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RESEARCH ARTICLE

NSPASES: CAN THOSE EXOGENOUS ENZYMES REALLY CONSTITUTE NATURAL GROWTH COFACTORS IN BROILER CHICKENS?

Sophia Derqaoui[1], Mohammed Oukessou[2] and Saadia Nassik[1]

1. Unit of Avian Pathology, Department of Veterinary Pathology and Public Health. Agronomy and Veterinary Medicine Institute Hassan II, Rabat, Morocco.
2. Unit ofPhysiology and Therapeutics, Department of Veterinary Biological and Pharmaceutical Sciences. Agronomy and Veterinary Medicine Institute Hassan II, Rabat, Morocco.

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Abstract

In the quest to improve public and animal health, white meat is considered to be one of the major causes of antibioresistance identified in medicine, through uncontrolled use of antibiotics in poultry farming as growth promoters (GPA). Thus, this use needs to be reconsidered at large scale. This reconsideration involves the substitution of GPAs by natural alternatives, particularly enzymes degrading non-starch polysaccharides (NSPases) which will allow:1) modulation of the intestinal microbiota, to attenuate the anti-nutritional effects of insoluble NSPs, 2)reduction of the non-digested portion of the substrate and 3) improvement of the zootechnical performance of the chicken. Non-starch polysaccharides contain two main families, namely: water-insoluble NSPs including cellulose and partially water-soluble NSPs. However, despite the fact that these components constitute the major part of cereal dietary fiber, they have an anti-nutritional effect associated with the viscous nature of these polysaccharides and their interaction with the intestinal microflora due to the fact that poultry does not produce enough endogenous enzymes to hydrolyze NSPs. The use of NSPases produced mainly by fungi and bacteria allows counterbalancing the anti-nutritional properties of dietary fibers by increasing the digestibility of starch and improving the zootechnical performance of broilers, particularly the conversion index.Thus, this literature review aims to shed light on the effects of NSPases on the zootechnical parameters of chickens, the intestinal microflora as well as on nutritional digestibility in order to use them as alternatives to GPAs and limit the aggravation of the phenomenon of antibioresistance. This approach is therefore, part of the world famous concept of "one world one health" and which applies to the design and implementation of programs, policies, legislations and research for which several sectors communicate and collaborate to improve public health outcomes.

Corresponding Author:- Sophia Derqaoui
Address:- Unit of Avian Pathology, Department of Veterinary Pathology and Public Health. Agronomy and Veterinary Medicine Institute Hassan II, Rabat, Morocco.

Introduction:-

In a world of perpetual scientific and intellectual evolutions, the awareness of today's consumer of the health risks of some foodstuffs, has led to the definitive abolition of some substances from production, in general, and the food industry, in particular. This approach has given way to a new concept: "the substitution" of these emblematic substances, subjectedto intensive debates, by other substrates of natural origin, having the same virtues without generating the same harmful effects.This approach extends to several sectors related to human food, including the poultry sector, as white meat is considered the cheapest and most consumed meat in the world. Indeed, its consumption has experienced an evolution of 58% in 20 years since it increased from 32.038 million tons in 2000 to 50.596 million tons in 2021 (FAO and OECD, 2020).

To meet the increasing human demand for white meat, the use of new farming techniques to intensify production has been imposed. The most commonly known is the use of growth-promoter antibiotics (GPA) in broiler farms (Chevalier, 2011). Thus, the rapid emergence of antibiotic resistance in human and animal health might be linked to the use of GPAs in poultry farming.Previous studies have established a direct relationship between the excessive and uncontrolled use of antibiotics in poultry farming and the antibiotic resistance reported in humans. Indeed, when a bacterium involvedis resistant to antibiotics, the animal-human passage of antibio-resistance is evident (Madec, 2013). In fact, the strain responsible for human salmonellosis wasidentified, for the first time, in cattle (Gayatri etal., 2017). Selection of resistant bacteria will be important as the number of simultaneously treated animals is greater (Sanders etal., 2017).

Therefore, thinking of GPAs use in chicken feed is highly encouraged to come up with cofactors, of natural origin,to substitute GPAs. Indeed, several alternatives to GPAs have been identified such as enzymes, out of which the most widely used are the so-called non-starch polysaccharide degrading enzymes (NSPases).

