In the results of Zanferari et al. **[51]**, changes in ruminal fermentation were observed when provided to cows with either chitosan or WRS including an increase in a ruminal molar proportion of propionate and a reduction in acetate proportion and acetate to propionate ratio. Changes in rumen microbial population (e.g., decreases in gram-positive bacteria and ciliate protozoa and an increase in gram-negative bacteria) and decreased OM and NDF digestibility can be observed when lipid supplementation exceeds 5% of dietary FA. In addition, the inclusion of lipids in diets increases the release of intestinal peptides regulators that suppress intestinal motility and feed intake of cows [79]. Although diets did not exceed 5% diet EE, decreases in digestibility of OM and NDF, and reduced NDF intake was observed in the current experiment. The WRS has a relatively lower ruminal availability of fatty acid [80] but seemed to affect fiber digestion. According to NRC [81], the adverse effects of oilseeds on ruminal fermentation depend on diet composition besides the inclusion level, whereas the negative effects of oilseeds are more evident in corn-silage-based diets with low forage proportions. Specific PUFA, such as linoleic acid (present in high amounts in WRS), have toxic effects on cellulolytic and butyrate-producing bacteria [82].

The combination of chitosan and WRS decreased the total tract digestibility of EE. Earlier studies evaluating CHI dietary supplementation did not report differences in EE digestibility of lactating cows [52]. Nevertheless, Zhang et al. [83] observed that CHI has hypolipidemic activity in high-fat diet-fed mice with a reduction in plasmatic and hepatic levels of lipids and an increase in lipids fecal excretion effects attributed to a decrease in intestinal fat absorption. Chitosan has high adherence capacity to fat in vitro due to its polycationic structure [84]. A similar response to chitosan was observed in broilers, which exhibited lower ileal digestion of fat, and no changes in CP and starch digestibility [85]. Authors from the latter study speculated possible effects of CHI on digestive viscosity that could impair the activity of intestinal lipases in feed particles or glucosamine groups in chitosan acting as a chelator of lipid micelles and the mode of action of chitin and chitosan in ruminants and their sources are mentioned in Figure 3.

