II. REVIEW OF LITERATURE

2.1 Nonstarch polysaccharides (NSPs)

Nonstarch polysaccharides (NSPs), as the name itself suggests, are polymeric carbohydrates differing in composition and structure from amylose and amylopectin (Gruppen, 1996). They are the plant structural analogues of the skeletal system of animal kingdom (Fig. 1 and 2). NSPs are \( \beta \)-linked polymers of pentoses and hexoses having high molecular weight ranging from 8000 to a million. NSPs are part of cell wall and are closely associated with other polysaccharide or non-carbohydrate material such as protein and lignin. These complex carbohydrates are present in high quantity in certain feedstuff. 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, whereas in starch the glucose molecules are joined mainly by \( \alpha \) \( (1-4) \) bond with a small number of \( \alpha \) \( (1-6) \) bonds (Smits and Annison, 1996). The nature of the bonds determines their susceptibility to digestion by avian enzymes. Glycosidic bonds other than \( \alpha \) \( (1-4) \), \( \alpha \) \( (1-6) \), \( \alpha \) \( (1-2) \), \( \alpha \) \( (1-1) \) and \( \beta \) \( (1-4) \) are not cleaved and are resistant to endogenous starch degrading digestive enzymes, but can be cleaved by microbially derived enzymes (Annison, 1993: Smits and Annison, 1996). NSPs are present both intra and extra cellularly but the majority originate from the cell wall. Most NSPs in poultry diets are complex polysaccharides and may vary in their complexity from simple \( \beta \)-glucans to very complex carbohydrates like arabinoxylans present in cereals. Among NSPs the nonsoluble portions like cellulose do not pose much of a problem while the partially soluble portions like \( \beta \)-glucans, arabinoxylans, etc., adversely affect the nutrition of birds by inducing physico-chemical changes in the gut.

2.1.1 Common NSPs of plant cell walls

Though commonly called NSPs, the plant cell wall polysaccharides include a wide range of chemically distinct compounds in different
combinations and proportions characteristic to and variable between different species of plants. Some of the common NSPs present in the plant cell walls, broadly classified into Cellulosic, Hemicellulosic, Pectic and/or Galactosidic substances are enlisted in Table 1.

2.1.2 Classification of NSPs

Figure 3 depicts the classification of NSPs based on solubility and further chemical composition. It is to be noted that cellulose is not soluble in water or even weak acids and alkalis and hence may not contribute towards an increase in the gut viscosity. This point is very important as it clearly suggests that cellulose, as a part of avian diet, may not have any direct adverse effect other than contributing to a lesser nutrient density unlike the soluble NSPs which are responsible for most of the problems associated with ‘dietary fiber content’

![Diagram](image)

**Fig. 3. Classification of nonstarch polysaccharides**

Based on chemical structure NSPs are further divided as follows

2.1.2.1 Cellulose
Made up of linear unbranched chain of β(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 (Fig. 4).

2.1.2.2 Hemicelluloses

Hemicelluloses are low molecular weight miscellaneous noncellulosic polysaccharides forming the major fraction of NSPs 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:

- **Pentosans (arabinoxylans, xylans):**
  The structure of cereal pentosans are composed predominantly of two pentoses, arabinose and xylose. Main chain is made of β(1-4) linked xylopyranose residues. Side chain made of α(1-3) linked arabinofuranosyl residues which, in turn, are attached to C-3 position of the xylan main chain (Fig. 5). The major substitutes are single arabinose residues, although in many instances hexoses and hexuronic acids are present in minor proportions.

- **β-D-glucans:**
  It consists of linear chain of glucopyranosyl residues (glucose units) joined by both β(1-3) and β(1-4) linkages (Fig. 6).

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

- **Arabinans and galactans:**
  The arabinans are polymers of (1-5)-α-L-arabinose residues branched through 02, 03 or both positions, whereas the galactans are polymers of (1-4)-β-D-galactose residues.
- **Galactomannan:**
  Made of $\beta(1\rightarrow4)$ linked mannan backbone to which D-galactose side chains are attached at C-6 positions.