According to the literature, NSPases are proteins with catalytic properties that metabolize non-amylaceous polysaccharides (NSP) mainly in viscous cereals (wheat, barley and triticale) into oligosaccharides millions of times faster than in their absence (Ravindranand Son, 2011; Pirgozliev and Bedford, 2013; Pirgozlievetal. 2019). Thus, this degradation allows poultry to metabolize cereal diets, based on an oligosaccharide and not a non-amylaceous polysaccharide (Bedford and Classen, 1992; Bedford and Schulze, 1998). As a result, the development and use of enzymes has gained momentum in poultry nutrition.

Definition And Classification Of Non-Amylaceous Polysaccharides

Dietary fiber is an important component of plants. Plant cell walls are highly ordered and consist of polysaccharides, polyphenolytics, glycoproteins and glycolipids. The components are arranged in three main patterns to give fibrillar polysaccharides (predominantly cellulose and arabinoxylans), matrix polysaccharides (predominantly arabinoxylans, pectin, and β-glucan) and encrusting substances (predominantly lignin) (Cone etal.,1996). The variation and structuring of these fibers are highly dependent on the type of plant, its stage of maturation and the part of the plant. Most poultry feed formulations are based on cereals and cereal-oil meal by-products, so they contain a significant amount of non-amylaceous polysaccharides (Ayoola etal., 2014).

Definition of NSP

Non-starch polysaccharides (NSP) are carbohydrate fractions except for starch and free sugars. They are polymeric carbohydrates whose composition and structure differ from starch and amylopectin. They are the plant structural analogues of the skeletal system in the animal kingdom (Sethy et al., 2015). The monosaccharide unit, namely glucose, is linked by α or β (1-4) bonds, with (1-6), β (1-3) and (1-4) bonds. The main characteristics that distinguish starch from NSP are the type, number, and position of these glucoside links (Hetland et al., 2004). These polymers have high molecular weights ranging from 8.10^3 to 1.10^6(Sethy et al., 2015). They can be branched or linear, with or without charged groups (Cone et al., 1996). NSP content in plants varies not only with plant species, but also between genotypes and cultivars of the same species. In addition, agronomic growing conditions such as pre-harvest environmental factors and post-harvest storage conditions can influence this content (Sethy et al., 2015).

Thus, NSPs are defined and classified based on the following structural considerations (Choc, 1997):

1. The identity of the monosaccharides,
2. The monosaccharide ring forms (6-membered pyranose or 5-membered furanose),
3. The positions of the glycosidic bonds,
4. The configurations (α or β) of the glycosidic bonds,

5. The sequences of monosaccharide residues in the chain and
6. The presence or absence of non-carbohydrate substituents.

Most recent poultry feed formulations are based on cereals, cereal meal by-products, and oilseeds, so they contain a significant amount of NSPs. These are classified according to several factors (Ayoola et al., 2014).

Classification Of NSPs

The term non-starch polysaccharides (NSPs) covers a wide variety of polysaccharide molecules except α-glucans (starch). The classification of NSPs was originally based on their methodology of extraction and isolation. The remaining residue after series of alkaline extractions of cell wall materials is referred to as cellulose and the fraction of this residue solubilized by alkali is referred to as hemicellulose (Choct, 1997). Thus, NSPs are seriated according to their water solubility and on the basis of their linkage.

Based on their solubility

- Water-insoluble NSPs including cellulose (Choact, 2002) are polysaccharides of the β-D-glucan series. Their repeating unit is cellobiose which consists of two β-D-glucopyranoses (glucoses) in their 4C 1 chair conformation joined by a β1-4 glycosidic bond (Stage, 2002).
- NSPs partially soluble in water (Choact, 2002):
- Non-cellulosic polysaccharides (also called hemicellulose): this is a mixture of non-cellulosic polymers-arabinoxylans, linked beta-glucans, mannans, galactans, xyloglucan, fructan.
- Pectic polysaccharides: these are poly-galactouronic acids that can be substituted by arabinan, galactan and arabinogalactan.

On the basis of their linkage

- Water-soluble or partially water-soluble NSPs: glycosidic beta 1, 4 linkage backbones with beta 1, 3 linkages (Sethy et al., 2015).
- Water-insoluble NSPs: long 1, 4 glycosidic beta unit sequence (Sethy et al., 2015).

Commonly known NSPs

Although commonly referred to as NSPs, plant cell wall polysaccharides include a wide range of chemically distinct compounds in different combinations and proportions that are characteristic and variable among different plant species. Some of the common NSPs present in plant cell wall are classified as cellulosic, hemicellulosic, pectic, and/or galactosidic substances (Bailey 1973; Choct, 1997; Bach Knudsen, 2001; Johansson et al., 2004; Sethy et al., 2015; Singh and Kim, 2021) (Figure1).

