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Marine by-products and insects as a potential chitosan source for ruminant feed additives

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Abstract: Chitosan is a hydrophilic polysaccharide produced from chitin that has a wide range of applications. Chitosan has several functions as an anti-microbial, methane reducer, and protein protective agent. Based on this function chitosan has been explored for its potential as a feed additive. Moreover, source and extraction technique have potentially affected the yield and degree of deacetylation (DD) of chitosan products. The present review provides information on various chitosan isolation processes in marine by-products and insects and the result of their DD and yield. Chemical isolation processes are still popular in industries compared with biological processes based on their DD and yield. Chitosan properties and yield from insects are comparable with those of commercial chitosan derived from a marine by-product. The application of chitosan as a feed additive is also highlighted in this review. Moreover, chitosan as a feed additive has the capability to decrease CH_4 production, increase propionate production, reduce the acetate/propionate ratio, and improve nutrient utilization efficiency, and animal performance. Chitosan has the potential to be a beneficial natural and plentiful feed additive, particularly for reducing enteric methane emissions.

Keywords: crustacean by-product; insect; chemistry extraction; biology extraction; methane mitigation; productivity enhancer; antimicrobial activity

N-acetyl-D-glucosamine and D-glucosamine are two repeating units of a linear polysaccharide linked by -(14)-linkages linked of *N*-acetylglucosamine (GlcNAc) units known as chitosan (Yadav et al. 2019; Hahn et al. 2020). The non-enzymatic process is discarding R–$NHCOCH_3$ residue and processing it at high temperatures with a strong alkali to obtain chitosan common as deacetylation (Goy et al. 2009; Vilar et al. 2016). Chitosan is prevalent in the exoskeletons of insects, molluscs, crustaceans, fungi, and some algae, but it is mostly acquired from marine crustaceans (Kaya et al. 2015a; Jimenez-Ocampo et al. 2019).

Conventional chemical extraction is a very common method to obtain chitosan. Because it can produce higher chitosan yield among other methods, it is also easy to hands-on (Beaney et al. 2005). This method consumes more time, it also

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uses a lot of energy and water, and it also produces a lot of caustic waste (Beaney et al. 2005; El Knidri et al. 2016). This process presents some drawbacks since it is expensive and environmentally unfriendly (Tan et al. 2020). Several methods of chitosan extraction have been developed with the concept of being more energy and water efficient and also environmentally friendly. Microbial activities, enzyme methods, and irradiation methods using microwave and ultrasonic chambers (sonicator) are some new extraction methods widely developed to replace conventional chemical extraction methods (Arbia et al. 2013). Until now, biotechnological chitin extraction has not been used on a large scale, however, this eco-friendly technique could be a useful pre-treatment for producing high-quality chitin by reducing the usage of corrosive reagents and trash disposal costs (Beaney et al. 2005; Arbia et al. 2013).

Chitosan with its various functions is being explored for its potential as a feed additive agent in ruminants. Due to its being non-toxic, biodegradable, and biocompatible, chitosan is commonly used as a component and encapsulant in food, biomedical, and agricultural fields. Chitosan and its derivatives have been used as a feed additive due to important intrinsic functional qualities such as anti-bacterial and anti-inflammatory activities (Abidin et al. 2020). Feed additives cannot be considered dietarily essential to the animal because there are no nutrients. They have been shown to increase the efficiency of feed intake, nutrient utilization, animal health, and growth (Van Saun 2013). Natural compounds must meet the following criteria: they must be safe for usage in humans and animals, be effective over time with various raw feedstuffs, be inexpensive to minimize ruminant emissions, and boost livestock output (Jimenez-Ocampo et al. 2019). Chitosan has been demonstrated to affect feed intake, digestion, ruminal fermentation, and the formation of enteric methane (Harahap et al. 2020). Several additives are added in the making of silage to improve the quality of silage, reduce methane emission, and prevent rot in the silage product (Gandra et al. 2016; Seankamsorn et al. 2019). Several kinds of research showed the ability of chitosan in decreasing methane emission (Zanferari et al. 2018; Seankamsorn et al. 2019; Harahap et al. 2020).

As a result, the present review compares the degree of deacetylation and yield as the primary quality parameters of chitosan produced by chemical extraction to chitosan extracted by a biological method, particularly from marine by-products and insects, and reveals the gap between these extraction methods. The paper also emphasizes chitosan applications as feed additives based on distinct chitosan functions and mechanisms.

Chitin-chitosan

Chitin is a structural homopolysaccharide whose structural formula contains nitrogen, with a polymer chemical structure of -*N*-acetyl-D-glucosamine. The units of -*N*-acetyl-D-glucosamine are in the form of pyranose, or β-(1→4)- linked 2-acetamido-2-deoxy-β-D-glucopyranose units and partially of β-(1→4)-linked 2-amino-2- deoxy-β-D-glucopyranose with the chemical formula $(C_8H_{13}O_5N)_n$ and can only be soluble in concentrated mineral acids (El Knidri et al. 2018). Chitin chains are associated with each other by strong hydrogen linkages between N–H group of one chain and C=O group of the neighbouring chain, which makes chitin insoluble in water (soluble only in concentrated mineral acids). Because of its hydrogen bonding between molecules and solid structure, chitin is insoluble in water and other ordinary solvents (Marei et al. 2016). In each ring of the chitin molecule, there is an acetyl group (CH_3–CO) on the second carbon atom (Figure 1). It has a rather high molecular weight. The polycationic polymer is found in insect, crustacean, mollusc, fungal cell

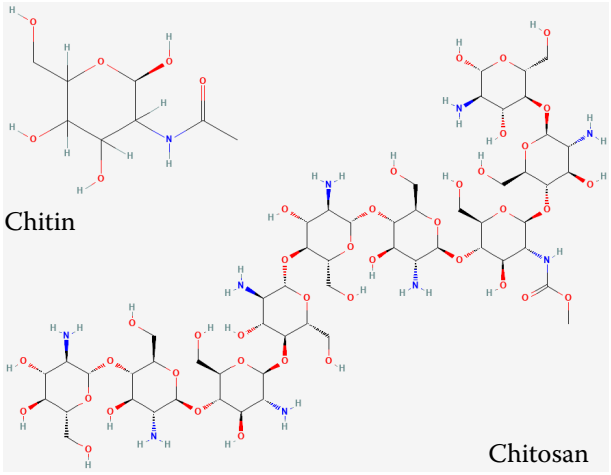

Figure 1. Chemical structure of chitin and chitosan
Source: Pubchem (https://pubchem.ncbi.nlm.nih.gov/; Accessed: August 14, 2022)

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walls, and some algae structural exoskeletons but it is mostly produced from the exoskeleton of marine crustaceans (Kaya et al. 2015b; Marei et al. 2016; Jimenez-Ocampo et al. 2019). X-ray diffraction can reveal chitin polymorphism, revealing three crystalline forms α, β, and γ, which differ in the degree of hydration, the number of chains per cell, and unit size (Santos et al. 2020). Furthermore, polymorphic forms of chitin with different orientations of the microfibrils are classified by intrinsic qualities including viscosity, molecular weight, and deacetylation degree (Liu et al. 2012; Marei et al. 2016; Jimenez-Ocampo et al. 2019). The most frequent form is -chitin; it is found in arthropod exoskeletons with antiparallel polymeric chains, allowing for the formation of multiple hydrogen bonding between and within chains, resulting in a dense substance like lobster, crab, krill, and insect cuticle (Yadav et al. 2019; Mohan et al. 2020). The disposition of β-chitin is parallel, and it is found in animals that are flexible and resistant, such as squid pen and *Aphrodite chaetae*, and can be transformed into the α-chitin form (Santos et al. 2020). The γ-chitin has a combination of both positions seen in *Ptinus* beetles and *Loligo* squid, and the main distinction between these allomorphs is their structure (Liu et al. 2012; Marei et al. 2016; Jimenez-Ocampo et al. 2019; Santos et al. 2020). It has been reported that the crystallinity, purity, and arrangement of the polymer chains of chitin extracted from a variety of sources are influenced by the source as well as the quantity of chitin in the source and that the crystallinity, purity, and chain arrangement of the polymer chain vary depending on the source origin (Liu et al. 2012; Marei et al. 2016) investigated related to the chitosan antibacterial properties (Shahidi et al. 1999).