- **Xyloglucans:**
  The structure of xyloglucan is made of $\beta(1\rightarrow4)$ linked D-xylopyronosyl (xylose) residues are attached at C-6 positions (Fig. 7).

### 2.1.2.3 Pectic polysaccharides

The term pectic polysaccharides refers to galacturonans and in that, more commonly rhamnogalacturonans.

- **Polygalacturonans**
  Main chain is made of $\alpha(1\rightarrow4)$ linked D-galacturonic acid and side chain consists of either $\beta(1\rightarrow3)$ linked D-xylose, $\beta(1\rightarrow6)$ linked D-galactose or $\beta(1\rightarrow4)$ linked $\alpha$-arabinose and less frequently $\alpha$-fucose (Fig. 8).

- **Rhamnogalacturonans**
  Similar to polygalacturonan except that the main chain in addition to D-galacturonic acid, contains $\alpha(1\rightarrow2)$ linked rhamnose residues resulting in a bent macromolecule (Fig. 9).

- **Oligosaccharides ($\alpha$-galactosides)**
  Successive addition of $\alpha$-galactosyl residues to sucrose primer leads to formation of raffinose, stachyose, verbascose and ajugose (Fig. 10).

### 2.1.3 Properties of NSPs

#### 2.1.3.1 Solubility
Solubility is an important property determining the antinutritional effects of NSPs. The solubility of NSPs is determined by their primary structure and their binding to other cell wall components. The degree of solubility is directly proportional to the degree of branching of the NSP molecule (Annison, 1993). In arabinoxylan, side chain with arabinose is soluble and in pectins, side chain with arabinose and xylose are soluble whereas in both, the main chain is insoluble. Among Beta-glucans those with (1-3) linkages are soluble and those Beta (1-4) linkages are insoluble whereas cellulose is completely insoluble. That is to say, cellulose is not soluble in water while the hemicellulosic and pectic substances are partially soluble in water and other aqueous solutions.

2.1.3.2 Viscosity and water holding capacity

Many polysaccharides when dissolved in water result in viscous solutions. Viscosity is dependent on several factors including the size of the molecule, branching nature, presence of charged groups, the surrounding structure and the concentration of polysaccharides. NSPs increase viscosity by directly interacting with the water molecule. At higher concentration, the molecules of the NSPs interact themselves and become entangled in a network further increasing the viscosity (Morris and Ross Murphy, 1981). Because of the formation of network with water, the viscosities and water holding capacities of soluble NSPs are relatively high compared to insoluble NSPs.

2.1.3.3 Binding of ions and molecules

Some NSPs such as pectins may have high charge density at a given pH value because of presence of acidic groups. The carboxyl groups can bind to cations influencing the mineral absorption in intestines.

2.1.4. NSP content of various feed ingredients.

The NSP contents of different feed ingredients as per various reports and NSPs commonly found in feed ingredients are given in Tables 2 to 4.

The NSP in cereal grains are composed predominantly of arabinoxylans (pentosans), β-glucans and cellulose. 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 NSPs. The main soluble NSP in these grains
and their byproducts is arabinoxylan, whereas in barley, oats, rye etc., it is β-glucan. Most of the cereals and by-products commonly used in Indian poultry diets have arabinoxylans as the main soluble NSP, almost an equal proportion of cellulose and much lower proportions of pectins, almost at one third levels of former two. By far, wheat tops the list among the cereals in arabinoxylans with around 7.25 per cent content closely followed by maize with 5.25 per cent content.

Oilmeals, on the other hand, tend to be higher in the content of all the three types of NSPs viz., arabinoxylans, pectins and cellulose. Among these, the cereal by-product, de-oiled rice bran (DORB) tops the list with a maximum reported NSP content of 60 per cent (Malathi and Devegowda, 2001). Nevertheless, rice bran samples across different places are highly variable and sometimes the total NSP contents could be as low as 22 per cent (Choc, 1997). Among oilseed cakes, sunflower meal tops the list with highest content of total pentosans, cellulose and total NSP, followed by soybean meal, which is higher in pectin content.

2.1.5 Adverse effects of NSPs in poultry

NSPs have a large number of adverse effects on the performance and well-being of poultry. The antinutritive properties of NSPs are broadly grouped into two types.