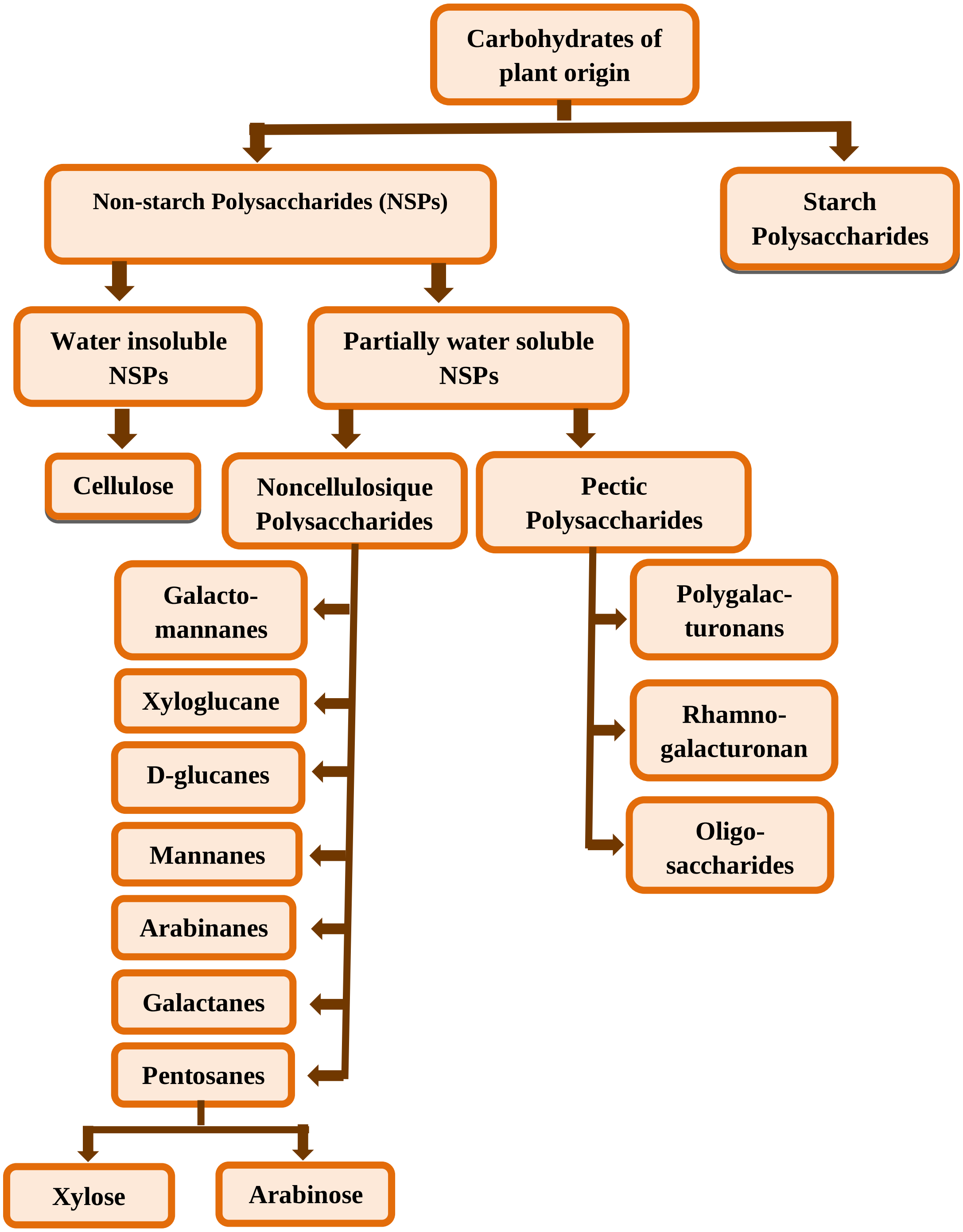

Figure 1:- Scheme summarizing the classification of commonly known non-amylaceous polysaccharides.

Moreover, the non-amylaceous polysaccharide content of food ingredients varies among products (Table 1). From 10%to 30% of cereals are NSP-based, the majority of which are composed of arabinoxylans, cellulose and β-glucans. Nevertheless, cereal grains can be classified into two groups:

1. Viscous cereals: rye, wheat, barley, triticale and oats and
2. Non-viscous cereals: corn, sorghum, rice and millet.

This classification is based primarily on the amount of soluble NSPs present in the grain (Table 1)(Choct, 1997; Choct, 2015; Englyst, 1989; Graham and Aman, 2014).

