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Constraints and amelioration of non-starch polysaccharides (NSP) in broiler diets: A review

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Abstract
Poultry is an imperative domesticated livestock species that provides high quality protein and micronutrients as meat and eggs. In poultry production, feed is the single major input constituting 70-75% of total production cost, mainly consists of cereal grains providing energy to the birds. Grains contain higher proportions of non-starch polysaccharides (NSP). Non-starch polysaccharides (NSP) contain ß-glucans, cellulose, pectin and hemicellulose. NSP consist of both soluble and insoluble fractions. Soluble NSP of cereals such as wheat, barley and rye increases intestinal viscosity there by interfere with the digestive processes and exert strong negative effects on net utilization of energy. NSP cannot be degraded by endogenous enzymes and therefore reach the colon almost indigested. Insoluble NSP make up the bulk in the diets. NSP are known to possess anti-nutritional properties by either encapsulating nutrients and/or depressing overall nutrient digestibility through gastro-intestinal modifications. In order to counteract the adverse effect of such substances feed enzymes are added to the diet not only to improve the nutritive value but also to economize poultry production and reduce environmental pollution.

Keywords: Arabinoxylans, β-glucans, NSP (Non starch polysaccharides), viscosity

1. Introduction
Livestock plays a key role in alleviation of poverty as well as food scarcity. Among livestock, poultry is one of the significant commodities that provide high quality protein and micronutrients through meat and eggs those are more easily taken up by the human body than plant based foods. Total poultry population in the India is 851.81 million as per 20th livestock census. India is the 5th largest broiler meat producing country in the world with an estimated production of 4.34 million metric tons and contributed 50.50% of total meat production in India during 2019-20 (Anonymous, 2020) [8]. In poultry farming, feed contributes 70-75% of total production cost. Poultry feed is based primarily on cereal grains mainly corn/maize, wheat, sorghum and vegetable protein meals which are supplied to meet most of energy and protein requirements in the poultry diet. Recently, these grains are also being used for bio-fuel production. Due to this paradigm shift of farming from the food industry to bio-fuel industry and increase in the prices of these raw ingredients in international market open the ways to find out less costly and alternative sources of energy and protein for animal feed. The nutritionists are trying to formulate the diets alternatively with the agro-industrial by products and non-conventional feed resources. The more affordable ingredients including barley, oat, triticale, rye, olive cake and sunflower meal (Al-Harthi, 2017; Teymouri *et al*., 2018; Waititu *et al*., 2018) [3, 46, 47], play important role in substitution of corn, wheat and soybean but have some anti-nutritional factors which may affect growth performance of the birds. Non-starch polysaccharides (NSP; soluble and insoluble), one of anti-nutritional factors, which are present in large amount in feed ingredients such as wheat, barley, sunflower meal, canola meal etc., and are indigestible by birds as they lack the endogenous enzymes necessary to digest the beta type of linkages present in these ingredients. These complex non-starch polysaccharides (NSP) are a heterogeneous group of compounds that include cellulose, pectins, β-glucans, pentosans, heteroxylans, and xyloglucan (Kumar *et al*., 2012) [30]. Owing to lack of endogenous enzymes, that degrade dietary fibers (structural carbohydrates or non-digestible components that make up the plant cell wall) including high molecular weight soluble NSP, intestinal viscosity increased which slows down the migration and absorption of nutrients. Consequently, this affects bird's health and increases the production cost. In order to counteract the adverse effect of such substances, feed enzymes are added to the diet not only to improve the nutritive value but also to economize poultry production and to reduce environmental pollution

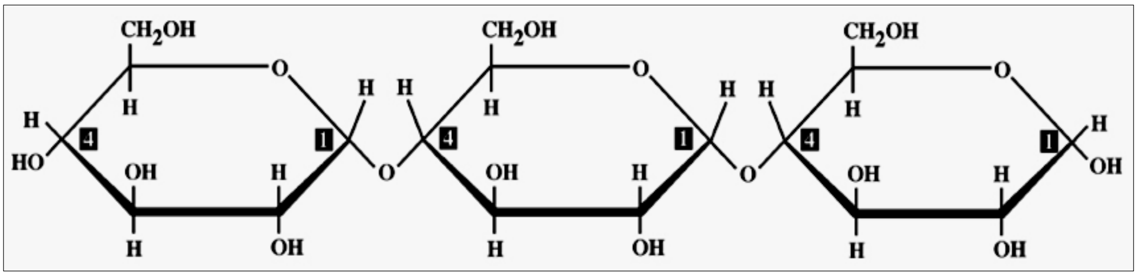
2. What are non-starch polysaccharides (NSP)?