2.1.5.1 NSPs are not available directly to the system

Most NSP are part of cell wall and closely associated with other polysaccharides or non-carbohydrates like protein and lignin (Selvendran et al., 1987). NSPs are not available to the system as they are resistant to endogenous enzymatic digestion and monogastric animals do not secrete the enzymes to degrade the complex bonding of NSPs.

2.1.5.2 NSPs induce physico-chemical changes hindering nutrient utilization

The association of NSPs with other fractions in feedstuffs determines the antinutritive activity. Solubility is an important property, which increases the viscosity of ingesta in birds (Annison and Choc, 1991). Higher viscosity of ingesta would lead to reduced digestion and absorption of feed nutrients and hence poor performance. A few factors contributing to the anti-nutritional effects of NSPs are discussed below.
2.1.5.3 Water holding capacity (WHC)

Soluble NSPs have property of adsorbing or imbibing water molecules to give viscous solutions. The viscosity is dependent on size of molecule, branching character, presence of charged groups, surrounding structure and concentration. As the concentration increases, the molecules interact and become entangled in a network and increases the viscosity (Choct and Annison, 1992).

The primary important negative effect of increased gut viscosity is reduction in contact intensity between potential substrates and digestive secretions resulting in depressed digestibilities of fat, protein and carbohydrates and overall reduction in AME of diet (Smits and Annison, 1996). In addition, high viscous digesta increase the amount of sticky droppings (Marquardt et al., 1994). Sometimes NSPs complex with intestinal enzymes leading to lesser enzyme availability for digestion and also causing reduced fat digestibility by entrapping the bile salts.

In addition, viscosity forming agents increase the thickness of unstirred water layer resulting in increased resistance for absorption of fatty acids and monoglycerides. Further, viscosity increases the proliferation rate of enterocytes and change in morphology of villi and microvilli resulting in poor absorption (Smits and Annison, 1996).

2.1.5.4 Effect on intestinal viscosity

Highly viscous properties of soluble NSPs reduce the digesta passage rate and impair the diffusion of digestive enzymes to their substrates and mixing with gut contents (Antonion and Marquardt, 1982). Ingestion of soluble NSPs like arabinoxylans, β-glucans, pectins, etc. increases the digesta viscosity in broilers. The insoluble polysaccharides like cellulose pass through the GIT unchanged and are biologically inert (Annison and Choct, 1991).

The increased intestinal viscosity caused by the soluble NSPs present in grains depends on:

- Age of the bird – viscosity falls with age (Bedford, 1996).
Molecular weight of NSP – higher the molecular weight of the NSP, higher will be the viscosity (Smits and Annison, 1996).

Geographical area of production (Willingham et al., 1960).

Variety or genotype of the grain (Campbell et al., 1989).

Maturity at harvest – solubility and viscosity decreases with aging (Hesselman et al., 1981).

Processing – pelleting increases foregut and hindgut viscosity (Bedford et al., 1991).

Thus increased viscosity decreases physical contact between endogenous enzymes and nutrients by acting as a barrier thus decreasing movement of enzymes and substrate molecules thereby reducing the digestibility of starch, protein and lipids and reducing the performance of broilers.

Also, increased viscosity reduces rate of passage of digesta and increases retention time leading to stasis of food for long time, stimulates secretion of digestive juices in more than the required amounts. This causes increased endogenous nitrogen loss besides increasing thickness of unstirred water layer adjacent to intestinal mucosa, reducing the diffusion of nutrients (Smits and Annison, 1996).

2.1.5.5 Interaction between viscosity and gut microflora

The increased gut viscosity reduces the passage rate of digestion. The digesta retained for a prolonged period provides a good stage for bacterial fermentation resulting in colonization of unwanted bacteria in anterior parts of small intestine. The microflora may compete for nutrients with the birds causing irritation and thickening of gut mucosa, additionally increasing proliferation of enterocytes, which changes the morphology of villi and microvilli. Also, the dimensions and the function of fatty acid binding protein present at the villi surface will change leading to further damage. Finally, the microbes may cause deconjugation of bile acids from fat globules thus reducing emulsification of fat and its uptake (Choc't and Annison, 1992).