Chitin has numerous applications and is more effective when it is converted into chitosan (through partial deacetylation under alkaline conditions) (Younes and Rinaudo 2015). Chitosan is a natural biopolymer in the form of a linear polysaccharide composed of β-(1-4-linked D-glucosamine and *N*-acetyl-D-glucosamine), which is the result of chitin deacetylation by a strong base (usually using 50–60% NaOH), by removing the acetyl group (CH_3–CO) from the molecule, allowing it to disintegrate in acid and water solutions. Chitosan is produced by eliminating a number of acetyl groups (CH_3–CO) from chitin to make the molecule soluble in most dilute acids. The acetyl component of the polymer is what distinguishes chitin from chitosan. The most useful chitin derivative is chitosan, which has a free amino group. The distinction between chitosan and cellulose is that chitosan has an amine ($–NH_2$) group in the C-2 position, whereas cellulose has a hydroxyl (–OH) group (Hajji et al. 2014; Abidin et al. 2020). In each ring of chitosan there is an amine group (NH) on the second carbon atom (Jimenez-Ocampo et al. 2019; Yadav et al. 2019; Hahn et al. 2020) (Figure 1). Each D-glucosamine unit has a free amino group, which can take on a positive charge and provide chitosan essential features including solubility and antibacterial activity. These groups form an excellent chelating ligand that can bind to a variety of metal ions and electrostatically adsorb the dye anions. Furthermore, these amino groups may be protonated, resulting in chitosan solubility in dilute acidic solutions (El Knidri et al. 2018). Chitosan is a non-toxic, biodegradable biopolymer that is made from the deacetylation of chitin (Pereira et al. 2019; Harahap et al. 2020). Chitosan is a naturally occurring, positively charged polysaccharide with a pH of 6.3–7 (Chung et al. 2004). Chitosan is the most common derivative, which is produced by partially deacetylating chitin to make it soluble in acidic aqueous solutions and act as a cationic electrolyte when the degree of deacetylation (DD) is greater than 0.5 and DD has also been used to differentiate chitosan from chitin (Marei et al. 2016; Cheng et al. 2020). It is also utilized in biomedical research, agriculture, genetic engineering, food business, pollution control, water treatment, paper manufacturing, and photography, among other things (Casadidio et al. 2019). Previous *in vitro* research in ruminant nutrition indicated that chitosan could alter ruminal fermentation by altering the VFA profile and raising the propionate concentration (Goiri et al. 2010).

Potential chitin-chitosan source (marine by-product and local insect) and its extraction

Chitin-chitosan is the second most prevalent polysaccharide in nature according to a prior study. Insects, crustaceans, especially marine crustaceans, molluscs, fungi, and certain algae all have chitin-chitosan in their structural exoskeletons (Kaya et al. 2015b; Jimenez-Ocampo et al. 2019). In this paper, we focused on insect and marine crustaceans as potential sources of chitosan and its isolation method.

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The seafood processing industry generates a huge amount of scrap due to the low biodegradation rate (Yadav et al. 2019). If not treated properly, it might harm human health, biodiversity, and the environment (Allegretti et al. 2018; Dicke 2018) while the insect is widespread throughout the world (Kaya et al. 2015c). In some countries like Thailand, Uganda, Mexico, China, and some regions of Indonesia, especially grasshoppers *Valanga nigricornis* (Burm.) commonly became a daily menu of dishes, except for the head and feet. But in some areas insects are known plant pests. On the other hand, increased demand for animal-derived goods necessitates a rise in feeding raw materials, and sustainable future raw materials are required to suit the needs of a market that is increasingly concerned about environmental issues (Allegretti et al. 2018; Dicke 2018). This marine by-product and also insect can be explored as a chitin substrate and chitosan production. The most important step in obtaining chitin is the extraction procedure from natural sources. The degree of deacetylation, purity, molecular weight, and polydispersity index of purified chitin are all influenced by the parameters and conditions of extraction. All of these features have a significant impact on the use of chitin in a wide range of applications (Yadav et al. 2019). Figure 2 illustrates the extraction of chitin derived from natural resources and its transformation into chitosan.

There are two main processes of chitin extraction, demineralization, and deproteinization (Philibert et al. 2017). Chemical demineralization is a minerals removal procedure, mainly calcium carbonate to calcium chloride, with carbon dioxide released (Mohan et al. 2020). Acid treatment with hydrochloric acid, nitric acid, sulphuric acid, acetic acid, and formic acid is often used (Yadav et al. 2019; Abidin et al. 2020; Hahn et al. 2020; Mohan et al. 2020). While deproteinization is a step breakdown of chemicals between chitin and proteins to depolymerize the biopolymer, chemicals are used. Due to the breakdown of chemical links between proteins and chitin, the deproteinization procedure is highly challenging (Mohan et al. 2020). At various temperatures and treatment times, NaOH (a preferred reagent) was used (Yadav et al. 2019; Abidin et al. 2020; Hahn et al. 2020; Mohan et al. 2020). The efficiency

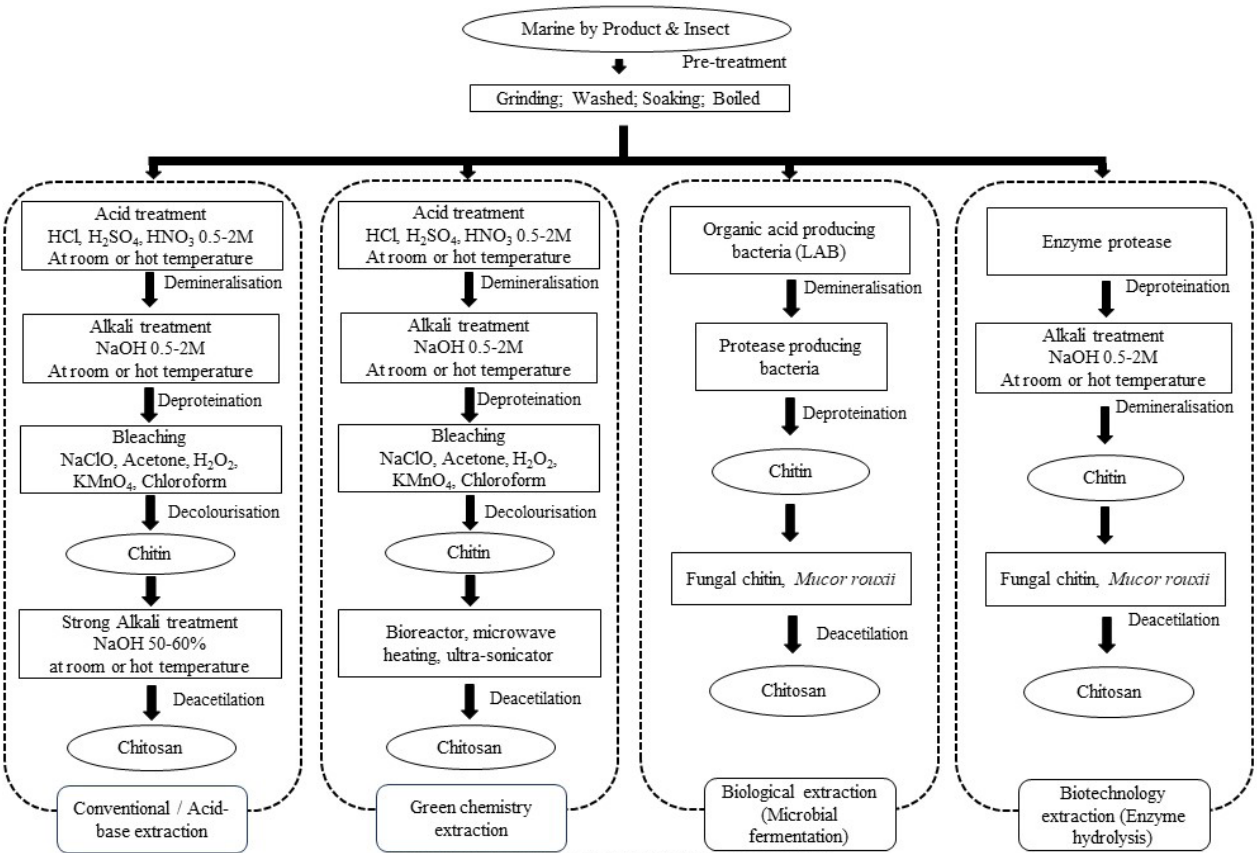

Figure 2. Producing chitosan by several processing methods

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of the deproteinization process is influenced by several elements such as temperature, NaOH concentration, and reaction time (Paulino et al. 2006; Kaya et al. 2014a; Mohan et al. 2020). If a colourless result is desired, an additional step called decolourization is required. To remove colours like carotenoids, an organic solvent mixture of sodium hypochlorite, acetone, potassium permanganate, or hydrogen peroxide was used (Yadav et al. 2019; Abidin et al. 2020; Hahn et al. 2020; Mohan et al. 2020). Chitosan is made from chitin that has been deacetylated (deleting the acetyl groups from the chitin polymer). Deacetylation is also a stage in the functionalization of chitin into a variety of derivatives for use in the chemical and pharmaceutical industries (Mohan et al. 2020). The resulting chitosan contains a high percentage of free amino groups (NH_2), which serve as active sites for a variety of chemical reactions, making it a flexible polymer that may be modified and used in a variety of ways (Hahn et al. 2020). Process alkalis or acids are used to deacetylate chitin in the chemical procedure of deacetylation. Because glycosidic linkages are sensitive to acid, a strong alkali is suggested as a superior option (Yadav et al. 2019; Abidin et al. 2020; Hahn et al. 2020; Mohan et al. 2020).