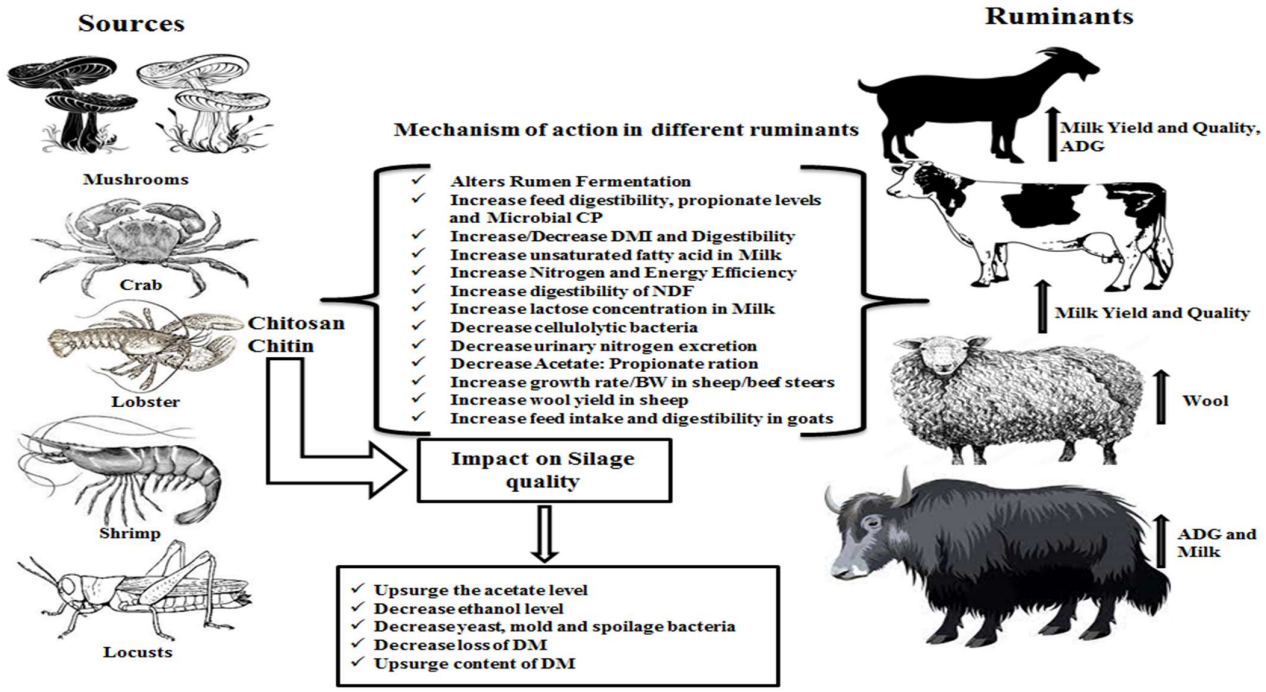

Figure 3. Schematic diagram of chitin and chitosan sources and their mode of action in ruminants and quality of silage.

4.2. Bacterial Population and Methane Mitigation

Chitosan decreased or tended to decrease bacterial populations related to ruminal biohydrogenation (*Butyrivibrio* group and *B. proteoclasticus*), whereas WRS supplementation decreased *F. succinogenes* and *Butyrivibrio* group, which corroborates with the decreased digestibility of NDF in cows fed WRS. Feeding WRS increased the *S. bovis* population. Although the latter specie is often associated with lactate production in the rumen, the study of Hudson et al., 2000 [86] indicated that *S. bovis* easily hydrate linoleic acid to 13-hydroxy-9-octadecenoic acid. The FA hydration competes with biohydrogenation processes in the rumen [86], which could be related to the relatively low concentration of intermediates of biohydrogenation in the milk of cows fed WRS. Zanferari et al. [51] concluded that the addition of chitosan in the diet of dairy cows with WRS changes rumen fermentation and microbiota community, up surging the content of unsaturated fatty acid in milk, however, reduces feed intake and feed digestibility, milk production, and microbial protein synthesis. But research also shows that chitosan supplementation in the diet of dairy cows with no lipid improves feed efficiency, in addition to surging content of unsaturated fatty acid milk content and *cis*-9,*trans*-11 CLA. Supplementing WRS as an alternative to soybean oil as a fat source did not evade the negative relation impact of chitosan on the milk performance of dairy cows and the unchanged fatty acid profile in milk.

Belanche et al. [56] reported that in-vitro trial chitosan reduced the methane emission (42%) however, it changes the mode of action. Chitosan has antimicrobial qualities that alter the structural bacterial community and transferred the rumen fermentation pattern to the production of propionate acid which clarified and reduces the emission of methane. The greater activity of amylase noted in fermenter-supplemented chitosan recommended chitosan could also be moderately hydrolyzed and used as a bacterial population of the rumen. In addition, the 5% inclusion of chitosan in dairy cows increased methane production and also increased the volatile fatty acid concentration and this was escorted by reducing ammonia levels and pH. In dairy cows, samples obtained 2 h afterward diet had greater levels of total lactate, L-lactate, and acetate molar proportion, along with lesser proportions of propionate and butyrate concentrations. Furthermore, the 5% chitosan inclusion reduced the molar proportion of the acetate and upsurge the molar proportion

of the valerate. Furthermore, chitosan increased propionate proportion and reduced the butyrate, isobutyrate, and isovalerate proportions. The addition of chitosan in the diet upsurges the concentration of ammonia, mainly at 2 h after feeding.

qPCR results showed that chitosan alters DNA levels of microbiota and methanogens, and lowest with the lowest levels were noted at 2 h subsequently feeding. However, no other effect of feed was observed. Furthermore, when compared with the control group IVY supplemented group reduced levels of anaerobic fungi, whereas relative methanogens abundance decreased compared to the total population of bacteria when chitosan was supplemented. The concentration of the protozoa in the fermenters endured below (500 cells mL^{-1}) and did not influence by the research treatments. The population of protozoa was chiefly composed of subfamily Entodiniinae 96.1% $\pm$ 4.1%, followed by Diplodiniinae 3.5% $\pm$ 4.2% and Holotrichs 0.4% $\pm$ 0.7%, and these proportions were not influenced by the treatment groups.