Table 1:- Concentration of non-cellulosic polysaccharide fractions in cereal grains [g/kg DM] (Rodehutscord et al., 2015).

Cereals	Type	Arabinoxylane	β-glucanes	Cellulose	Soluble NSP	Total NSP
Wheat	Soluble	13.9	2.0	-	19.1	98.2
	Total	63.7	6.1	14.4		
Barley	Soluble	9.7	24.1	-	50.6	172
	Total	77.4	46.7	27.5		
Rye	Soluble	30.9	6.6	-	41.2	139
	Total	85.4	20.1	11.9		
Triticale	Soluble	12.6	0.9	-	20.6	103
	Total	55.3	6.6	19.3		

Anti-Nutritional Properties Of Partially Water-Soluble NSPs

It is generally admitted that the main adverse effects of NSPs are associated with the viscous nature of these polysaccharides, their physiological and morphological effects on the gastrointestinal tract and their interaction with the gut microflora. These propertiesgenerate a considerable decrease in growth parameters of broiler chicken and are considered as anti-nutritional factors (Choct and Annison, 1992; Choct, 1997; Topping, 2007; Chutkan et al.,2012; Mateos et al., 2012; Choct, 2015). Indeed, the anti-nutritional effect of NSPs is due to the fact that poultry does not have the capacity to produce enough endogenous enzymes to hydrolyze the glycosidic bonds of its polysaccharides (Carré, 1993). Since polysaccharides are the major caloric substrate in poultry nutrition, itsavailable metabolizable energy and impact on zootechnical parameters are directly related to the dietary level of NSPs (Santos et al., 2004a,b).In addition, NSPs undergo rapid fermentation in the gut and act as an energy source for anaerobic pathogens, including Clostridium perfringens, the bacillus responsible for necrotic enteritis in poultry, and aerobic agents, namely: Escherichiacoli (Hetland et al, 2004; Józefiak et al., 2006; Timbermont et al., 2011; Sethy et al., 2015; Hashemipour et al., 2016).Thus, the anti-nutritive effects of non-amylaceous polysaccharides include:

Viscosity

The viscosity of the digest is one of the main factors influencing digestibility. Indeed, viscosity interferes with efficient diffusion of nutrients including fat-soluble vitamins and minerals, subsequently reducing their degradation by endogenous enzymes at the mucosal surface and their transport (Montagne et al., 2003; Bederska-Łojewsk et al., 2017). Their poor absorption is probably due to a reduction in the formation of micelles mediating their transfer to the brush border of the intestinal epithelium for absorption. This processleads to a low metabolizable energy level of feed and consequently, reducedpoultry growth (Kluth and Rodehutscord, 2007; Slominski, 2011; Chutkan et al., 2012; Latorre et al., 2015), which in turn, causes an increase in feed consumption to compensate for the lack of nutrients and therefore, an increase in the production cost (Boros et al., 1998; Goncharenko et al., 2011; Cordoso et al., 2014).

NSPs increase viscosity also by directly interfering with water molecules allowing the formation of a "cage" type structure that traps nutrients. At high concentrations, the NSP molecules interact with each other and become further entangled in a network consecutively increasing the viscosity of the digest (Simon, 1998; Mudgil and Barak, 2013). Thus, the viscosity and water retention capacity of soluble NSPs are relatively high compared to insoluble NSPs (Thebaudin et al., 1998; Sethy et al., 2015; Whiting et al., 2017). Indeed, supplementation with 15 g/kg of wheat arabinoxylan decreased the apparent average amino acid digestibility by 17% and increased the average of endogenous amino acid loss by 23.5 g/kg DM intake (Angkanaporn et al., 1994). Similarly, the loss of lysine and methionine in a low-fiber diet (Raw Fiber = 30 g/kg) was 0.4 and 0.17 g/kg, respectively, and 0.59 and 0.19 g/kg, respectively, in a high-fiber diet (FC = 80 g/kg) (Kluth and Rodehutscord, 2009). These proprieties of NSPsreduce digestion and nutrient absorption efficiencies.