Poultry feed is primarily composed of cereal grains, protein meals and minerals/vitamins to meet energy and protein requirement of birds. Carbohydrates (polysaccharides) present in these ingredients broken down into mono-saccharides (glucose, etc.) to serve as energy source and have been classified on the basis of their location (structural/cell wall and storage/cell contents), nutritional/physiological values (starch and NSP) and analytical methods. Based on the analytical methods these dietary fibers are mainly classified as crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF). These dietary fibers have also been classified as insoluble dietary fibers and soluble dietary fibers based on their digestibility. Among these fibers, non-starch polysaccharides (NSP) are carbohydrate fractions excluding starch and free sugars. These are polymeric carbohydrates differing in composition and structure from amylase and amylopectin (Chesson, 2001) [13]. They are the plant structural analogues of the skeletal system of animal kingdom. NSP are α-linked polymers of pentoses and hexoses having high molecular weight ranging from 8000 to a million. NSP constitute the major fraction of cell wall polysaccharides and are closely associated with other polysaccharide or non-carbohydrate material such as protein and lignin. Under NSP cellulose, hemicellulose, pectins and oligosaccharide (alpha-galactosidase *etc.*) are included. The dietary fibre is mainly formed by NSP and lignin. Chemically, NSP consists of macromolecular polymers of monosaccharides joined by a specific type of linkage called glycosidic bond formed between hemiacetal group of one sugar and the hydroxyl group of another (Hesselman, 1989)[22]. NSP are present both intra and extra-cellularly but the majority originate from the cell wall. The endogenous enzymes can't hydrolyse it. In cereal grains the NSP present are mostly xylans, arabinoxylans (pentosans), beta-glucan and cellulose. The stem and leaves contain small amount of pectic polysaccharides. In the leguminous plants cellulose and xylans are present only in the hull and husk portion. The cotyledon of legumes contains pectic polysaccharide. The NSP content of plants varies not only with the plant species but also between genotype and cultivar of the same species. Furthermore the agronomic cultivation conditions such as environmental factors prior to harvest and storage conditions after harvest can influence NSP content.

3. How NSP difference from starch

Starch is Intra-cellular plant polysaccharides-Non cell-wall components. It composed entirely of glucose monomers, which are linked by α-glycosidic bonds, digested in the small intestine (90-95%) through endogenous enzyme activity (Panda and Kumar, 2006) [37].

3.1 Structure of starch



Starch is made up of a long chain of glucose molecules bonded together by an α-1,4 glycosidic bond (carbon 1 of one glucose is bonded to carbon 4 of another glucose).

3.2 While NSP is

Extra-cellular or Cell wall polysaccharides, Composed of different kinds of monomers, which are linked predominantly by β-glycosidic bond. Providing rigidity and forming the fundamental structure of plant cell walls, Cannot be degraded by endogenous enzymes (Panda and Kumar, 2006) [37].

4. Classification of NSP

4.1 On the Basis of Solubility

4.1.1 Cellulose: Insoluble in water, alkali or dilute acids.

4.1.2 Non-cellulosic polymers: Arabinoxylans mixed linked beta glucans, mannans, galactans, xyloglucan, fructan. These are partially soluble in water.

4.1.3 Pectic polysaccharides: Poly galactouronic acids which may be substituted with arabinan, galactan and arabinogalactan. These are partially soluble in water. (Choact, 2002) [15]

4.2 On the Basis of Linkage

4.2.1 Water soluble or partially water soluble: Beta 1, 4 glycosidic linkage backbones with beta 1, 3 linkages.

4.2.2 Water insoluble: Long sequence of beta 1, 4 glycosidic unit.

5. Common NSP of plant cell walls

NSP present in the plant cell walls, broadly classified into Cellulosic, Hemicellulosic, Pectic and/or Galactosidic substances.