In terms of bacterial diversity, chitosan did not affect the Good's coverage and Chao index, representing that the depth of sequencing was similar in all groups. Chitosan reduced indexes of the Shannon and Simpson showing a reduction in the diversity of bacteria. Furthermore, the lesser Evenness values in chitosan feed showed the availability of very plentiful species together with few species, though in control and there was a higher resemblance in the abundance across bacterial species. At the family level in the phylum Firmicutes supplementation of chitosan increased the family *Veillonellaceae* abundance and included the *Mistsuokella, Schwartzia*, and *Megasphaera* generaas. The *Escherichia coli* species were not affected by supplementation of the chitosan which has antimicrobial properties meanwhile levels of the post-inoculation pathogen in the vessels followed the same decay pattern to all feeds [56].

The activities of chitosanase and chitinase have been defined in some ruminal protozoa and bacteria [87]. Especially, for the degradation of the chitosan, *Clostridium tertium ChK5* two strains of the bacteria in the rumen have been recognized as the utmost active species of bacteria [88].

Moreover, Kong et al. [48] reported that chitosan and chitooligosaccharides have vital antimicrobial properties. Because of these properties, chitosan is not recommended in high doses in order to avoid any side effects on the normal function of the rumen [55].

In rumen fermentation chitosan upsurge the concentration of the lactate and the bacteria which utilize the lactate including *Veillonella, Selenomonas*, and *Magasphaera* genus could elucidate a certain degree of upsurge propionate production as a fermentation product. Therefore, chitosan did not change the production of total volatile fatty acid but considerably transferred the pattern of fermentation from acetate to propionate [89]. This fermentation change pattern was the chief anti-methanogenic motorist for chitosan amplifying 2/3 of the noted reduction in methanogenesis. Consequently, chitosan reduced the methane and volatile fatty acid ration showing that high energy was taken for products of fermentation. Araujo et al. [54] concluded that up surging of chitosan addition (150 mg/g/BW) in steer's feed diet stimulated a linear upsurge in propionate production in the rumen, glucose level in blood and digestibility of dry matter excluding dry matter intake. However, it has been reported that intraruminal infusion of propionate can harm feed intake and milk fat concentration [90].

Belanche et al. [56] stated that chitosan addition to the diet did not affect the population of bacteria, protozoa, and methanogens. In addition, no impact of the chitosan was observed on the volatile fatty acid production and the activity of different enzymes, proposing that rumen fermentation was not affected by the inclusion of the chitosan in feed. The chitosan polymers which have a chief antimicrobial mode of action are defined due to their effect on the cell permeability because of the interaction between the polycationic chitosan ($R\text{-}NH_3^+$), and the electronegative charges on the microbial surfaces when the pH of the rumen is below the molecule's pKa (6.3–6.5) [48]. These electrostatic relations endorse peptidoglycan hydrolysis in the wall of the microorganism and eventually cell lysis [91]. Subsequently, the layer of the peptidoglycan is more reachable in gram-positive

than in gram-negative bacteria, chitosan microbial property tended to cause a reduction in the abundance of the former bacterial group courtesy of the latter one. Consequently, chitosan alters important shifts in the bacterial community structure, encouraging a less diverse community. Certainly, chitosan reduced the abundance of *Fibrobacter* and *Firmicutes*, however, upsurge in *Proteobacteria* (+0.71 log) and *Bacteroidetes* (+0.14 log) abundances, such as most of the bacteria belonging to amylolytic bacteria. The change of amylolytic bacteria to fibrolytic bacteria could elucidate the higher activity of amylase noted in vessels fed chitosan feed, and therefore, the greater production of the abundance of propionate and lactate. Furthermore, the results showed that variations in bacterial community structure persuaded by chitosan were positively associated with upsurging lactate levels and low pH, proposing that the chitosan mode of action depends on pH. The characteristics of hydrophobic and chelating reactions have been defined as key facets of the antimicrobial activity of chitosan when pH is higher than pKa [48].