Furthermore, the degree of solubility is directly related to that of branching and polymerization of the non-amylaceous polysaccharide, in that, the greater the branching is, the higher the viscosity and degree of water retention are (Sethy et al., 2015). In addition, soluble NSP generates sticky fecal material that increases moisture content of the litter and therefore promotes the development of pathogens in the chicken environment (Bach Knudsen, 2014). These conditions are so far favorable for the development of plantar pododermatitis causing intense pain in the musculoskeletal system reducing the individual’s movements for feed intakewhich ends into decreased growth (Meluzzi et al., 2008). In addition to the viscosity, anNSP rich diet generates a high bacterial translocation to the portal system, causing systemic infections that can lead to death in the most severe cases (Latorre et al., 2015).

Gut microflora:

The microflora is a key link influencing the health status of poultry, especially intestinal development, digestion, nutrients' absorption and immune system (Matin et al., 2012; Sánchez et al., 2017).It is present throughout the poultry tract, with a progressively increasing population along the intestines. The vast majority of which resides in the cecum, ranging from 10^{11} to 10^{12}Colony-Forming Units (CFU)/g of luminal content (Apajalahti et al., 2004). Therefore, it is crucial to maintain balancedpopulations of beneficial bacteria (Lactobacillus, Enterococcus, and Bifidobacterium) and potentially pathogens (Clostridium perfringens, Escherichia coli,Salmonella spp....) present in the gastrointestinal tract(Matin et al., 2012; Bederska-Łojewska et al., 2017).

Commensal bacteria in the gastrointestinal tract are able to ferment not digested nutrients by endogenous enzymes including those resulting from the digestion of NSPs. As a result, lactic acid and short chain fatty acids (SCFAs), mainly acetate, propionate and butyrates, are formed. These compounds play an important role as energy sources for chicken (Józefiak et al., 2004). In addition, SCFAs reduce the number of pathogenic bacteria by lowering the pH of the gastrointestinal tract, which creates unfavorable conditions for their proliferation, while such an environment promotes the development of the commensal anaerobic population (Bederska-Łojewska et al., 2017). Indeed, the ability of SCFAs to readily penetrate the lipid membrane is considered one of the mechanisms of antimicrobial action of SCFAs (Ricke, 2003; Singh and Kim, 2021). Studies have confirmed a reduction in the population of Salmonellatyphimurium in poultry (Van der Wielen et al., 2000; Józefiak et al., 2004) and Campylobacter jejuni in broiler chicken (Guyard-Nicodème et al., 2016). Soluble dietary fiber has the ability to inhibit the adherence of C. difficile, enterotoxigenic E. coli, and Salmonella to the intestinal epithelium both in vitro and in vivo (Roberts et al., 2013).

However, there is no doubt that a high level of ileal viscosity slowing down the gastric passage rate and leading to impaired digestion, has a negative effect on the gut microbiota as undigested nutrients become a fertile ground for pathogen proliferation (Bedford and Cowieson, 2012). Indeed, the overgrowth of certain anaerobic organisms can lead to toxin production and the deconjugation of bile salts essential for fat digestion (Choct, 1997). In addition, when digestion is compromised, the flow of unabsorbed nutrients into the cecum increases dramatically (Choct et al., 1996) causing dramatic changes in bacterial population (Chen et al., 2010).The increase in the concentration of undigested substrates in the gastrointestinal tract generates proliferation of the local microflora, causing the competition phenomenon for nutrients with the host, thus accentuating the risk of microbial disruption if the balance is in favor of pathogen growth (Kiarie et al., 2013). Indeed, excessive levels of dietary NSPs can tip the balance of the host's symbiotic commensal microflora towards the more pathogenic that ultimately harm gut health, animal welfare, and jeopardize the microbial safety of products (Ayoola et al., 2014). Furthermore, the host counteracts increased undigested substrates by increasing gut mass, which increases health and maintenance requirements and compromises growth efficiency resulting in increased cost price per kilogram of live weight (Ferrell, 1988; Agyekum et al., 2012).

The anti-nutrient effects of partially soluble NSPs depend on the polymeric nature of the considered NSPs, once the polymers are cleaved into smaller fragments, their activity is amply limited. Therefore, it is appropriate to ensure that these long-branched chains of non-starch polysaccharides are cleaved to better take advantage of their nutritional value (Singh and Kim, 2021). This denaturation is achieved through exogenous enzymes that help to amend the zootechnical performance of a healthy individual through improving digestibility or stabilizing the gut flora or maintaining a healthy environment for growth (Pirgozliev et al., 2019).

Definition Of Exogenous Enzymes

Enzymes are biological catalysts that activate reactions and act on specific substrates or reactants. Their effectiveness in poultry feeding depends on some criteria based on their mechanism of action (Bedford and Schulze, 1998).