Physicochemical and/or spectroscopic data can be used to make a broad comparison with commercialized sources in terms of structure, chemical content, and purity. The primary methods for the characterization of marine by-product and insect-based chitin and chitosan are summarized in Table 1. Table 1 shows the chitin-chitosan source, the extraction method of chitosan, also yields and DD. One of the most important aspects of extracting chitin and chitosan from the source is yield (Mohan et al. 2020). Chitin-chitosan yield has different results among species. The highest chitosan yield was in the short-horned grasshopper (*Schistocerca gregaria*), cicada slough, *Portunus pelagicus,* shrimp waste, Colorado potato beetle adults, *H. piceus*, *A. bipustulatus*, *R. linearis*, *N. glauca*, *A. imperator*, crab (*Carcinus mediterraneus*), grasshopper, Colorado potato beetle larvae, house cricket, *A. aquaticus*, silkworm chrysalis, mealworm, and cuttlefish (*Sepia officinalis*) bones with conventional acid-base reaction methods (chemistry extraction) for almost any method, while some of them use a biological extraction method with a fairly high yield using this method. Some insect results in a chitosan yield of 2.5–36.4%, while the marine source results in 1.2–22.4%. Research shows that chitin crystallinity, purity, and polymer chain arrangement change depending on the source origin and the amount of chitin contained in the source, and this has been found for chitin isolated from a variety of sources (Liu et al. 2012; Marei et al. 2016). A similar amount of chitin may be extracted from crustacean shell fragments and aquatic creatures to make chitosan, which is produced from insects. It is safe to say that chitosan derived from insects is not the only option (Mohan et al. 2020).

To differentiate chitosan from chitin, the degree of deacetylation (DD) has also been used. When the DD exceeds a predetermined threshold (such as 50%), it is referred to be chitosan (Cheng et al. 2020). For completely soluble materials, several methods, such as potentiometric titration, conductometric titration, acid-base titration, and FT-IR, have been developed for the measurement of DD in chitin and chitosan (Mohan et al. 2020). The DD ranged from 44.11 to 98% depending on the species. Short-horned grasshopper (*Schistocerca gregaria*), honey bee (*Apis mellifera*), beetles *Calosoma rugosa*, Pacific white leg shrimp (*Litopenaeus vannamei* Boone) shells, and the larvae of blowfly (*Schistocerca gregaria*) are all examples of the highest DD. Mealworm, mealworm chrysalis, *Chrysomya megacephala*, *Penaeus kerathurus* shrimp, cicada slough, female Rocky Mountain potato bug, *Litopenaeus vannamei*, crab (*Carcinus mediterranean*) shells, Colorado potato beetle larvae, *Portunus pelagicus*, cuttlefish (*Sepia officinalis*) bones, and black soldier fly (*Heredia illucens*) are other examples. Commercial chitin derived from aquatic invertebrates and crustaceans has features and yields that are similar to those of insect-produced chitin with similar properties (Abidin et al. 2020).

Even though it is less environmentally friendly and less cost-effective, chemical extraction is the most often used industrial method (Dhillon et al. 2013). Depolymerization of chitin may result in uneven physiological qualities if high acid treatments and high NaOH concentrations are used. This can be avoided by using lower NaOH concentrations and lower deproteinization temperatures. Stirred bioreactors and ambient temperature have been shown to increase quality and shorten the procedure to prevent the risk of chitin depolymerization (Philibert et al. 2017). The deacetylation process

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Table 1. Chitosan source, extraction method, and chitosan yield-characteristic

Chitosan source	Extraction method	DD (%)	Chitin yield (%)	Chitosan yield (%)	Characterisation	Reference
Silkworm chrysalides	open reactor using a heating plate with stirring	83	3.90	–	FTIR, NMR, SEM, TGA, DSC	Paulino et al. 2006
Cricket *Gryllus assimilis*	exoskeleton removal	–	3.5	–	chitin yield	Jayanegara et al. 2017
Cricket *Gryllus assimilis*	chemical extraction	–	nd	–	chitin yield	Jayanegara et al. 2017
House cricket	chemical method modifying the methods of Song et al. (2013), Jarolimkova (2015)	80.5	4.3–7.1	2.4–5.8	FTIR, SEM, elemental analysis, XRD	Ibitoye et al. 2018
Larvae of blowfly *Chrysomya megacephala*	chemical extraction method	87.9–89.6	–	–	halogen adsorption capacity, molecular weight, elemental analysis, antioxidant activity, FTIR, NMR, SEM	Song et al. 2013
Beetle *Holotrichia parallela* Motschulsky	conventional acid-base reaction methods	–	15	–	elemental analysis, XRD, IR, SEM	Liu et al. 2012
Butterfly *Argynnis pandora* Murat	conventional acid-base reaction methods	–	20 (butterfly wings), 8 (another body part)	–	FTIR, XRD, TGA, SEM, elemental analysis	Kaya et al. 2015c
Colorado potato beetle adults and larvae	conventional acid-base reaction methods	82 (adult)	20 (adult)	14.4	FTIR, XRD, TGA, SEM, elemental analysis, molecular weight, antioxidant and antimicrobial activity	Kaya et al. 2014a
		76 (larvae)	7 (larvae)	4.69		
Cicada slough, silkworm chrysalis, mealworm, grasshopper,	conventional acid-base reaction methods	84.1 (cicada)	–	28.2	FTIR, XRD, TGA, SEM, elemental analysis, rheological measurement	Luo et al. 2019
		85.5 (silkworm)		3.1		
		85.9 (mealworm)		2.5		
		89.7 (grasshopper)		5.7		
Male and female grasshopper *M. desertus*	conventional acid-base reaction methods	–	4.71–11.84	–	FTIR, XRD, TGA, SEM, elemental analysis, chitinase digestive activity chitinase	Kaya et al. 2015a
Shrimp *Litopenaeus vannamei*	chemical extraction by dilute sulfuric acid	63	33	–	FTIR, DD, MBC, MIC	Vilar et al. 2016
Shrimp *Litopenaeus vannamei*	chemical extraction response surface method	81.7	36	–	–	Vilar et al. 2016
Shrimp *Penaeus kerathurus* waste > crab *Carcinus mediterraneus* shells > cuttlefish *Sepia officinalis* bones	chemical demineralization; deproteinization using protease, chemical deacetylation	88.5 (shrimp)	20 (shrimp)	14.9 (shrimp)	FTIR, NMR, XRD, elemental analysis	Hajji et al. 2014
		78.5 (crab)	10 (crab)	5.3 (crab)		
		70.1 (cuttlefish)	5 (cuttlefish)	1.2 (cuttlefish)		

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Table 1 to be continued

Chitosan source	Extraction method	DD (%)	Chitin yield (%)	Chitosan yield (%)	Characterisation	Reference
Crab, crayfish, and shrimp shells	fast chitin extraction chemistry method	–	13.4, 15.3, 14.8	–	TGA, FTIR, XRD, and elemental analysis	Kaya et al. 2015b
Shrimp *Penaeus monodon*, desert locust *Schistocerca gregaria*, honey bee *Apis mellifera*, and the beetles *Calosoma rugos*a	conventional acid-base reaction methods	74	10	–	FTIR, XRD, SEM, DD, moisture-ash content, water-fat binding capacity	Marei et al. 2016
		98	12.2			
		96	2.5			
		95	5.0			
Shrimp shells	Biologically purified chitin isolated by microbial fermentation and deproteinization	83.2	25.4	–	total titratable acid, ash-moisture, protein content, proteolytic activity, DA, viscosity, colour analysis	Ploydee and Chaiyanan 2014
Shrimp waste	biological extraction: co-fermented with *Lactobacillus plantarum* subsp. *plantarum* ATCC 14917 and *Bacillus subtilis* subsp. *subtilis* ATCC 6051, with 10 carbon sources: sugarcane molasses, light corn syrup, red grape pomace, white grape pomace, apple peel, pineapple peel and core, potato peel, mango peel, banana peel, and sweet potato peel	53.39–75.19	–	10–17.8	FTIR, XRD, elemental analysis	Tan et al. 2020
Pacific white leg shrimp (*Litopenaeus vannamei*) Boone shells	conventional acid-base reaction methods	90	–	–	sulphated ash and insoluble content, FTIR, DA, SEM, viscosity average molecular weight viscosity, XRD	de Queiroz Antonino et al. 2017
Aquatic invertebrates: *Ranatra linearis*, *Anax imperator*, *Hydrophilus piceus*, *Notonecta glauca*, *Agabus bipustulatus* > *A. sellus aquaticus*	conventional acid-base reaction methods	74–78	15–16 (*R. linearis*)	10.5–11.2 (*R. linearis*)	FTIR, XRD, TGA, SEM, elemental analysis	Kaya et al. 2014b
			11–12 (*A. imperator*)	7.37–8.04 (*A. imperator*)		
			19–20 (*H. piceus*)	14.06–14.8 (*H. piceus*)		
			10–11 (*N. glauca*)	6.9–7.59 (*N. glauca*)		
			14–15 (*A. ipustulatus*)	9.94–10.65 (*A. bipustulatus*)		
			5–6 (*A. aquaticus*)	3.3–3.96 (*A. aquaticus*)		

