A MINI REVIEW OF THE EFFECTS OF NSP AND EXOGENOUS ENZYMES IN BROILER DIETS ON DIGESTIBILITY AND SOME INTESTINAL FUNCTIONS

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SUMMARY

NSP (non-starch polysaccharides) are a large group of compounds, which vary greatly in their chemical structure and properties. They are linked with the use of some of the alternative proteins or energy feed ingredients in broiler nutrition. Exogenous enzymes which act on NSP are sometimes in broiler production with the purpose of increasing digestibility of nutrients and consequently increasing broiler performance in production. This paper will attempt to review changes occurring in the digestive tract as a result of the use of feed with NSP soluble and insoluble in water, and the effect of NSPase on them, in terms of how an animal organism is burdened or helped to overcome the problems.

Key words: NSP, exogenous enzymes, broilers

Abbreviations: NSP – non-starch polysaccharides

INTRODUCTION

In intensive broilers production, producers tend to compose diets at the minimal cost with all necessary nutrients, minerals, and energy for a given age. The main sources of energy are carbohydrates, the most common of which is starch. Starch is highly digestible (about 96%) (Weurding et al., 2001), but feed ingredients contain a number of carbohydrates, polysaccharides which are not so highly digestible. Traditionally, broiler production is based on the use of corn and soybeans as a source of energy and proteins. In these feeds, the content of “undesirable” polysaccharides is low. The term non-starch polysaccharides (NSP) is commonly used to include a large group of very different polysaccharides compounds. Inclusion of some alternative feed ingredients as a source of proteins or energy is usually accompanied with an increased level of NSP and consequently depressed digestibility and a drop in production (Choct et al., 1992; Vukić Vranješ et al., 1994; Fuente et al., 1995; Classen et al., 1985; Polovinski-Horvatić et al., 2015; Glamočić et al., 2011). Based on water solubility, they can be divided into two fractions: water soluble and water insoluble fraction (Choct & Kocher, 2000). The fraction insoluble in water is almost fully unavailable for poultry, while with the fraction of soluble NSP the situation is more complex. Although soluble NSP are somewhat digestible for poultry, this fraction has antinutritive effects through a range of mechanisms.

Alternative feed ingredients which can be used as a source of proteins or energy, such as sunflower meal, wheat, rye or sorghum, contain a relatively high amount of NSP (Sredanović et al., 2005). Exogenous enzymes (NSPase) are used as an attempt to overcome problems caused by increased levels of NSP in broiler diets and to enhance digestibility and consequently broiler performance (Campbell & Bedford, 1992; Choct & Kocher, 2000; Cowieson et al., 2006; Polovinski-Horvatić et al., 2015; Glamočić et al., 2012). However, while some feeds contain

significant amounts of soluble NSP causing reduced digestibility, some diet ingredients contain (also) high amounts of insoluble NSP causing reduced energy density of diets. In this paper, we will try to review some of the most important changes caused by both fractions of NSP and the use of exogenous enzymes.

Digestibility

The existence of a large variety of NSP and great diversity in their physico-chemical properties mean that they have different effects on digestibility in animals. It is important to note that NSP do not exist as entirely separate entities in the feed. Most of these compounds make the cell wall and are closely associated with other polysaccharides or other biological compounds such as proteins and lignin (Smits & Annison, 1996). This fact is important because it influences the effects of NSP on digestibility in broilers.

It is well-known that inclusion of large amounts of soluble NSP causes depression in digestibility of starch, protein and fat (Campbell et al., 1983; Choct et al., 1992; Choct et al., 1995). Consumption of feed containing higher amounts of soluble NSP was connected with increased viscosity of intestinal contents (White et al., 1983). The amount of NSP in feed ingredients varies greatly. It is very small in corn, while it is high in barley and rye, which significantly affects the nutritional value of these ingredients. The amount of NSP varies significantly among different varieties of certain cereals, such as corn or wheat. It can even be different for the same varieties depending on the season, storage conditions, etc. (Scott et al., 1998). The age of broilers is important for the effects of NSP on digestibility. It seems that these effects are more profound at the younger age, while in older animals they are much less obvious (Almirall et al., 1995).

Starch is the main source of energy and it is a significant part of AME of feed. However, starch digestibility can vary greatly among feed (Weurding et al., 2001). NSP soluble in water are among the factors which can influence digestibility of starch in feed by increasing viscosity of intestinal contents (Carre, 2004).

NSP, especially NSP insoluble in water, are largely inaccessible for monogastrics because there are no endogenous enzymes for their digestion. Digestibility of both fractions of NSP by the action of pepsin and amylase is very low (Castañón et al., 1997). However, a certain part of the energy contained in these compounds is still available for poultry (Jamroz et al., 2002).