Exogenous enzymes facilitate specific chemical reactions and target specific substrates. These enzymes are produced primarily by fungi and bacteria and have been used since the late 1970s (Ravn et al., 2016). They belong to the group of zootechnical feed additives that favorably affect poultry performance through improvement of digestibility or stabilization of gut flora (Pirgozlievetal, 2019). Enzymes widely used in feed industry are non-starch polysaccharide cleaving enzymes (NSPases namely xylanase, β-glucanase, β-mannanase, α-galactosidase and pectinase) which are safe feed additives designed to improve average weight gain and feed conversion in poultry (Pirgozliev and Bedford, 2013; Ravn et al., 2016)

NSPase enzymes hydrolyze non-digestible bonds in plant substrates allowing improved digestibility (Bedford and Classen, 1993; Mathlouthi et al., 2002; Coppedge et al., 2012,). Indeed, the increase in gut viscosity causes alterations in the microflora and reduction in nutrient utilization; however, the use of a suitable NSPase counteracts these anti-nutritional properties (Choct et al., 1999). Furthermore, the inclusion of NSPases in broiler diets also improves growth performance (Azaro et al., 2003; Meng and Slominski, 2005; Coppedge et al., 2012) and this can be attributed to an increase in apparent metabolizable energy, digestible ileal energy, and dry matter retention (Meng and Slominski, 2005; Leslie et al., 2007; Cowieson and Ravindran, 2008; Olukosi et al., 2010). The majority of diets contain a variety of NSPs; thus, the most effective means is supplementation with an enzyme cocktail that varies in specificity and mode of action. This approach has the advantage of increasing starch digestibility and improving broiler performance, especially feed conversion ratio (Meng and Slominski, 2005). Nevertheless, each substrate requires different enzymes and the enzymes required afterwards depend on the final objective namely: viscosity reduction or pre-biotic generation (Langfelder, 2014).

Effects Of Nspase Enzymes

Poultry is not fully capable of digesting fiber in plant-based feeds since this species does not produce enough digestive enzymes (Boros et al.,2004),supporting the major interest of using exogenous enzymes that increase fiber digestibility (Classen, 2014).

On nutritional digestibility

Insoluble NSPs constitute the essential component of total fiber present in the poultry diet. Indeed, they promote the evacuation of the gut and its maintenance in good health (Francesch et al., 2012; Mendes et al., 2012). Nevertheless, a high concentration of dietary fiber shortens the digest transite time leading to reduced digestion and consequently absorption (El-Wafa et al., 2013; Sethy et al., 2015). Thus, the use of NSPases in viscous grain diets has been almost ubiquitous for decades. Indeed, the low level of endogenous enzymes in poultry has led to the widespread adoption of exogenous enzyme supplementation to take full advantage of the nutritional value of each feed (Flores et al., 2019). NSPases prevent the accentuation of viscosity in the gastrointestinal tract and its associated problems (Santos et al., 2013; Munyaka et al., 2015; Tellez et al., 2015) which will allow better digestion and absorption of nutrients, reduced microbial proliferation and healthier gut (Pirgozliev et al., 2010; 2015; Abdulla et al., 2017). Indeed, when xylanase is used, for example, with rye, triticale, and wheat-based diets and glucanase with barley-based diets, the positive effect of enzyme supplementation is mainly due to the reduction of the viscosity of the digest and the partial hydrolysis of the soluble NSP fraction (Gao et al., 2007; Aftab, 2014). In addition, incorporation of a cocktail of NSPases namely xylanase, β-glucanase and pectinase resulted in a significant decrease in jejunal viscosity of digest in broilers fed a corn-based diet (Dunaway and Adedokun, 2021).

Due to their ability to partially hydrolyze polysaccharides, NSPases soften the "cage effect" of NSP (Pirgozliev and Bedford, 2013; Ravn et al., 2016) which allows pancreatic enzymes to better digest nutrients, and improve their absorption by intestinal villi, thereby increasing digestible energy rate and consequently, nutritional value of the grain (Masey O'Neil et al., 2014).Indeed, NSPases supplementation of 75g/ton of feed with a cocktail of 203 IU of xylanase, 60 IU of cellulase, and 53 IU of glucanase per kilogram of supplement improves the apparent ileal digestibility of polysaccharides and proteins by 2% and 23%, respectively(Woyengo et al., 2019). Similarly, the inclusion of NSPases, namely: α-galactosidase and xynalase significantly improved the total ileal digestibility of amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and tryptophan) by 3.8% compared to the unsupplemented group (Jasek et al., 2018). Also, supplementation with xylanase improves starch digestibility in the jejunum and ileum by 2.4%, leading to increased energy in broilers (Stefanello, 2015). An inclusion of this enzyme in the diet of laying hens reduces their intestinal viscosity allowing a better benefit from nutrients (Bederska-Łojewska et al., 2019).