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Table 1 to be continued

Chitosan source	Extraction method	DD (%)	Chitin yield (%)	Chitosan yield (%)	Characterisation	Reference
Prawn shell (*Nephrops norvegicus*)	biological extraction: inoculant (Sil-All 4 × 4® silage additive: *Lactobacillus salvarius, Enteroccus facium,* and *Pediococcus acidilactici*)	67			protein, inorganic content, FTIR, molecular weight, viscosity	Beaney et al. 2005
	chemical extraction	71.3				
Shrimp shells	microwave irradiation method	82.37			FTIR, XRD, NMR, SEM, molecular weight	El Knidri et al. 2016
Fish waste (*Labeo rohita*), shrimp shells (*Crangon crangon*), and crab shells (*Crangon crangon*)	conventional acid-base reaction methods	75, 78, 70			DD, FTIR, XRD, SEM, TGA, elemental analysis, solubility, fat binding capacity, water binding capacity, ash, moisture, molecular weight	Kumari et al. 2017
Swimming crab (*Portunus trituberculatus*) shells	subcritical water pretreatment	62.2–88.5		11.2–13.2	yield, ash, DD, FTIR, XRD, SEM, molecular weight, UV absorption spectrum, DSC	Hao et al. 2021b

DA = degree of acetylation; DD = degree of deacetylation; DSC = differential scanning calorimetry; FTIR = Fourier transform infrared ray spectrometer; IR = infrared spectroscopy; MBC = minimum bactericidal concentration; MIC = minimum inhibitory concentration; NMR = nuclear magnetic resonance; SEM = scanning electron microscope; TGA = thermogravimetric analysis; XRD = X-ray diffraction

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heated by microwaves produces the same quality of chitosan (similar DD characteristic) as the traditional ones, also time of heating on its process. The molecular vibration in microwave heating increased the contact between the solid and liquid, and the alkali solution makes chitin more accessible and promoting deacetylation (Cheng et al. 2020). Biological extraction, on the other hand, has recently attracted attention as a chitin retrieval technique that is both safer and less expensive (Yadav et al. 2019). Minimizing chitin degradation and introducing contaminants to a level where they are useful in the biological isolation process is the goal (Philibert et al. 2017). A degree of deacetylation similar to 71% was found after combining enzymatic deproteinization with a chemical demineralization procedure. This integrated strategy utilizing seawater has the potential to turn crab waste into commercially viable chitin products (Pachapur et al. 2016). It is now possible to extract biological materials utilizing fungi, proteolytic bacteria, or pure enzymes in a study by Arbia et al. (2013). When compared to the chemical technique, the biological process has not yet achieved the expected yields (Arbia et al. 2013). The chemical method of obtaining chitin-chitosan is therefore still preferred, particularly by the industry. Bio and biotechnological extractions, on the other hand, need to be improved as their environmentally friendly yields are even less chitosan.

Application of chitosan as animal feed additive

Additives are non-nutritive materials or ingredient combinations that can be added to the basic feed mix or animal nutrition (Van Saun 2013). To put it in another way, feed additives are any substances that enhance the digestion, absorption, and assimilation of nutrients, as well as the health of an animal (Van Saun 2013; Watts et al. 2020). According to research, additives in feed have been shown to increase the utilization efficiency of feed, as well as to maintain it, for example by increasing the digestibility of the feed ingredients (Van Saun 2013). Nutritional requirements are not met by feed additive additions (Watts et al. 2020).

Some examples of feed additives involve feeding attractants, immune stimulants, prebiotics, probiotics, acidifiers, essential oils, or other additions (Watts et al. 2020). In the United States, the Food and Drug Administration (FDA) and analogous authorities in other countries have strict regulations on the use of additives that act as a preventative or therapeutic measure in animal feed products. The non-drug ingredients include yeast or yeast extract probiotics, glycosaminoglycans, aloe vera, yucca, kelp, omega-3 fatty acids, oligomannosaccharides, mineral oil, and bentonite, artificial flavours or colours, and ethoxyquin (Van Saun 2013).

Animals' immune systems, stress tolerance, and reproductive systems are all affected by the addition of feed additives (Watts et al. 2020). Because of its antimicrobial properties, chitosan accelerated the uterine repair after parturition in dairy cows (Okawa et al. 2021). Moreover, chitosan has an anti-inflammatory ability to tolerate heat stress and increase immunity, particularly in the suppression of serum inflammatory cytokine response, and increased organ weight, blood parameters, reduced mRNA expression of TLR4 and its downstream gene expression, as well as decreased p65, IL-10, TNF-α, and increased the expression of occludin and claudin-2 (Mohyuddin et al. 2021). Mineral oil, bentonite, ethoxyquin, flavouring, and colouring compounds are all examples of additives that serve specialized objectives. Other ingredients, including yeast, yucca, probiotics, and chondroitin sulphate, have been shown to aid digestive and joint health (Van Saun 2013). The use of feed additives prevents and controls infections in the feed. To prevent *Salmonella* infection and subsequent livestock colonization of ingested bacteria, various chemical and physical interventions have been used on feed (Getabalew et al. 2020). In animal agriculture, feed additives have been employed to limit pathogen colonization in animals' guts (Jeong et al. 2010). Broiler chickens have been administered organic acids such as formic and propionic acids to minimize or eliminate infections from their intestines and prohibit the bacteria from being shed, as well as to diets and feed additives. Additives such as prebiotics and probiotics can also be used to minimize or prevent the colonization of infections in the intestines of ruminants and poultry. Antibiotics have been employed as a feed supplement to eradicate disease colonisation (Alali and Ricke 2012; Getabalew et al. 2020).

It is a common practice to utilize a wide range of feed additives to keep the animal metabolic and health state in check while also boosting their

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performance index. These include feed enzymes, organic acids, herbal extracts, and pre- and probiotics. In the world of feed additives, chitosan is one of the more recent and less often utilized ones. β-(1-4)-2 acetamido-D-glucose and β-(1-4)-2 amino-D-glucose units make up this non-toxic polyglucosamine, which is present in several fungi. Antimicrobial, antioxidative, anti-inflammatory, anticancer, immunostimulatory, and hypocholesterolaemic activities can be found in chitosan because it comprises functional groups that are reactive, such as amino acids and hydroxyl groups. Chitosan benefits suggest that it could be an excellent pro-health feed supplement for cattle and an antibiotic-free feed option (Swiatkiewicz et al. 2015). Resource productivity can be increased by using chitosan from waste materials as an animal feed ingredient (Figure 3).

Chitosan as an antimicrobial agent

Chitosan can be used alone or in combination with other natural polymers in a variety of applications, including food processing and preservation, silage inoculants, textile, biotechnology, biomedical industry, pharmaceutical water treatment, tissue engineering, and cosmetics (Jimenez-Ocampo et al. 2019). Chitosan has a wide range of applications since it serves multiple roles, one of which is as an antibacterial agent. The minimum inhibitory concentration (MIC) values of chitosan nanoparticles ranged from 200 to 400 µg/ml for *S. aureus* strains, and from 400 to 800 µg/ml for coagulase-negative *Staphylococcus* (CNS) (Orellano et al. 2019).