2.1.5.6 NSPs causing flatulence

Oligosaccharides along with other NSPs are carried to lower part of gut without being hydrolysed, but fermented by established microflora to liberate volatile fatty acids like butyric acid which results in flatulence and intestinal disorders (Iresh et al., 1995).
2.1.5.7 Effect on endogenous enzyme activity

Viscous polysaccharides may complex with digestive enzymes and reduce their activity. Danaif and Schneenan (1981) reported decreased activity of pancreatic enzymes viz., amylase, lipase, trypsin and chymotrypsin *in vitro* upon incubation with cellulose, pectin and xylan.

Isaksson *et al.* (1982) have shown *in vitro* that in a viscous environment, the activities of lipase and other enzymes are reduced. Almirall *et al.* (1993) reported a significant reduction in amylase and lipase activity in chicks fed barley based diets compared to the control group fed maize based diets. Larsen *et al.* (1994) reported no significant effect of fibre viscosity on proteolytic enzyme activity in the gut of rats fed on low fermentable carboxymethyl cellulose.

2.1.6 Methods of NSP estimation

Dietary fibre, usually defined as NSPs plus lignin can be analysed by various methods. The choice of method depends on the extent of information on the composition required.

2.1.6.1 Gravimetric methods

These methods measure the insoluble residue after chemical or enzymatic solubilisation of non-fibre constituents. Gravimetric methods are easy to handle and do not require any special equipments.

a. **Crude fibre method**

This method implies sequential extraction with dilute acid and alkali and isolation of the insoluble residue by filtration. Disadvantage of this method is that 40 per cent of the unavailable carbohydrates will be lost due to alkali treatment (Williams and Olmsted, 1935).

b. **Detergent method**

Suggested by Van Soest (1963) and includes treating with a detergent and either acid (acid detergent fibre, ADF) or neutral buffered solution (Neutral detergent fibre, NDF). Ideally, ADF determines cellulose and lignin, but residues of pectin and hemicellulose have been reported (Morrison, 1980). NDF method measures cellulose, hemicellulose and lignin, solubilises protein efficiently and
fat to a limited extent. Difference between NDF and ADF gives hemicellulose content.

c. **Enzymatic methods**


### 2.1.6.2 Colorimetric methods

In strong acid solution, carbohydrates undergo condensation reaction with a large number of substance giving coloured products that can be measured spectrophotometrically. Three such reactions are those with anthrone, orcinol and carbazole, which are relatively specific for hexoses, pentoses and uronic acid, respectively. Mutual interference between groups of sugars is possible in gravimetric methods. However, by colorimetry, it is possible to measure individual dietary fibres separately.

### 2.1.6.3. Chromatographic methods

a. **Thin layer chromatography**  Usually used for separation of individual sugars, either monosaccharides or oligosaccharides, and most commonly for raffinose series of oligosaccharides Viz, sucrose, raffinose, stachyose, verbascose, etc.

The ethanol extract containing mixture of sugars and spotted on the TLC plate (cellulose coated), developed using suitable solvents, individual sugars are identified and recovered and then estimated spectrophotometrically (Tanaka et al., 1975).

b. **Gas liquid chromatography (GLC):** The monomeric constituents of the NSPs are liberated by acid hydrolysis, derivatized usually to their corresponding alditol acetates (Englyst and Cummings, 1988). GLC gives more accurate results than a routine technique, its use needs considerable training and attention on the various
steps involved. High performance liquid chromatography (HPLC) is an alternate to GLC method of screening NSPs.

2.1.7 Methods to improve utilization of NSPs

i. **Water treatment:** Grains are soaked in water to remove soluble fractions of NSPs and dried, but this method is found to be only partially effective and impractical in poultry.

ii. **Germination:** Plant cells contain an endogenous enzyme, which gets activated during the process of germination, and hydrolyse the NSPs completely. This is very effective method but not practical.

iii. **Heat treatment:** Autoclaving breaks some of the NSPs like oligosaccharides into smaller units.

iv. **Acid treatment:** Treating with dilute acids would result in extraction and hydrolysis of different NSPs.

v. **Inclusion of Salt:** Sodium ions of salt bind with anions of uronic acids and other NSPs.

vi. **Radiation:** May damage NSPs molecules and reduce the molecular weight (Lee and Campbell, 1983).

vii. **Antibiotic supplementation:** The response to antibiotic supplementation has been hypothesized as the result of inhibition and elimination of intestinal microflora, which compete with the host for available nutrients thus alleviating the antinutritive effects of the NSPs. Classen et al. (1985) noticed improvement in the quick growth rate and fat absorptions in hulless barley fed birds supplemented with 100 ppm lincomycin.