Indeed, digestive viscosity was reduced with dietary supplementation with a mixture of 2500IU of xylanase and 250IU of β-glucanase per kilogram of feed as well as in jejunal digest viscosity to a greater extent in wheat-based diets (-31%) than in maize-based diets (-10%) compared to the group without enzymes. Starch digestibility was also higher in the supplemented diet group (3.5%) (Munyaka et al., 2015). In addition, supplementation of carbohydrase in vitro, namely: xylanase and arabinofuranosidase increased digestibility of dry matter and solubilized arabinoxylan, in particular,from 6% to 41% (Vangsøe et al. 2020). Certainly, supplementation with NSPases improves total digestibility coefficient values by 9 to 11 units in broilers compared to both positive and negative controls (Maharjan et al., 2019).

On growth performance

In an attempt to reduce the anti-nutritive effects of NSPs, exogenous enzyme supplementation in poultry has been shown to be effective, especially in high fiber diets. This eternal quest concerns both nutritional digestibility and growth parameters of broilers.

Indeed, the administration of β-mannanase and NSPases (carbohydrate cocktail: xylanase, β-glucanase and α-galactosidase) at 363.2 g/t of feed (159.5 × 10^3IU/g product) and 113.5 g/t feed (2700 IU/g product), respectively, in an energy-reduced diet (88 or 132 kcal/kg of apparent metabolizable energy (AME) depending on the growth stage) improves zootechnical performance and reduces broiler mortality to levels similar to those of the positive control (PC) (Williams et al., 2014).

In a similar context, supplementation of β-mannanase in an energy-reduced diet of 132 kcal/kg MEA in broilers reduces conversion index and increased body weight of individuals compared to CP while maintaining similar weight (Klein et al., 2015). Use of a 0.01% mixture of NSPases (cellulase, glucanase, and xylanase) in broilers significantly optimizes weight gain by 3.04% (Horvatovic et al., 2015).

Xylanase supplementation also appears to improve average daily gain in 20–25-day old broilers by 2.5 g/day and to decrease the conversion index by 6 points compared to the negative control (Singh and Kim, 2021). The same enzyme in combination with protease at 0.25g and 0.20g of a commercial product per kg of feed respectively, improved the conversion index as well as the body weight gain of broiler (Barekatain et al., 2012). Xynalase had as well a positive effect on zootechnical parameters as a whole as well as on apparent metabolizable energy in the finishing phase and this at 2000 IU/kg feed (Perón et al., 2012). Similarly, the incorporation of a commercial product at a rate of 500g/t of feed containing a cocktail of NSPases namely α-galactosidase, β-mannanase, protease, amylase, β-glucanase, xylanase and cellulose significantly improves the conversion index compared to the negative control (Bilal et al., 2016). Also, enzyme B supplementation (with 1500 IU/g of α-amylase and 300 IU/g of amylopectase) as well as administration in another group of individuals of enzyme C(with 1500 IU/g of α-amylase, 300 IU/g amylopectase and 10,000 IU/G protease) reduced body weight gain to a higher level than that displayed by broilers supplemented with enzyme A (with 1,500 IU/g α-amylase) or enzyme D (with 1,500 IU/g α-amylase, 300 IU/g amylopectase, 10,000 IU/G protease and 15,000 IU/g xylanase) (Yin et al. , 2018).In laying hens, incorporation of NSP-degrading enzymes effectively improves growth performance including egg size in a diet fixing linoleic acid level in small egg layers by 2% and 1.5% in large egg strains compared to negative control (Elliot, 2012).