5.1 Cellulose

Made up of linear unbranched chain of (1-4) linked D-glucose molecules. Cellulose is believed to be identical in chemical composition regardless of sources. Cellulose is insoluble in water, alkalis and dilute acids.

5.2 Hemicelluloses

Hemicelluloses are low molecular weight miscellaneous non cellulosic polysaccharides forming the major fraction of NSP. It is partially soluble in water. They are found most often as heteropolymers and less commonly as homopolymers of monosaccharides and mainly include D-xylose, D-mannose, D-galactose, L-arabinose, D-glucuronic acid, D-glucose etc. The commonly occurring hemicelluloses include:

5.3 Pentosans

Pentosans are composed predominantly of two pentoses, arabinose and xylose. Main chain of the pentosans is made of (1-4) linked xylopyranose residues. Side chain made of (1-3) linked arabinofuranosyl residues which, in turn, are attached to C-3 position of the xylan main chain. The major substitutes are single arabinose residues, although in many instances hexoses and hexuronic acids are present in minor proportions.

5.4 D-glucans
It consists of linear chain of glucopyranosyl residues (glucose units) joined by both (1-3) and (1-4) linkages.

5.5 Arabinans and Galactans
The arabinans are polymers of (1-5)-L- arabinose residues branched through 02, 03 or both positions, whereas the galactans are polymers of (1-4) - D-galactose residues.

5.6 Mannans
Glucomannans have been found as a minor component of cereal grains. The glucomannans are comprised of (1-4) -linked glucose and mannose units.

5.7 Xyloglucans
The structure of xyloglucan is made of (1-4) linked D-xylopyronosyl (xylose) residues are attached at C-6 positions.

5.8 Galactomannan
Made of (1-4) linked mannan backbone to which D-galactose side chains are attached at C-6 positions.

5.9 Pectic Polysaccharides
The term pectic polysaccharides refer to galacturonans and rhamnogalacturonans.

5.10 Polygalacturonans
Main chain is made of (1-4) linked D-galacturonic acid and side chain consists of either (1-3) linked D-xylose, (1-6) linked D-galactose or (1-4) linked arabinose and less frequently with-fructose.

5.11 Oligosaccharides (α-galactosides)
Successive addition of α-galactosyl residues to sucrose primer leads to formation of raffinose, stachyose and verbascose etc.

5.12 Rhamnogalacturonans
Similar to polygalacturnonan except that the main chain in addition to D-galacturonic Acid contains (1-2) linked rhamnose residues resulting in a bent macromolecule.

6. NSP content of various feed ingredients
The NSP in cereal grains are composed predominantly of arabinoxylans (pentosans), glucans and cellulose (Table 1). NSP content of rice, jowar and maize is comparatively low. Cereal by-products, which are obtained after separating away the starchy portion, have very high values of NSP.

7. Anti-nutritive effects of NSP
7.1 Changes in digesta viscosity
The soluble NSP are generally viscous in nature and therefore they enhance the viscosity of the diet as well as intestinal digesta. The magnitude of viscosity development in gut with response to dietary NSP varies among animals and also depends on the source of NSP (Montagne *et al*., 2003) [34].
Cereals contain high proportions of their energy in the form of NSP and soluble indigestible polysaccharides like arabinoxylan (wheat), mixed linked β-glucans (in barley and rye) and lignin. This NSP can bind a large amount of water increasing viscosity in the gut affects the rate of passage of digesta, rate of mixing of bile, pancreatic and intestinal secretions with digesta, rate of absorption of digesta and increases the number of sticky droppings (Svihus *et al*., 2005) [45]. Viscosity is highly dependent on several factors including the size of the molecule, whether it is branched or linear and the concentration present. (Caprita *et al*., 2010) [11]. Moreover, the binding of NSP with the intestinal brush border increases the thickness of the unstirred water layer adjacent to the mucosa, leading to impaired nutrient digestion and absorption (De Lange, 2000) [18]. NSP gel may acts as a physical barrier which limit the mixing of nutrients with pancreatic enzymes and bile acids, leads to impaired digestion and absorption (Petersson and Aman, 1989) [39]. NSP may bind the brush border digestive enzymes that limit digestion and absorption of fats, proteins and carbohydrates (Silva and Smithard, 2002) [43].