Although the antibacterial activity of chitosan is not fully understood, a more plausible hypothesis is that it is generated by interactions between the biopolymer chitosan and the cell permeability (pH less than 6.5), and when the negatively charged microbial cell walls release proteinaceous and other intracellular components (Rabea et al. 2003; Goy et al. 2016; Vilar et al. 2016). Additionally, chitosan serves as a chelating agent, binding trace metals selectively and inhibiting the formation of toxins and microbial growth (Rabea et al. 2003). According to Goy et al. (2016), the decrease in antibacterial activity with increasing polymer concentrations can be explained by the configuration of the polymer chains spatially: lower polymer concentrations result in a more homogeneous molecular distribution in the solvent, with a low number of contacts between nearby chains, increasing the available charged sites for external coupling. Added by Rabea et al. (2003), at low concentrations (0.2 mg/ml), the polycationic chitosan attaches to the negatively charged surface of the bacteria, causing agglutination; at greater concentrations, the increasing multitude of posi-

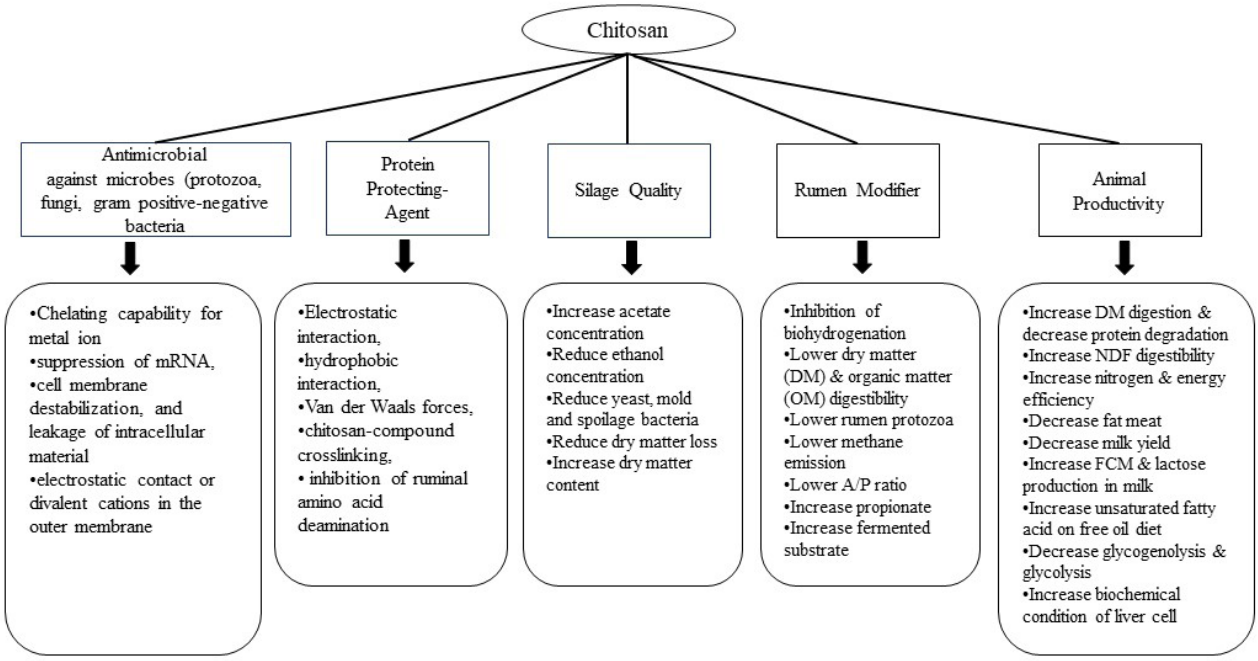

Figure 3. Applications and effect of chitosan as feed additive in ruminants

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tive charges may have imparted a net positive charge to the bacterial surfaces, keeping them suspended.

The antimicrobial activity of chitosan *in vitro* is affected by a variety of internal and external factors, including the chitosan itself (type, molecular weight, DD, viscosity, solvent, and concentration) and the environmental elements (test strain, physiological state, and bacterial culture medium, pH, temperature, ionic strength, metal ions, EDTA, and organic matter), respectively (Raafat and Sahl 2009). According to No et al. (2002) chitosan has stronger antibacterial properties and significantly inhibits the growth of the majority of bacteria when compared to chitosan oligomers. Chitosan had a greater bactericidal effect on gram-positive bacteria (*Bacillus megaterium*, *Bacillus cereus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Lactobacillus brevis*, and *Lactobacillus bulgaricus*) than on gram-negative bacteria (*Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella*). In contrast, Vilar et al. (2016) discovered that the minimum inhibitory concentration (MIC) and minimum inhibitory bactericidal concentration (MBC) of Gram-negative bacteria were significantly higher than those of Gram-positive bacteria, and the hydrophilicity of the cell wall and negatively charged cell surface were greater in Gram-negative bacteria than in Gram-positive bacteria. Chung et al. (2004) discovered that chitosan was more readily adsorbable to Gram-negative bacteria than it was to Gram-positive bacteria. The adsorbed amounts of chitosan were found to be connected to the environmental pH (pH 4.0 was found to be more adsorbed than pH 5.0) and the degree of chitosan deacetylation (a higher degree of deacetylation resulted in a bigger adsorbed amount). The antibacterial properties of chitosan were tested using Gram-negative bacteria and Gram-positive bacteria. It was discovered that the addition of 1 g/l chitosan was the most effective dosage against Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria (Goy et al. 2016). Antimicrobial activity of the chitosan film solution supplemented with essential oils was demonstrated against two harmful bacteria (*S. aureus* and *E. coli*), two beneficial bacteria (*Enterococcus faecium* and *Lactobacillus rhamnosus*), and two mould fungi (*Aspergillus niger* and *Alternaria alternata*). In comparison with beneficial bacteria, pathogenic bacteria are more vulnerable to antibacterial agents. When the chitosan film solution was supplemented with thyme and oregano essential oils, antimicrobial activity was improved compared to when the chitosan film solution was used alone (Raphael and Meimandipour 2017).

Chitosan may act as an antimicrobial, possibly due to an alteration in the cell permeability produced by interactions with the biopolymer chitosan; additionally, chitosan is effective at suppressing both positive and negative gram bacteria growth, although there are some discrepancies in the effectiveness of chitosan against the two types of bacteria (Goy et al. 2009). According to the findings of Rajasekaran and Santra (2015), chitosan can be employed as a carrier for copper and zinc nanoparticles that act as an antibacterial feed addition when hydrothermally processed. Additionally, hydrothermal treatment results in the loading of chitosan hydrogels with zinc (800 g/ml) and copper (57.6 g/ml) metal particles, resulting in the reduction of a load of model gut bacteria (target organisms of antibiotic growth promoters), such as *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,* and *Lactobacillus fermentum*, which exhibited significantly (Rhazi et al. 2002) the stability and regulated release of micronutrients from chitosan gels and copper chelate matrices (functional matrix for the preparation of copper and vitamin). In simulated biological fluids, they appear to be a promising alternative for overcoming copper shortage in ruminants such as cattle (Kofuji et al. 2005; Duffy et al. 2018).

Chitosan as proteolysis inhibitor agent

The rumen is the principal site for microbial fermentation of ingested grain in ruminants and is home to a complex microbiome composed of approximately 7 000 species of protozoa, archaea, bacteria, and fungi. Although amino acids and peptides created by proteolysis may be useful nutrients for rumen bacteria, they are very likely to be degraded to ammonia and expelled by the rumen. Bacteria are in charge of digestion of food protein, while ciliate protozoa are in charge of breaking down particulate feed protein of adequate size as well as bacterial protein (Hart et al. 2018). Rumen proteolytic activity varies significantly between animals and diets, which are the main determinants. Protease hydrolyses the protein into amino acids and peptides, and then microbial deamina-

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tion converts some of the amino acids into ammonia (Hao et al. 2021a). Ruminal proteolysis results in the loss of high-quality food protein that would otherwise be digested and absorbed in the small intestine of the ruminant animal as a result of the ruminant animal digestion and protein absorption. It was attempted to increase protein utilization in the rumen by minimizing the apparent breakdown of protein through the use of inclusion additives, chemical treatment of feed, and defaunation, among other methods (Brock et al. 1982). The use of chitosan as a coating material significantly reduces the proteolytic activity of samples containing protein (Yu et al. 2018).

Chitin and chitosan have long been used as animal additives. Researchers discovered that chitin deteriorated slowly in the rumen, whereas chitosan did not. Based on this finding, they hypothesized that chitosan could be utilized to protect proteins from degradation in the rumen (Fadel El-Seed et al. 2003). Chitosan was unable to be removed from the faeces of animals using formic acid or aqueous acetic acid due to the formation of strong polyelectrolyte complexes, as proven by chitosan-heparin complexes. The cationic amino group in chitosan has been shown to impede acid hydrolysis of glycosidic bonds. Chitosan is probably digested via the enzymes chitinase and chitosanase, which are released by gut microorganisms (Hirano et al. 1990). The chitosan polycation is positively charged, which enables it to bind negatively charged molecules such as protein, polysaccharides, nucleic acid, and heavy metals. Chitosan inhibited the deamination of ruminal amino acids. This happened because the rumen concentration of branched-chain fatty acids like isobutyrate and isovalerate in the rumen is reduced, which are formed in the rumen by isoleucine, leucine, and valine deamination (de Paiva et al. 2016). This fact may increase amino acid delivery to the duodenum, hence increasing N usage efficiency. Chitosan increased nitrogen excretion in milk without affecting nitrogen intake and boosted nitrogen usage efficiency. According to Slottner and Bertilsson (2006), adding chemicals to the crop and boosting its DM content can help minimize proteolysis during the ensilage process (Slottner and Bertilsson 2006). Gandra et al. (2016) observed that 1% chitosan increased the DM content of sugarcane silages, increased lactic acid bacteria, and decreased ethanol concentrations compared to other treatments.