Fengler et al. (1988) observed an increase in the fat retention and excreta dry matter along with reduction in excreta viscosity in chicks fed rye containing diets when supplemented with penicillin.

viii. **Enzyme application:** Exogenous microbial enzymes are supplemented to poultry diets based on the type of substrate present in the feed.
2.1.8. Enzymatic approach to tackle NSPs

2.1.8.1 Introduction to enzymes

Enzymes are highly substrate specific class of bioactive compounds aiding the biochemical reactions. By what is known as the so called ‘lock and key’ principle enzyme attach on to substrate firmly and facilitate the bioconversion reaction.

Enzymes are proteins with highly complex three-dimensional molecular structure. They are biological catalysts and act under very specific reaction conditions (temperature, pH and humidity) only with their specific substrates. They accelerate chemical reactions by many folds. According to international Enzyme Commission (EC), enzymes are divided into six main classes based on type of the reaction they catalyse.

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Synthetases (ligases)

Enzymes used in animal nutrition are exclusively hydrolases with the EC No.3, which break down C-O, C-N, C-C bonds (Shaw and Pack, 1998).

2.1.8.2 Mechanism of action

Enzymes used in animal nutrition have both exo and endo activities. Exoenzymes breakdown only the terminal monomers one by one while endoenzymes degrade bonds within the molecular strand randomly. Thus, endoenzymes are able to break large molecules into smaller fragments of molecular weight, which is mainly responsible for reducing digesta viscosity.

2.1.8.3 Characteristics of enzymes

In addition to the specific degradation site in the molecule, the effectiveness of an enzyme is further determined by conditions prevalent at the site of reaction like pH value, temperature, water content and presence of activators or inhibitors, as well as the substrate concentration and strain of organism producing the enzyme.
2.1.8.4 Source of enzymes

Various fungi, bacteria and to some extent yeast are employed in enzyme production. It is essential for these micro-organisms to produce enzymes as they sustain their own viability by producing enzymes to break down substrates for further metabolism. Fungi represent largest group among enzyme producing micro-organisms. The most important genera are; *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp. and *Huminicola* sp. Among the bacteria *Bacillus* sp., mainly *B. subtilis* and *B. licheniformis* are most commonly employed as sources of enzymes.

### Enzymes commonly used in animal feeds

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
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<tbody>
<tr>
<td>Cellulase</td>
<td>Cellulose</td>
</tr>
<tr>
<td>Pentosanase (Xylanase)</td>
<td>Pentosans (Arabinoxylans)</td>
</tr>
<tr>
<td>Beta-glucanase</td>
<td>Beta-glucans</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Pectin</td>
</tr>
<tr>
<td>Amyloglucosidase</td>
<td>Resistant starch</td>
</tr>
<tr>
<td>Protease</td>
<td>Proteins</td>
</tr>
<tr>
<td>Alpha-galactosidase</td>
<td>Oligosaccharides(Alpha-galactosides)</td>
</tr>
<tr>
<td>Phytase</td>
<td>Phytic acid/Phytates</td>
</tr>
<tr>
<td>Tannase</td>
<td>Tannins</td>
</tr>
<tr>
<td>Lignase</td>
<td>Lignin</td>
</tr>
</tbody>
</table>

2.1.9 Effect of enzymes in laying hen diets

A comparison of the effect of enzymes to layer diets across different parameters such as intestinal viscosity, egg production, egg weight, feed intake, feed conversion efficiency, litter moisture, gut bacterial profile and nutrient digestibility etc., has been tabulated in Table 5. A few relevant cases have been reviewed in depth in the current chapter.