7.2 Alteration in passage rate of digesta
Dietary soluble NSP increase the viscosity of digesta in monogastric animals and decrease the rate of passage, whereas the insoluble NSP, such as cellulose and hemicellulose, increase the passage rate (Johansen *et al*., 1996) [27]. Dietary inclusions of soluble NSP reduce the rate of gastric emptying in birds, which can delay the intestinal absorption of glucose (Knudsen, 2001) [29] and possibly of other nutrients.

7.3 Alteration in gut morphology
Prolonged consumption of soluble NSP is associated with increased size and length of the digestive organs in chickens (Iji *et al*., 2001) [24] accompanied by a decrease in nutrient digestion. Prolonged accumulation of undigested feed material in the gut could cause an increase in size of gastrointestinal tract and organs as a response to intestinal motility and digestive excretions (Shakouri *et al.*, 2009) [41].

7.4 Alteration in gut physiology
Apart from increasing the gut viscosity, the soluble NSP elicit an anti-nutritive effect by modifying the gut functions. These hamper the endogenous secretion of water, proteins, electrolytes and lipids (Angkanaporn *et al*., 1994)[7]. Non-starch polysaccharides can enhance bile acid secretion and subsequently result in significant loss of these acids in the faeces (Ikegami *et al*., 1990)[25]. This can result in increased hepatic synthesis of bile acids from cholesterol to re-establish the homeostasis, which may ultimately influence the absorption of lipids and cholesterol in the intestine, resulting in lower blood cholesterol levels (Hossain *et al*., 2001) [23].

7.5 Alteration in native gut microflora
The delay of digesta passage in the intestinal tract, as a result of increase in viscosity, may stimulate microbial fermentation in the intestine. The fermentation of NSP produces volatile fatty acids (VFA) as an end product (Amirkolaie *et al*., 2006) [6]. The production of these organic acids in the intestinal tract may lower its pH, which in the long term may disturb the normal microbiota prevailing in the gut. Moreover, increase in the residence time of digesta in the intestine following intake of soluble NSP may decrease oxygen tension and favour the development of anaerobic microbiota (Choct, 1997) [14].

7.6 Alteration in gut mucus layer
The mechanisms by which dietary NSP modify mucin characteristics are not well understood. The physical scraping and proteolytic breakdown of mucus gels are the main factors releasing mucins into the gut lumen (Allen, 1981) [4]. Therefore, it could be hypothesised that the erosion of mucus layer may be due to:
- An increase in the bulk of digesta,
- Stretching the intestinal mucosa and
- Scraping mucin from the mucosa as they pass through the digestive tract.

8. Amelioration of NSP
8.1 Role of NSP degrading enzymes in poultry diet
Commercial use of carbohydrases/feed enzymes in poultry diet started in late 1980’s and early 1990’s due to their ability to correct wet litter conditions, digestion and apparent metabolizable energy problems owing to high fiber diets. The enzymes are being used to balance the adverse effects of NSP on gut health/performance of poultry (Aftab and Bedford, 2018) [1].
Various kinds of NSP-degrading enzymes have been reported in animal feed, which include cellulase, hemicellulase, xylanase, pectinase, β-glucanase and α-galactosidase. β-glucanase and xylanase have been successfully used in monogastric diets to hydrolyse NSP, such as barley β-glucans and arabinoxylans (Bhat, 2000) [10]. Besides, addition of NSP degrading enzymes during feed production was found to degrade NSP and markedly improved the digestion and absorption of feed components as well as growth performance in broiler chickens (Ghorbani *et al*., 2009) [21].

8.2 Improvement of NSP Utilization
Various methods are used for improvement of NSP utilization *viz*. Enzyme supplementation, removal of water soluble NSP, antibiotic supplementation, milling and gama radiation (Sethy *et al*., 2015)[40]. Among them enzyme supplementation is most fesible, economic and practical method.

8.3 Enzyme production
Various moulds, bacteria and yeast are used to produce enzymes (Table 2), among the microorganisms, most commonly used to produce enzymes are *Aspergillus*, *Trichoderma* and *Bacillus* (Sikka and Singh, 2008) [42].