Chitosan combines with some compounds such as biopolymer or nano-particle have a great result in terms of protein protection. Chitosan beads bovine serum albumin (BSA) was significantly protected by 30 g/l alginates and 1 g/l from acidic and alkaline *in vitro* enzymatic hydrolysis (Saez et al. 2015). The functional groups of graphene oxide reacted with chitosan and BSA, increasing thermal resistance while increasing BSA stability (Emadi et al. 2017). Chitosan coating of amino-acid-entrapped fat particles could greatly boost amino acid retention (Chiang et al. 2009). Covalent cross-linking in alginate-chitosan polymers appears to overcome the chemical and enzymatic variables that threaten protein structural integrity in the gastrointestinal tract evaluated on sea bream (*Sparus aurata*) (Saez et al. 2015). Furthermore, Van der Waals forces, electrostatic and hydrophobic interactions, and hydrogen bonds all play a role in protein-chitosan interactions. Non-covalent protein loading on nanostructures has certain advantages over covalent binding since covalent immobilization might produce steric changes in the protein, reducing its functionality and activity (Emadi et al. 2017). Chiang et al. (2009) postulated that chitosan may be protonated under acidic circumstances to generate a positively charged NH_3 group, which caused electrostatic repulsion to relax polymer chains. The positively charged component may also interact electrostatically with negatively charged fatty acids in fat or carboxyl groups in amino acids. Those electrostatic interactions thus kept the chitosan coating on the amino-acid-entrapped fat particles in place.

Based on past studies, we believe that chitosan has the ability to act as a proteolysis inhibitor, protecting high protein feed from rumen degradation and allowing the protein to bypass, digest, and absorb in the small intestine. Alternatively, chitosan can be added to ensilage processing to inhibit proteolysis and enhance the nutritive content of silages.

Chitosan as methane reducer agent and rumen modifier

Recent animal nutrition research has focused on its ability to regulate rumen fermentation and nutrient digestibility in beef or dairy cattle (Table 2) (Jimenez-Ocampo et al. 2019). Manipulation of the rumen environment can be used to inhibit CH_4

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Table 2. The effect of chitosan application on ruminal characteristic and animal productivity

Level of chitosan	Method	Ruminal characteristic	Animal productivity	Reference
21% glycerin on TMR, supplementation 2% chitosan (self-extraction) DM of substrate	*in vitro*	depressed 53.67% CH_4	–	Seankamsorn et al. 2019
		increased C_3 26.41%		
		reduced 31% C_2:C_3		
5 g/kg chitosan – Polymar Indústria e Cia. Imp. And Exp. LTDA, Fortaleza, Brazil (0.5%) fresh matter soybean silage	*in vitro*	increased in vitro degradation of DM, CP, and NDF	–	Gandra et al. 2018
		decreased non-fibre carbohydrate content		
		increased NH_3–N and lactate concentrations		
		decreased ethanol content on soybean silage		
		highest C_3 content		
4.47–6.34 g/kg chitosan (Fagron, São Paulo, Brazil) DM sugarcane silage	*in situ*	increased C_3 and NH_3	–	Del Valle et al. 2020
		N concentration		
6 g/kg DM chitosan (Fagron-São Paulo, Brazil) sugarcane silage	*in situ*	decreased OM, NDF, ADF content	–	Del Valle et al. 2018
1% chitosan (Polymar Industria, Ceara, Brazil) of fresh matter sugarcane silage	*in vitro*	improve NDF degradation	–	Gandra et al. 2016
136 mg/kg chitosan of BW lambs	*in vivo*	increased N balance and MCP synthesis	improved DM, CP, NDF and digestibility	Pereira et al. 2019
0.75–36.36 g/kg chitosan of DM	*in vitro* (meta-analysis)	reduced enteric methane emissions	–	Harahap et al. 2020
		decreased C_2		
		increased C_3, DM-CP, NDF digestibility		
75–225 mg/kg chitosan (Polymar Industria, Ceara, Brazil) of BW	*in sacco*	increased crude protein digestibility, propionate concentration	enhanced milk yield, fat-corrected milk, protein, and lactose production	de Paiva et al. 2016
	in vivo	decreased C_2/C_3 ratio	increased milk nitrogen content	
			no effect on DMI	
50, 100, 150 mg/kg chitosan (Polymar Industria, Ceara, Brazil) of BW	*in vivo*	enhanced total-tract digestibility of dry matter, neutral detergent fibre, and crude protein	increased plasma glucose concentration	Araujo et al. 2015
	in sacco	decreased C_2/C_3 ratio, C_2	no effect on DMI	
	in vitro	quadratic effect on NH_3 concentration, C_3, and C_4 molar proportion		
136 mg/kg chitosan (Trades S.A., Barcelona, Spain) of BW	*in vivo*	increased C_3	no effect on DMI, apparent total tract digestibility of OM, CP, EE, and NDF	Goiri et al. 2010
	in vitro	decreased C_2/C_3 ratio, NH_3–N concentration, CH_4 production		
10 g/kg chitosan (A and Z Food Additives Co., Ltd, Zhejiang, China) of DM	*in vivo*	reduced apparent total tract digestibility of DM, OM, and CP	increased urinary N excretion	Kirwan et al. 2021
	in vitro	increased ruminal pH	increased N excretion in faces	

Table 2 to be continued

Level of chitosan	Method	Ruminal characteristic	Animal productivity	Reference
800 mg/kg chitosan (Polymar, Fortaleza, Brazil) of DM concentrate	*in vivo*	the highest value of allantoin, absorbed nitrogen, microbial nitrogen (g/day), and retained nitrogen	increased DM, CP, NDF intake	Dias et al. 2017
		increased C_3 concentrated		
4 g/kg chitosan (Polymar, Fortaleza, Brazil) of dry matter	*in vivo*	raised ruminal pH and reduced acetate-to-propionate ratio	increased milk concentration [g/100 g of fatty acids (FA)] of 18:1 *trans*-11, 18:2 *cis*-9,*cis*-12, 18:3 *cis*-9,*cis*-12,*cis*-15, 18:1 *cis*-9,*trans*-11, total monounsaturated FA, and total polyunsaturated FA	Zanferari et al. 2018
	in sacco	decreased the *Butyrivibrio* group proportion in bacterial population		
1.5 g/kg chitosan (Alfadelta, Estado de Mexico, Mexico) and naringin	*in vitro*	increased the concentration of propionic acid	did not affect rumen fermentation	Jimenez-Ocampo et al. 2021
	in vivo	reduction in methane production	DM intake and digestibility	
			enteric methane emissions	
5% from DM chitosan (Nitta Gelatin India Ltd. Cocin, Kerala, India) and ivy fruit extract	*in vitro*	decreased methane emission (42%) with a different mechanism	–	Belanche et al. 2016
		increase C3, C5		
		decrease C2, C4		
150 mg/kg (Polymar, Fortaleza, Brazil) body weight and whole raw soybean	*in vivo*	interaction effect for the ruminal ammonia nitrogen concentration (NH_3–N), total VFA and propionate concentrations	no interaction effect on feed intake, apparent digestibility, nitrogen balance, blood metabolites and feeding behaviour	Dias et al. 2020
		no interaction effect on microbial protein synthesis, rumen kinetics and flow of nutrients		
100 mg/l chitosan (Sigma-Aldrich Co., St. Louis, MO, USA) of culture fluid, rapeseed oil and sunflower oil (35 g/kg DM) per culture fluid	*in vitro*	decrease in *in vitro* dry matter digestibility, total gas, methane emission and total protozoa	–	Wencelova et al. 2014
		increased short-chain fatty acid		
0, 1 625, 3 500, or 7 500 mg/kg chitosan (Polymar, Fortaleza, Brazil) of DM	*in vitro*	decreased *in vitro* dry matter digestibility	–	Jacauna et al. 2021
		no change in total short-chain fatty acid and acetate/propionate ratio		
		increased total gas production		
1 380 mg chitosan (Trades S.A., Barcelona, Spain)	*in vitro*	decreased gas production, methane, disappearance of DM, OM, CP, NDF	–	Goiri et al. 2009
		increased ratio of propionate to acetate		