Earlier research concluded that chitosan (136 mg/kg-1BW in vitro experiment forage diet) inclusion in the diet has a negative impact on the total tract NDF and dry matter digestibility in sheep [53,62]. The main cause could be an adverse effect of chitosan on protozoa in the rumen [62]. Belanche et al. [56] further stated that chitosan has also a negative effect on the abundance of cellulolytic bacteria in the rumen, including *Butyrivibrio, Ruminococcus* and *Fibrobacter*, and hemicellulolytic bacteria including *Eubacterium*. The finding stated that this change in the bacterial community tended to be positively related to the level of total bacteria and could recompense the lower abundance of fibrolytic bacteria. So, the noted reduction of gram-positive bacteria and upsurge in the gram-negative belongs to fibrolytic bacteria and amylase activity appears to support the idea that the mode of action of chitosan depends on electrostatic interaction with the cell wall of bacterial [91]. Otherwise, the potential chitosan hydrolysis occurred by amylases [92] could also favor the proliferation of those bacteria able to use chitosan as an energy source leading to alter in rumen fermentation product and bacterial community structure. Therefore, more research is required to investigate which bacteria species in the rumen are efficiently capable of utilizing chitosan as an energy source.

5. 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 [52]. 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 [93]. 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 [94]. Goiri et al. [55] 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 [48]. Goiri et al. [53] 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. [53] 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. [95] 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. [57] 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 [73]; lately, chitosan can be used in silage preparation as an inoculant due to its antimicrobial property [37] and provided as a rumen modulator to confined beef cattle [54] and dairy cows [58] 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 [48]. Dias et al. 2017 [57] 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. [96] 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. [56] 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. [55] 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 study 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 (Table 1).

Table 1. Effects of the chitosan/chitin inclusion in diets of ruminants on dry matter intake, digestibility, and rumen fermentation.

Chitosan/Chitin	Trial Animal Model and Duration	Dose	Substrate/Feed	Results	Reference
Chitosan	Sheep lambs Santa	0, 136, and 272 mg/kg of BW	Diet	No effect on feed intake, BW, and FC. Improved pH and fatty acid composition in meat, improved meat quality	[97]
90% deacetylation	Dairy cows	0 and 2%	TMR	No effect on nutrient digestibility, affect rumen fermentation pattern, reduced methane production	[98]
Chitosan	Balady male goats (120 days)	0.2% (2 kg/ton concentrate)	Concentrate diet 3%	Chitosan increases ruminal ammonia nitrogen, no effect on VFA levels, no effect on total protozoal count	[99]

Table 1. *Cont.*

Chitosan/Chitin	Trial Animal Model and Duration	Dose	Substrate/Feed	Results	Reference
Chitosan sucinate deac.75%	black and white breed dairy cow	0% and 2%	Feed	Improved blood physiological condition and metabolism	[100]
Chitosan Deac. 85%	Dairy cows	0.500, 1000, 1500, 2000 mg/kg	TMR	Improved DMI intake, milk production, antioxidant capacity,	[101]
Chitosan	Sheep (45 days)	0 and 136 mg/kg of BW	Alfalfa hay and concentrate at 50:50	Chitosan reduce NDF apparent digestibility, ruminal NH_3-N concentration and modulates ruminal and fecal fermentative activity	[52]
Chitosan	Feedlot lambs Santa Inês crossbred sheep (90 days)	136 mg and 272 mg chitosan/kg BW	Roughage to concentrate ratio at 50:50	Chitosan did not affect the DM intake, improved digestibility. No effect was observed on weight gain, carcass weight not influenced	[102]
Chitosan	dairy cow (84 days)	0, 50, 100 and 150 mg/kg BW	Corn Silage-concentrate 60:40	Chitosan shifted rumen fermentation, improved nutrient digestibility andpropionate concentrations	[53]
Chitosan	dairy cow (84 days)	0 and 4 g/kg ofDM	Corn silage-to con-centrateratio50:50	Improved feed e ciency, increased milk UFAconcentration	[51]
Chitosan	Cattle (25 days)	0, 2.0 g/kg chitosan (CH)of DM. Wholeraw soybean(WRS) 163.0 g/kg DM; andCH + WRS	Corn silage toconcentrate ratio50:50	Chitosan improved nutrient digestion anddecrease DMI and reduce nitrogen excreted infeces	[26]
Chitosan	Cattle (105 days)	0, 400, 800, 1200 or 1600 mg/kg DM	Grazing Urochloabrizantha andconcentrate at 150 g/100 kg of LW	Chitosan increased DMI and digestibility, propionate concentration and microbial crudeprotein	[56]
Chitosan	Dairy cow (84 days)	50, 100 and 150 mg/kg BW	Corn silage toconcentrate ratio50:50	Improved nutrient digestibility withoutaltering productive performance of dairy cows	[58]
Chitosan	dairy cow (98 days)	0, 75, 150, 225 mg/kg BW	Corn silage toconcentrate ratio63:37	In dairy cattle works like a modulator ofrumen fermentation, increasing milk yield, propionate and nitrogen utilization	[51]