9. Mechanism of action for enzymatic degradation of NSP
Addition of enzymes to feed functions in various ways in bird’s body thus improving its nutrients utilization and overall performance.

9.1 Disruption of cell wall integrity
The cell wall in cereals and legumes is constructed mainly of small amounts of cellulose, hemicellulose and arabinoxylan with minor β-glucan components. The activity of exogenous NSP degrading enzymes creates holes in the cell wall. This allows water hydration and permits pancreatic proteases and amylases to act, enabling better digestion of the starch and protein. Xylanases and to a lesser extent cellulases have been proven most effective in broilers (Leslie *et al*., 2007) [31]. Supplementation of enzymes will break down the cell walls of the particles feed, so it will be easier to digest by enzyme digestion, improve nutrient availability is higher by as in vitro capable of lowering the viscosity in the feed, led to result from gastrointestinal tract homogenous and makes the process of absorption of food to be better (Mathlouthi *et al.*, 2003) [33].

9.2 Reducing the gut viscosity
The addition of suitable multi-enzyme preparation improves animal performance in the following ways. Breaking down the gel form characteristic of soluble fibers allows the bird’s digestive enzyme to function more efficiently. This improves starch, protein, fat, amino acids and energy digestibility. The enzymes reduce the water holding capacity of the gut contents thus increasing the dry matter content stimulating feed intake. The addition of enzymes also reduces the dry matter outside the body, due to protein digestibility lowering of excreta output, reducing the viscosity, improves nutrient digestion, lower water intake and helps to reduce litter problems. (Cowieson *et al*., 2006) [17].

9.3 Stimulation of bacterial population
Addition of NSP-degrading enzymes in feed breaks down plant cell wall carbohydrates and reduces chain length, producing smaller polymers and oligomers. These fragments further become small enough to act as a substrate for bacterial fermentation that can be beneficial with VFA production and altering the bacterial population.

9.4 Increase in available energy
One of the main reasons for supplementing wheat and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt, 1995) [38].

10. Effects of NSP degrading enzymes in broiler diet
Commercial use of feed enzymes in poultry diet started in late 1980’s and early 1990’s due to their ability to correct wet litter conditions, digestion and apparent metabolizable energy problems owing to high fiber diets. Various kinds of NSP degrading enzymes have been reported in animal feed, which include cellulase, hemicellulase, xylanase, pectinase, β-glucanase and α-galactosidase. β-glucanase and xylanase have been successfully used in monogastric diets to hydrolyse NSP such as barley β- glucans and arabinoxylans (Bhat, 2000) [10]. Enzymes hydrolyse the NSP thereby reducing water holding capacity, breakdown the gel formation process and allows bird’s digestive enzymes plus digestive juices to function more efficiently which improves digestion and absorption of nutrients leads to improved growth performance in broiler chickens (Aftab and Bedford, 2018) [1]. Cardoso *et al*. (2018) [12] reported that exogenous enzyme supplementation improved the nutritive value of wheat based diet with high extract viscosity and low endogenous endoxylanase activity in poultry. Yildiz *et al*. (2018) [48] observed that xylanase based enzyme supplementation improved egg production and decrease intestinal viscosity regardless of the inclusion rate of distillers dried grains with soluble. Some other recent studies on the use of feed enzymes, inclusion rate and their effects against various feed ingredients reported by different scientists are presented in Table 3.

Table 1: NSP contents of some cereals on % DM basis (Irish and Balnave, 1993) [26]

Cereal	Arabinoxylan	ß-Glucan	Cellulose	Total
Wheat Soluble	1.8	0.4	2	2.4
Insoluble	6.3	0.4	-	9.0
Barley Soluble	0.8	3.6	3.9	4.5
Insoluble	7.1	0.7	-	12.2
Rye Soluble	3.4	0.9	1.5	4.6
Insoluble	5.5	1.1	-	8.6
Triticale Soluble	1.3	0.2	2.5	1.7
Insoluble	9.5	1.5	-	14.6
Sorghum Soluble	0.1	0.1	2.2	0.2
Insoluble	2.0	0.1	-	4.6
Maize Soluble	0.1	-	2.0	0.1
Insoluble	5.1	-	-	8.0
Rice Soluble	T	0.1	0.3	0.3
Insoluble	0.2	-	-	0.5
Soya bean Soluble	2.7	-	4.4	2.7
Insoluble	16.5	-	-	16.5