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Table 2 to be continued

Level of chitosan	Method	Ruminal characteristic	Animal productivity	Reference
24 g/40 ml micro chitosan (Sigma-Aldrich Co., St. Louis, MO, USA) on distilled water and 6.6 mg/kg ceftiofur crystalline-free acid	*in vivo*	–	reduce curing the metritis in cows	de Oliveira et al. 2020
			greater proportion of cows culled within 60 days in milk (DIM)	
			lower first 60 DIM	
			lesser hazard of pregnancy up to 300 DIM	
0 and 4 g/kg chitosan (Polymar, Fortaleza, Brazil) of dietary dry matter and soybean oil, 0 and 33 g/kg of dietary dry matter	*in vivo*	–	On an oil-free diet, chitosan enhanced DM and CP digestibility.	Del Valle et al. 2017
			Both soybean oil and chitosan raised total serum cholesterol levels.	
			The urea plasma concentration was increased with the chitosan diet.	
			In oil-diets, chitosan reduced milk output, nitrogen consumption, and feed conversion efficiency.	
			In meals including soybean oil, chitosan had no effect on long-chain milk fatty acids.	
			In oil-free diets, chitosan enhanced milk polyunsaturated fatty acid content, nitrogen, and energy efficiency.	
			The addition of chitosan to oil-free diets boosted feed efficiency and raised the concentration of milk unsaturated fatty acids.	
			Cottonseed processing method and chitosan in the lamb feed had no effect on carcass characteristics or meat quality.	
			Increase in palmitoleic (C9–C16), conjugated linoleic fatty acids and the concentration of unsaturated fatty acids when pulverized cottonseed was combined with chitosan in the meal of feedlot lambs.	
			Irradiated chitosan lowers glycogenolysis and glycolysis activity while improving the biochemical conditions of liver cells.	

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Table 2 to be continued

Level of chitosan	Method	Ruminal characteristic	Animal productivity	Reference
Combination of 0 and 136 mg/kg chitosan (Polymar, Fortaleza, Brazil), body weight with cottonseed processing (whole and ground)	*in vivo*	–	–	da Silva Magalhaes et al. 2020
0 ppm irradiated chitosan (IC) from self-extraction; 350 ppm IC; 400 ppm IC; 450 ppm IC; and 500 ppm IC	*in vivo*	–	–	Mushawwir et al. 2020

ADF = acid detergent fibre; C_2 = acetate; C_3 = propionate; C_4 = butyrate; C_5 = valerate; CH_4 = methane; CP = crude protein; DM = dry matter; DMI = dry matter intake; FA = fatty acid; MCP = microbial cell protein; N = nitrogen; NDF = neutral detergent fibre; NH_3 = ammonia; OM = organic matter; TMR = total mixed ratio; VFA = volatile fatty acid

generation in ruminants. Numerous natural antimicrobial substances can be employed to alter the rumen microbial environment, one of which is chitosan (Harahap et al. 2020). The addition of 21% crude glycerine to TMR decreased CH_4 production by up to 53.67% and raised C_3 content by 26.41%, while decreasing the C_2 to C_3 ratio by 31% when compared to the non-supplemented group (Seankamsorn et al. 2019). Certain properties of chitosan, such as its high degree of deacetylation, may alter the permeability of the methanogen cell wall, hence lowering CH_4 generation (Harahap et al. 2020). Additionally, chitosan, which is positively charged, may interfere with negatively charged methanogens resulting in cytosolic protein and other intracellular components leakage (Zanferari et al. 2018). When chitosan is given to the rumen, changes in carbohydrate digestion occur without a change in DMI, resulting in an increase in propionate and a decrease in acetate (Araujo et al. 2015). The addition of chitosan (3 000 molecular weight at 16 mg/g DM dosage) to fermentation could alter the fermentation route, favouring propionate and amylolytic bacteria (Jimenez-Ocampo et al. 2021). Fermentation of crude glycerine and chitosan may affect the creation of an H_2 sink and may help transition carbohydrate fermentation from C_2 to C_3 production (Seankamsorn et al. 2019). Chitosan may have a mechanical impact similar to monensin, which was associated with alterations in the VFA profile, namely lowering C_2 and increasing C_3, even at low levels of dietary inclusion (Goiri et al. 2010; Zanferari et al. 2018). The conversion process may alter the overall electron equilibrium in the rumen, reducing the amount of hydrogen available for CH_4 production. Chitosan has been found to have an effect on feed intake, digestion, fermentation, and the formation of enteric methane (Goiri et al. 2009; Wencelova et al. 2014; Seankamsorn et al. 2019).

In vitro degradation of dry matter, crude protein, and neutral detergent fibre was increased by the addition of chitosan, which also increased NH_3–N and lactate concentrations and decreased ethanol concentration in soybean silage and sugarcane silage (Gandra et al. 2016; Del Valle et al. 2018; Gandra et al. 2018; Del Valle et al. 2020). Increased concentrations of NH_3–N could be due to N contained in chitosan, which is primarily transformed into a soluble protonated form when the pH of the environment is lower than the chitosan pKa (potentially hydrogenated form) (6.3) (Goy et al. 2009). This pro-

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tein-rich fibre can contain up to 10.8% crude protein and has been used as a nitrogen source for cattle and other ruminants (Fadel El-Seed et al. 2003). When chitosan is present in an aqueous acid solution, the glucosamine units (NH_2) are transformed into a soluble protonated form (NH^{3+}), which is more readily soluble (Goy et al. 2016). The administration of chitosan at a concentration of 900 mg/kg DM resulted in increased digestibility of DM, NDF, and CP. Chitosan has been shown to reduce the digestibility of DM and NDF in rich forage diets by up to 50%. The addition of chitosan to a meal will cause an increase in ammonia concentrations approximately 2 h after feeding. The breakdown of amine ($R–NH_2$) into ammonia (NH_3) is responsible for the increased ammonia concentrations found in chitosan diets (Belanche et al. 2016). More specifically, by using a reverse mechanism, chitosan decreased the fibre concentration of silages, while simultaneously having a beneficial effect on NFC concentration and DM decomposition (Del Valle et al. 2018). Chitosan altered the rumen fermentation pattern and boosted propionate synthesis, while simultaneously lowering cellulolytic bacteria such as *Fibrobacter*, *Butyrivibrio*, and *Ruminococcus*, hemicellulolytic bacteria such as *Eubacterium*, and increasing amylolytic bacteria (Goiri et al. 2010; Dias et al. 2017; Harahap et al. 2020; Jimenez-Ocampo et al. 2021). Chitosan can alter the profile of volatile fatty acids (VFA) by increasing propionate concentration (C_3) and thereby reducing the production of CH_4, decreasing rumen NH_3 concentration and gas production. Furthermore, the reduction in CH_4 is connected with the amount of deacetylation present in chitosan, which has the potential to alter the permeability of methanogenic archaea cell walls (Harahap et al. 2020). As an added benefit, chitosan will reduce rumen bacteria, which will result in a fall in feed digestibility. This will result in a decrease in gas production.

Chitosan treatment increases the propionate content while decreasing the acetate level, resulting in changes in hydrogen synthesis, which is the major substrate for methane production (Jayanegara et al. 2017). Chitosan will increase energy savings especially on the effect of hexose fermentation, this is reflected in the changes observed in molar proportions of acetic, propionic, and butyric acids, so the ruminant will have more energy available for metabolism from the feed consumed since the energy in the feed is being used more efficiently (Goiri et al. 2010). The electrostatic interaction of chitosan with the cell membrane hindered methanogens, or metabolic pathways involved in methane synthesis, and reduced methane production by 10% to 42%, while increasing propionic acid and lactate as fermentation products as a result of the rumen microbiota use of chito-oligosaccharides as carbon sources, according to the results of this study. Jimenez-Ocampo et al. (2019) found that chitosan was extremely successful in suppressing biohydrogenation *in vitro* by boosting C18:1 *trans*-11 and conjugated linoleic acid (CLA) proportions, regardless of the fatty acid composition of the diet (Harahap et al. 2020). With the addition of chitosan, vaccenic acid and conjugated linoleic acid were shown to be enhanced significantly. The capability of chitosan to block *Butyrivibrio* could explain the rise in CLA and vaccenic acid levels in the blood (Toral et al. 2018).