On the gut microflora

The gastrointestinal tract hosts a variety of microbiota that play a critical role in the overall well-being of the chicken, in general, and its digestive health, in particular (Kiarie et al., 2013). In broilers, 16S rDNA gene sequence analysis revealed thirteen, eleven, fourteen, twelve, nine, and fifty-one operational taxonomic units in the proventriculus, gizzard, duodenum, jejunum, ileum, and cecum, respectively (Jong et al., 2007). The diversity and abundance of the gut microbiota is affected by the composition of the diet as well as its digestibility (Kiarie et al., 2013; Kiarie et al., 2014). NSPase supplementation improves digest transit and nutrient digestion rate, leading to fewer opportunities for pathogen-substrate interaction (Huyghebaert et al., 2011). Indeed, broilers supplemented with xylanase hosta negligible number of C. perfringens compared to control individuals (Choct et al., 2006) as well as reduced numbers of coliforms and Salmonella in the ileum (Nian et al., 2011). Dietary supplementation with xylanase in broilers challenged with Salmonella spp. effectively induced a 61% reduction in Salmonella in positive cecal samples compared to the control group (Amerah et al., 2012). Similarly, the abundance of enterobactreacea was reduced in caeca following incorporation of NSPases into the cereal diet (Rosin et al., 2007; Jòzefiak et al., 2011). NSPases are also able to decrease the susceptibility of broiler chicken to Salmonella, Campylobacter jejuni and Brachyspira intermediainfections (Montagne et al., 2003). Nevertheless, high numbers of Campylobacter, Helicobacter, Butyricicoccus, Anaerostipes Bifidobacterium, SutterellaandOdoribacter were detected in broilers aged between 16 and 23 days and supplemented with enzymes B (with 1500IU/g of α-amylase and 300IU/g of amylopectase), C (with 1500IU/g of α-amylase, 300IU/g of amylopectase and 10000 IU/g of protease), D (with 1500IU/g of α-amylase, 300IU/g of amylopectase, 10000 IU/g of protease and 15000 IU/g of xylanase) and E (with

1500 IU/g of α-amylase, 300 IU/g of amylopectase, 10000 IU/g of protease, 15000 IU/g of xylanase + 200 IU/g of cellulase + 1000 IU/g of pectinase) respectively (Yin et al. , 2018).

In addition to their effects on pathogens, NSPases have an extensively researched pre-biotic effect. Indeed, the cleavage of non-starch polysaccharides by exogenous enzymes, including NSPases, allows the release of oligosaccharides into the gastrointestinal tract. When these compounds reach the cecum, they, selectively, stimulate growth and activity of intestinal bacteria such as Bifidobacterium and Lactobacillus (Thammarutwasik et al., 2009) to act as pre-biotics in the poultry gut (Masey O'Neill et al., 2014b; Choct, 2015). Xylanase, for example, does not produce pre-biotics certainly but rather a signaling molecule stimulating bacterial species that can degrade xylan to produce xylanases (Bedford, 2018). NSPase supplementation thus, influences ileal and cecal microbiota (Gonzalez-Ortiz et al., 2016) and results in increased cecal fermentation (Masey O'Neill et al., 2014a).

Economic Coating ofNspase

Current broiler strains are characterized by rapid growth and high feed conversion rates. To utilize this promising potential, it is imperative to adopt a balanced nutrition that allows expressing their full genetic potential (Kubis et al., 2020). However, the cost of grain feedstock is a critical component of production costs in poultry production (Zentek and Boroojeni, 2020). Indeed, feed costs for chickens account for up to 80% of total production costs (Zhang et al., 2020). Therefore, in order to maintain production profitability while ensuring product quality, it is crucial to improve utilization of nutrients contained in feeds through feed additives. (Kubis et al., 2020). The majority of feed costs (95%) is for energy and protein requirements, about 3 - 4% for minerals and vitamins and about 1 - 2% only is spent for various food additives (Zentek and Boroojeni, 2020).

In addition to the beneficial effects of NSPases, these enzymes save energy in feed formulations in poultry, contributing to reduce the AME content of corn-based diets by up to 100 kcal/kg, therefore saving about 7.00 USD per ton of feed (Gomes, 2016). Hence, a cocktail of xylanase, β-glucanase and phytase saved 0.045 USD per kilogram of weight gain even with very poor quality feed material (Olnood and Liu, 2012).

As discussed above, the effects of NSPasesseem to be multidirectional. They limit the increase in digestive viscosity by hydrolyzing soluble polysaccharides, enhance the proliferation of beneficial microflora by providing substrates for fermentation, increase the availability of nutrients, thus improving the nutritional value of NSP-rich grains and ultimately increasing poultry performance while reducing production cost.

Conclusion:-

Use of such exogenous enzymes is therefore an effective method to eliminate the nutrient encapsulation effect of plant cell walls, generate oligomers, support the gut microbiota, and limit the use of GPAs to maintain gut health in poultry (Singh and Kim, 2021). With the existing challenges of the anti-nutritional effect of non-amylaceous polysaccharides, further investigations are required to explore the possibilities of improving the utilization of these products to, thereby, limit extensive use of growth promoting antibiotics.

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