T- Traces

Table 2: Microorganisms and enzymes produced by them

Types	Species	Enzymes
Fungi	*Aspergillus niger*	α- amylase, β- glucanase, Cellulase
	Aspergillus candidus	
	Aspergillus sydowi	
	Aspergillus oryzae	α- amylase, neutral protease
	Trichoderma viridae	Xylanase, β- glucanase, cellulase
Bacteria	*Bacillus licheniformis*	α- amylase
	Bacillus subtilis	α- amylase, neutral protease, β- glucanase

Table 3: Feed enzymes, their inclusion rates and effects using various feed ingredients

Enzyme	Substrate	Enzyme inclusion rate	Response	Reference
B-glucanase, xylanase, cellulases	Hull-Less Barley	0.5 g/kg	Supplementation of Enzyme Cocktail in the finisher diet can decrease the adverse effects of high level of Hull-Less Barley on performance of broiler chickens.	Teymouri *et al.* (2018)[46]
Glucanases	Barley	52.5 U/kg of barley	Improve the nutritive value of barley based diets.	Fernandes *et al.* (2016)[19]
Xylanase	Corn-soybean meal	50-200 U/Kg	Energy utilization and digestibility of crude protein and dry matter increased with xylanase.	Stefanello *et al.* (2016)[44]
Xylanase, amylase, protease	Corn/soy/wheat	2,000 U/kg, 200 U/kg 4,000 U/kg	Enzymes with direct fed microbial improved caloric efficiency by reducing the amount of energy needed to produce one kg of body weight gain.	Flores *et al.* (2016)[20]
Glucanase, Amylase, Protease	Corn/soybean meal	250 g/ton	Improve nutrient digestibility.	Allouche *et al.* (2015)[5]
Multi- glycanase	Wheat and barley	180 unit/g	Improve growth rate and carcass traits, blood parameters and gut physicochemical properties of broiler chickens.	Kalantar *et al.* (2015)[28]
Xylanase	De-oiled rice bran	10 g/100 kg feed	Breast muscle, thigh muscle and giblet weight (percentage) was significantly increased.	Anuradha and Roy (2015)[9]
Xylanase, cellulase and b- galactosidase	Rice bran	4520 U, 4060 U, and 2700 U	Enhance cell wall hydrolysis of rice bran with increased nutrient digestibility.	Liu *et al.* (2015)[32]
Xylanase, glucanase, mannanase	Wheat-soybean meal	500 mg/kg diet	Enzyme supplementation increase body weight, decrease digesta viscosity, lowered ileal *C. perfringens* and *Lactobacillus* colonization.	Agboola *et al.* (2015)[2]
Xylanase, glucanase, cellulase	Wheat	0.5 g/kg	Improve growth performance, histomorphology and gut microbiota.	Munyaka *et al.* (2015)[35]
Glucanase	Barley	1500 U/kg	Enzymes stimulate performance in young birds and significantly improve ileal and cecal microbial profile.	Costa *et al.* (2014)[16]
Xylanase, Cellulase, Glucanase	Wheat offal	20% inclusion level	Improve the nutritive value of barley-based diets	Ohiain and Ofongo (2014)[36]

11. Conclusion

Poultry diets with high soluble NSP increase viscosity of digesta, altered intestinal morphology, physiology, reduce nutrient digestion, nutrient utilization and thereby decrease the performance of broiler birds. Intestinal viscosity hampered the nutrient digestibility and has deleterious effects on the bird's performance. NSP degrading enzymes supplementation cleave β-glycosidic bond, thereby decrease the viscosity of digesta, resulting in improvements in nutrients digestibility and performance when added to broiler diets.

12. Future prospects
More research are required to find out the cheapest sources of enzyme to economize the cost of supplementation and thereby production. Biotechnological interventions are required to modify the genes of cereals and legumes in a way to reduce the NSP or genetic modification in the structure of NSP to make it easily degradable by endogenous enzymatic digestion.

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