Chitosan as animal productivity enhancer

In addition to *in vitro* and *in sacco* research, *in vivo* experiments are required to determine the effect of chitosan directly on the animal (Table 2). There are a variety of findings regarding the influence of chitosan on dry matter intake (DMI); some research demonstrates no correlation between DMI and chitosan supplementation in the diet. When chitosan was introduced as a feed supplement, it boosted dry matter (DM), crude protein (CP), and neutral detergent fibre (NDF) digestibility and reduced methane production by improving feed efficiency (de Paiva et al. 2016; Jimenez-Ocampo et al. 2019; Pereira et al. 2019). Chitosan had a quadratic effect on neutral detergent fibre intake, with the highest values at 800 mg/kg DM chitosan dosages (Dias et al. 2017). Chitosan enhanced the overall apparent digestibility of DM, CP, and NDF linearly in steers (Araujo et al. 2015; Dias et al. 2017). Chitosan will affect ruminal fermentation, as well as the ruminal bacteria responsible for proteolysis and deamination because chitosan has been shown in prior research to enhance ruminal ammonia nitrogen (Araujo et al. 2015). This situation will result in an increase in CP consumption as well as a decrease in DM intake. Chitosan capacity to alter rumen microorganisms and digestive processes, mostly on Gram-positive bacteria, results in improved DM, CP, and NDF digestibility

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(de Paiva et al. 2016; Del Valle et al. 2017; Pereira et al. 2019). Chitosan can interact with intestinal components to promote drug epithelial permeability, hence boosting ruminant intestinal membrane permeability and nutritional digestion (Del Valle et al. 2017). Furthermore, the addition of chitosan to the fat-supplemented diet reduced feed conversion because of the ability of chitosan to chelate various metal ions, which, when combined with alterations in the absorptive mechanism of diets supplemented with soybean oil, such as calcium salts of fatty acid synthesis, could decide changes in intermediary metabolism and lower the nutrient utilization efficiency (Del Valle et al. 2017).

Contrary to a previous finding, chitosan addition decreased the perceived total tract digestibility of DM, organic matter (OM), and CP (Goiri et al. 2010; Kirwan et al. 2021). This occurs because chitosan alters the rumen microbial environment, particularly its cellulolytic bacteria, hence altering ruminal fermentative activity (Goiri et al. 2010). Chitosan has antibacterial activity against ruminal microbes (protozoa and fibrolytic bacteria), and because protozoa are involved in protein breakdown, reducing protozoa will result in a reduction in protein degradability (Kirwan et al. 2021). When chitosan was added to a diet containing whole raw soybeans, it had a detrimental effect on cows' nutrient intake and digestibility, resulting in decreased milk yield and solids production. This was due to changes in ruminal fermentation. When chitosan was added to a diet containing whole raw soybeans, fibre digestion decreased significantly in comparison with the control treatment (Zanferari et al. 2018).

Chitosan enhanced animal performance and food consumption efficiency, while also increasing the percentage of unsaturated fatty acids in milk (Jimenez-Ocampo et al. 2019) and also in lamb meat (da Silva Magalhaes et al. 2020). Additionally, chitosan enhanced the content of 18:1 *trans*-11, 18:2 *cis*-9,*cis*-12, 18:3 *cis*-,*cis*-12,*cis*-15, 18:1 *cis*-9, *trans*-11, total monounsaturated FA, and total polyunsaturated FA in milk (g/100 g of fatty acids) (Zanferari et al. 2018). In the *in vitro* analysis, chitosan hindered the complete biohydrogenation of polyunsaturated fatty acids from sunflower oil and canola meal and increased the amounts of *trans*-11 18:1 and CLA (Goiri et al. 2010), the same result as a study from Del Valle et al. (2017) when chitosan combines with oil-free diet. Contrary to the study by Del Valle et al. (2017) chitosan did not affect milk fatty acids when combined with fat-supplemented diets. Chitosan molecule linked with linoleic acid undergoes particular chemical changes. The association altered the molecule charge density and cationic functions, as well as the structure and conformational flexibility. Additionally, chitosan enhanced milk production, protein yield, and lactose yield in cows, owing to increased ruminal propionate production and CP digestibility, which resulted in increased energy and nitrogen for milk synthesis (de Paiva et al. 2016). The addition of ground cottonseed with chitosan (136 mg/kg BW) to the meal enhances the fatty acid profile (increased lauric and palmitic acid), as well as the content of conjugated linoleic acid (CLA). The increased CLA content was most likely due to the enlarged contact surface of ground cottonseed for ruminal bacteria and their combination with chitosan (da Silva Magalhaes et al. 2020). Chitosan microparticle addition tends to decrease curing of metritis in cows (de Oliveira et al. 2020). *In vitro* tests revealed that chitosan microparticles have broad-spectrum antibacterial activity (Jeon et al. 2014; de Oliveira et al. 2020).

Chitosan improves the nitrogen balance and microbial protein synthesis in feedlot lambs but has little effect on their production performance (Pereira et al. 2019). Chitosan, at 225 mg/kg body weight (0.02% BW), acts as a natural alternative modulator of ruminal fermentation, increasing ruminal propionate concentration, nitrogen utilization efficiency, and milk output in dairy cows (de Paiva et al. 2016). Because chitosan selectively binds to certain chemical molecules such as cholesterol, proteins, fats, and triglycerides, making them inaccessible and hence altering their absorption, the addition of chitosan caused a decrease in meat fat content in feedlot lambs fed ground cotton seed and chitosan (da Silva Magalhaes et al. 2020). The activity of glycogenolysis and glycolysis is reduced by irradiated chitosan, while the biochemical conditions of liver cells improve (reducing glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, creatinine, creatinine kinase). This is an ideal environment for the metabolism of Pasundan bulls to improve their growth and reproduction (Mushawwir et al. 2020). The rate of energy supply via other pathways, such as glycogenolysis, is slowed by IC administration (a decrease in glycogen breakdown in liver cells). Chitosan that has been irradiated has been shown to promote gluconeogenesis. Irradiated chitosan with a molecular weight of 30–50 kD in-

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teracted with cells well and created molecular signals. Irradiated chitosan can counteract the harmful effects of free radicals, particularly reactive oxygen species, in addition to signals that encourage the cell repair (de Paiva et al. 2016; Del Valle et al. 2017; Mushawwir et al. 2020).

Chitosan as silage additive

Silage is one of the strategies for conserving feed or forage through an ensilage process, in which forage is preserved through anaerobic lactic acid fermentation (McDonald et al. 1991). The physical properties of silage, such as colour, smell, texture, mould presence, and temperature, indicate its quality (Kung et al. 2018). Numerous additives are used in the manufacture of silage to increase the product quality, reduce methane emissions, and prevent rot. The addition of chitosan and a microbial inoculum to soybean whole plant silage enhances nutritional and fermentative quality, increases all bacteria, and decreases yeast and mould on the silage product (Gandra et al. 2018). Chitosan inhibits sporulation and spore germination of fungi, moulds, and yeasts. Because their inhibition occurs as a result of anaerobiosis and acidification (especially by lactic acid) of ensiled forage, reducing the amount of mould and yeast in soybean silage likely increases the nutrient availability for bacteria (Gandra et al. 2018). Gandra et al. (2016) and Del Valle et al. (2018) added that chitosan improves silage fermentation by lowering fermentative losses and improving the chemical composition and breakdown of silage. Gandra et al. (2018) explained that chitosan improved dry matter (DM) losses, hence increasing the DM content of sugarcane silage. However, the higher DM concentration of sugarcane silage could be a result of the DM content of chitosan applied to the sugarcane, which could potentially affect affluent and fermentation losses. In minisilos, chitosan at a 1% concentration reduces pH and ethanol concentrations while increasing acetate, butyrate, and lactic acid, NH_3–N concentrations (Gandra et al. 2016). Chitosan concentrations of 4.47–7.47 g/kg of dry matter (DM) reduce fermentation losses and increase the nutritional content of sugarcane silage (Del Valle et al. 2020). Additionally, these chitosan concentrations can help reduce mould, yeast, and ethanol condensation in sugarcane silage. The addition of crude glycerine at a concentration of 21% to total mixed ration (TMR) diets increased ruminal propionate content and decreased methane generation without impairing gas kinetics or nutrient digestibility (Seankamsorn et al. 2019).

CONCLUSION

In conclusion, the present review shows various methods for the extraction process and chitosan sources will result in different chitosan yields and degrees of deacetylation. Insect chitosan has the same properties and yield as commercial chitosan derived from marine waste. Furthermore, the chemical extraction process is still popular in industries compared with biological processes based on their yield and degree of deacetylation. However, the biological extraction method uses reagents and produces less waste so that it is more environmentally friendly, therefore biological extraction needs to be further optimized. Moreover, chitosan as a feed additive has the capability to decrease CH_4 production, increase propionate production, reduce the acetate/propionate ratio, contribute to improvements in animal performance, as well as increase nutrient use efficiency. Chitosan has the ability to be a great natural feed addition because of its abundance and availability especially to reduce enteric methane emission. Future research should focus on how to develop the biological and biotechnological extraction method process, which is still confined to the laboratory scale, to be a promising commercial-scale method for sustainable chitosan manufacture via green chemistry technologies. On the other hand, the effect of chitosan on *in vivo* trials needs to be further investigated to confirm some aspects that have both positive and negative effects, as well as to disclose synergistic or antagonistic effects with other compounds or feeds.

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Conflict of interest

The authors declare no conflict of interest.

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