2.1.9.1 Effect of enzymes on intestinal viscosity and nutrient digestibility
Solminski and Campbell (1990) reported that the addition of a commercial enzyme cocktail high in polygalacturonase to a laying diet with 40 per cent Canola Meal (CM) resulted in a significant increase in NSP digestibility. However, those researchers made no reference to any improvement in laying performance or egg production. Classen et al. (1991) observed that increased viscosity of digesta was mainly due to soluble pentosans, which acts as a barrier between endogenous enzymes and nutrients. Additionally, increased moisture of droppings leads to not only environmental pollution by release of ammonia, but also causes hock and breast damage of birds. Similar results were obtained by Bedford et al. (1991) who studied the effect of pelleting of rye based diet with crude pentosanase supplementation at zero to two per cent levels on viscosity of intestinal contents and performance of broilers. Enzyme supplementation in all groups increased weight gain and feed efficiency, the viscosity of foregut and hindgut were significantly reduced. Weight gain and feed efficiency correlated only with foregut viscosity but not with hindgut viscosity.

Choct and Annison (1992) examined the roles of digesta viscosity and microflora in broiler chicken after extracting pentosans from wheat and depolymerising them using β-xylanase which reduced the relative viscosity of polysaccharides by four folds. Inclusion of 35 g alkali extractable pentosans per kg diet significantly (P<0.05) depressed performance and the digesta viscosity of these birds was significantly (P<0.05) higher than that of control, whereas addition of the same amount of deploymerised pentosans had no effect on bird performance and had lesser effect on digesta viscosity. Further, supplementation of diet containing wheat pentosans with procaine penicillin did not improve bird performance and the researchers concluded that wheat pentosans elicit their antinutritive activity by predominantly increasing the digesta viscosity.

Further, Bedford (1995) reported a dose response study employing four levels of rye (0, 20, 40 and 60%) substituting for wheat and six levels of pentosanase (experimental product from Trichoderma longibrachiatum, 0, 0.1, 0.2, 0.4, 0.8 and 1.6%). He indicated that addition of pentosonase significantly reduced the intestinal viscosity and improved weight gain and feed conversion efficiency at each level of rye inclusion. Moreover, there was a significant relationship between digesta viscosity and weight gain and between digesta viscosity and feed conversion efficiency. Choct et al. (1996) observed that addition of soluble NSPs (extracted from wheat) resulted in
increased the gut viscosity, reduced AME and feed efficiency of diet. Enzyme (Avizyme-1300) supplementation reversed the adverse effects by increasing weight gain, AME and feed efficiency. Caecal VFA concentration was elevated, whereas ileal fermentation was inhibited by enzyme supplementation.

Crouch et al. (1997) determined the efficacy of xylanase (1000 units/g) supplemented with 40 per cent wheat and corn – soy based diets, and concluded that enzyme supplementation had lowered the intestinal viscosity and improved the performance of chicks. Bedford and Classen (1993) identified foregut digesta viscosity as a major determinant of performance in broilers fed wheat – barley based diets. Soluble and high molecular weight fibre polysaccharides were responsible for high digesta viscosity, which resulted in poor feed efficiency and depressed the body weight.

Steenfeldt et al. (1998) conducted a trial on broilers fed diets containing high amount of wheat (>80%) with enzyme preparation Bio-feed plus (xylanase, cellulase and \(\beta\)-glucanase). Effect of enzyme was significant in the whole period from 21 to 42 days, however, feed intake was not influenced but intestinal viscosity in both jejunum and ileum were reduced. Dusel et al. (1998) observed a strong relationship between NSPs and excreta viscosity in pelleted diets based on wheat fed to broilers. Addition to xylanase reduced the digesta viscosity in both jejunum and ileum. Further, enzyme supplementation tended to improve AMEn and nutrient digestibility.

Jaroni et al (1999) investigated the effect of a commercial poultry enzyme preparation (xylanase and protease) in two strains of Leghorn hens, DeKalb Delta and Hisex White on the digestibility of protein, fat, Ca, and P and to determine any changes in the relative size of the digestive tract, gut morphology, and gut viscosity of birds fed wheat middlings over an 18-wk period. Protein digestibility was improved by enzyme supplementation in all diets which was significantly higher at 50 weeks but at 60 weeks the responses were similar in enzyme supplemented and non-supplemented groups.