Table 1. *Cont.*

Chitosan/Chitin	Trial Animal Model and Duration	Dose	Substrate/Feed	Results	Reference
Chitosan	Cattle (21 days)	0.0, 0.5, and 1.0% of DM	High-concentrate (85%) Lowconcentrate (36%)	In vivo: No effect on enteric methaneemissions. In vitro: Low concentrate substrateincreased methane production	[95]
Chitosan deace. 95%	HF cross 10 days	10 g kg^{-1} DM	TMR	No effect on nitrogen excretion, reduced nutrient digestibility	[50]
Chitosan deace 92%	Cross breed Heifers	0, 1.5, 3 g/kg DMI	TMR	No effect on rumen fermentation, methane production	[61]
Chitosan	Cattle (84 days)	150 mg/kg BW	Maize silage:concentrate ratio50:50	Chitosan increase the digestibility and reduceacetate to propionate relation	[59]
Chitosan	Dairy cow (92 days)	0 or 4 g/kgchitosan (CH)or Whole RawSoy-bean(WRS) of DM	Corn silage:concentrate ratio50:50	Improved ruminal fermentation, increased milk content of UFA, decreasesnutrient intake, digestibility, microbial proteinsynthesis, and milk yield. CH in diets with nolipid supplementation improves feedefficiency of lactating cows	[50]

DM: Dry matter; DMI: Dry matter intake; OM: Organic matter; BW: Body weight; UFA: Unsaturated fatty acids; IVDMD: In vitro dry matter digestibility.

6. 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 [51,52]. In addition, Abd- Elkader et al. [99] 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. [62] reported that the inclusion of chitosan in the diet of sheep decreased the total ruminal protozoa and improved the rumen fermentation. Zhang et al. [102] 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 [103]. In vitro study conducted by Jayanegara et al. [104] 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.

7. 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 our knowledge, no study is conducted to study the supplementation of chitin or chitosan on metabolomics in dairy cows, beef steers, sheep, goats, and yaks.

8. Conclusions

Based on available evidence, there is a strong impression that the inclusion of chitin and chitosan in diets of ruminants including dairy cows, beef cattle, goats, and sheep has a beneficial effect on meat and milk production, feed intake, and digestibility, rumen fermentation, rumen pH, bacterial diversity, growth rate, and wool yield. Their effects can be remarkable from a nutritional point of view, especially their ability to protect against degradation in the rumen, production of less ammonia nitrogen, and greater ability to bypass protein to the lower-gut. These qualities add to improved fermentation in the rumen by producing more propionic acid and less methane. Most importantly, enhancing ruminant production i.e., meat, milk, as well as wool yield in small ruminants. Besides, chitin and chitosan have immune-modulatory and antimicrobial properties when used as feed additives.

There is promising evidence that chitosan and chitin can be used as additives in livestock feed and that well-designed feeding interventions focusing on these compounds in ruminants are highly encouraged.

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