Silversides and Bedford (1999) studied intestinal viscosity, AME and performance of broilers fed nine different varieties of wheat based diets. Results indicated that intestinal viscosity accounted for 50 to 90 per cent of the variation in AME. Poor performance and increased viscosity were reported to be due to soluble NSPs, which
reduced the availability of energy in all varieties and in turn the body weight and feed efficiency.

Kocher et al. (2000) studied the jejunal and ileal viscosity of broiler chickens fed diets containing CM or SFM with or without enzyme supplementation at 35 per cent level in the diet. He noticed digesta viscosity in the jejunum and ileum differed significantly (P<0.05) between diets containing CM and SFM. The addition of enzymes had no effects on digesta viscosity, however the concentrations of soluble NSP in the jejunum were significantly reduced when enzyme was added to the SFM diet.

Channegowda et al. (2001) conducted a trial of six-week duration to determine the effects of two enzyme mixtures (A and B) on sunflower Meal (SFM) based diets fed broilers. Birds were fed diet containing 0, 10, 20 per cent SFM supplemented with or without enzyme A (1 kg/ton) or B (2 kg/ton). The relative viscosity of intestinal contents was increased in parallel with SFM levels. Addition of enzyme A or B reduced the digesta viscosity considerably, but did not improve performance.

2.1.9.2 Effect of enzymes on litter moisture

It is a general phenomenon that higher dietary fibre increases the litter moisture content and the same has been reported by many researcher. NSP degrading enzymes have been claimed to reduce litter moisture by virtue of their ability to digest dietary NSP.

Ferket (1993) studied the effect of supplementing β-glucanase to barley based diet on turkey and found significant improvement in nine weeks body weight, feed efficiency and litter moisture. On contrary to this, Arunbabu and Devegowda (1997) found no improvement in condition of droppings between treatments, whereas improvement in litter condition due to enzyme supplementation was reported by Devegowda and Nagalakshmi (1992), Jayanna and Devegowda (1993), Mohandas and Devegowda (1993) and Rajeswara Rao and Devegowda (1996). Further significant reduction in moisture content of droppings was noticed in high fibre (9.62 and 11.13%) diets than low and medium fibre diets (Prakash and Devegowda, 1996) in layers.
Channegowda et al. (2001) observed significant increase in litter moisture with the increased level of SFM (0, 10 and 20%) in the broiler diets, but addition of enzyme reduced the litter moisture considerably and did not improve performance.

2.1.9.3 Effect of enzyme supplementation on egg production, feed intake and feed conversion

Mohandas and Devegowda (1993) observed no influence of protease containing enzyme mixture supplementation to varying levels of protein and energy with 5, 10 and 15 per cent inclusion of SFM based diets on egg production. However, significant (P≤0.05) improvement in feed efficiency was recorded only in low energy diets (2300 KCal/kg of ME). In a similar experiment, Jayanna and Devegowda (1993) observed no effect on performance of layers fed various levels of energy (2300 to 2600 K Cal/kg of ME) with 0.1 per cent multi enzyme mixture.

Francesch et al. (1995) studied the effect of a commercial enzyme (Grindazyme GP-5000) on productive parameters of shaver laying hens receiving 60 per cent barley, 20 per cent SFM diet with either 0, 0.50, 0.75 or 1.00 g/kg feed from 26 to 41 weeks. The highest dose of enzyme (1 g/kg) improved egg weight by more than 1.0 g and the percentage of eggs heavier than 60.0 g and enzyme supplementation also reduced the percentage of dirty eggs, water intake and feed: water ratio. However, there were no significant differences in rate of lay, daily feed intake and body weight gain due to enzyme addition.

Garcia et al., (1997) observed significant improvement in egg weights in 46-week old layers supplemented with beta-glucanase and xylanase combination. The improvement in egg weights at 34 and 56 weeks of age was just numerical and non-significant. Nevertheless, supplementation with enzymes resulted in more percentage of XL eggs allowing for classification of more eggs into the larger category.

Jackson (1999) studied the effect of supplementing beta-mannanase to laying hen diets given low (2790-2830 Kcal/Kg) and high (2890-2930 Kcal/Kg) energy diets at various age groups (18-30, 31-42, 43-54 and 55-66 weeks of age) on hen-day production and egg weight. He reported that enzyme supplementation significantly improved the
hen-day egg production at both the energy levels in all the age groups tested, except in
the 18-30 weeks group fed low-energy diet whose performance was below that of the
control group. Differences in egg weights as a result of enzyme supplementation, which
were non-significant, were not in a predictable pattern but in general, the low energy diet
showed better positive response to enzyme supplementation.

In another trial, studying the effect of three different xylanase or mannanase based
enzyme combinations on molted hen performance (from 72-100 weeks of age) Jackson
(1999) found that all the enzyme combinations improved the hen-day egg production and
the enzyme combination which had additional cellulase, beta glucanase and alpha
galactosidase improved the production significantly. Egg weight was also improved by
enzyme supplementation while feed consumption was increased in all the three enzyme
groups.

In yet another study aimed at studying the interaction of dietary lysine level and
enzyme supplementation at 17-37 weeks of age, Jackson (1999) found that Enzyme
supplementation improved the hen-day egg production at normal levels of lysine (0.70
and 0.78%) while it had a negative response at high dietary lysine levels (0.87 and
0.94%). Egg weights were reduced at all the lysine levels except the highest and feed
intake increased at all the lysine levels except the lowest.

These three inter-linked studies bring out a pattern of birds’ response to enzyme
supplementation. Lower the energy level, higher the age group, and lower the other
nutrient levels, better would be the response to enzyme supplementation. However, egg
weight has not shown a particular predictable trend. This could be so because, it is
mainly a function of digestible protein, digestible amino acids and dietary protein-amino
acid contents, which might have varied due to dietary and enzyme combination
variations.

Observing the response of two different strains of birds, HyLine W-36 and
Babcock B-300 birds between 20 and 40 weeks of age at two different energy levels
(normal and low) Schiedeler et al (1998) has reported that the egg production and egg
weight response to enzyme supplementation were better in low energy diets in W-36
birds while egg production showed improvement in normal energy diet in B-300 birds but
dipped down in low energy diet. Feed intake and egg weight responses were not in any specific pattern, suggesting the interaction of other factors which might have influenced the results.

Hughes et al (1999) conducted a study progressively replacing wheat with triticale at 20, 40, 60, 80 & 100 per cent levels, which resulted in significantly reduced egg production and significantly increased feed intake, while not affecting egg weight, egg shell thickness and excreta moisture. Adding enzyme to wheat diet reduced the feed intake by about 3 per cent. Adding enzyme to triticale included diets improved the egg production and reduced to feed intake to the extent that they were non-significantly different from their wheat based counterparts.

2.1.9.4 Enzyme effects on gut bacterial population

The soluble fractions of the NSPs are known to increase the gut viscosity and decrease the digesta transit rate. This favours the colonization of anaerobic bacteria affecting the gut health and brings down the nutrient absorption in the intestine (Annison and Choc, 1991; Choc and Annison, 1992; Annison, 1993). The addition of enzymes to diets rich in NSPs has been shown to significantly reduce the intestinal viscosity and gut bacterial loads. (Kocher et al., 2000; Bedford, 2000) Several researchers have reported reduced pathogen profiles in the gut with or without improvement in beneficial microbial count upon enzyme supplementation to poultry diets (Apajalahti and Bedford, 1999; Bedford, 2000; Tan and MIlan, 2003; Engberg et al, 2004; Murphy et al., 2004, Ramesh and Devegowda, 2004).

2.1.9.5 Enzyme effects on nutrient digestibility

Drawing up a conclusion from a large number of studies conducted under the supervision of Animal Feed Analytical and Quality Control Laboratory, Namakkal, Chandrasekaran (2001) reported that enzyme supplementation improved the ileal digestibility values of hemicelluloses by 6.1 to 8.1 per cent, cellulose by 18.9 to 21.2 per cent, crude protein by 4.2 to 10.1 per cent and that of ME by 75 to 128 kilocalories per kilogram of diet.