The effect of exogenous enzymes acting on NSP and on AME can be accounted for by increases in starch digestibility due to improved conditions in the digestive tract and increased NSP digestion in the digestive tract via microbial fermentation, which is most intense in the cecum of chickens. Carboxylases among other things increases starch digestion in the small intestine, primarily by reducing viscosity in the upper gastrointestinal tract (Boros et al., 1998). It has the effect on starch digestibility in the ileum, while this effect is not visibly determined by the excreta (Meng & Slominski, 2005). The division between soluble and insoluble NSP is not as strict. The effect of carbohydrases is to reduce the amount of soluble NSP, splitting them into smaller polysaccharide chains and free sugars. A part of insoluble NSP fraction, owing to the effect of exogenous enzymes, becomes soluble and is subject to further degradation as soluble NSP (Castañón et al., 1997). The effect of enzymes on digestibility of NSP in vitro depends on their concentration. Moreover, at low concentrations, exogenous enzymes can increase the concentration of soluble NSP. This could be accounted for by the fact that a part of NSP insoluble in water was solubilised, while the soluble fraction was not hydrolysed to low-molecular weight NSP or sugars (Castañón et al., 1997).

Increase in AME which occurs by using exogenous enzymes is largely derived from enhanced digestibility of starch. However, although there was a visual decrease in digestibility in cecotomized chickens from eating wheat pentosan, the effect is significantly more pronounced in noncectomized chickens, suggesting that the antinutritive effect of NSP is largely maintained over the gut microflora (Choct et al., 1992). Microflora has its nutritional needs for nutrients and space. At one point, it could be a competitor with the host for them (Bedford & Apajalahti, 2000). However, some of the energy is provided by microbial fermentation. The question is how much of the energy chickens manage to get through the increased microbial fermentation in the lower part of the digestive tract. It was noticed that in organic farming, when using forage with addition of enzymes in broiler nutrition, there was an increase in AME of the mixture due to the effect of the enzymes as well as an increase in AME of the forage separately (Buchanan et al., 2007). It is known that the microflora plays an important role in digestion of fiber fractions in the cecum (where the process is the most intense) and the colon via short chain fatty acids (acetate, propionate and butyrate) and various gases (CO_2 , H_2 , CH_4). The effect of exogenous enzymes on NSP is to reduce the polysaccharide chains and thus increase availability of this fraction for the intestinal microorganisms. Too much fiber can cause hypertrophy of the cecum (Redig, 1989). Even small changes in the fiber can cause large changes in fermentation. During identification of the microbial species in the digestive tract, a direct link between certain types of microbial species and increased AME was established. It is possible that the growth of bacteria contributing to suppression of unwanted microbial species leads to an increase of AME (Torok et al., 2000). An experiment carried

out on three poultry species fed with a diet containing 137 g/kg NSP showed that 3.7% of the energy of the total amount of ME was derived from NSP fraction (Jamroz et al., 2002). The diet containing different grains (triticale, rye and wheat) most significantly affected the extent of fermentation in the cecum, but also fermentation in the upper parts of the digestive tract, while the effects of xylanase on the fermentation processes was mainly in the lower part of the digestive tract (Jozefiak et al., 2007). In broiler diets based on wheat, the amount of enzymes (xylanase and β -glucanase) linearly increased, which led to linearly increased amount of short-chain acids in the cecum, followed by an increase in AME of the mixture (Wang et al., 2005).

Insoluble NSP reduced the energy density of feeds. However, as previously mentioned, a certain amount of insoluble NSP under the influence of exogenous enzyme becomes soluble and partly available to animals. In the experiment on broilers fed with five levels of energy (energy density was reduced by inclusion of wheat bran), the effect of the enzyme supplementation on the energy increase was the largest in the diets with the lowest AME (Zhou et al., 2009). Adding coarse insoluble fibers in broiler diet stimulated the increase in stomach, increased consumption, and although the conversion was slightly increased, there was undoubtedly a positive effect on production (Sacranie et al., 2012). Increased inclusion of insoluble NSP also leads to appearance of increased peristaltic movements, which could be useful (extending the period of exposure of the digest to the digestion conditions) or harmful (displacement of the content of the cecum, and perhaps undesirable microorganisms in the upper part of the digestive tract) (Hetland et al., 2003; Sacranie et al., 2005). The fact is that insoluble NSP improve digestion when the content in the broiler diet is moderate. The assumption is that strong antiperistaltic movements led to increased availability of bile acids, which consequently enhanced emulsification of lipids. Due to increased emulsification of lipids, lipid cannot create a protective layer around the digest, which reduces digestibility of nutrients (Hetland, et al., 2004).

In animal nutrition, the dietary fibers including any polysaccharides reach the hindgut and so include resistant starch (RS) and soluble and insoluble non-starch polysaccharides (NSP). Routinely, there are two procedures used for chemical determination of the amount of fiber in animal diet. The Weende method determines the quantity of crude fiber. This fraction contains various amounts of several fiber classes which have different influence on the processes of digestion. The Van Soest procedure (Van Soest et al., 1991) obtains three fiber fractions, NDF (neutral detergent fiber), ADF (acid detergent fiber) and hemicelluloses (NDF-ADF), while only NDF corresponds to dietary fiber. Certain authors investigating the effects of fiber and the effects of exogenous enzymes acting on NSP on the digestion of nutrients estimated digestibility of fiber fraction through digestibility of NDF, ADF and hemicellulose (Vukić Vranješ et al., 1994; Jamroz et al., 2002; Ao et al., 2009; Zhang et al., 2010; Nian et al., 2011). However, some parts of soluble NSP are lost through dissolution during the aqueous treatment. The best solution for determination of NSP in diets and for digestibility should be based on methods measuring soluble and insoluble fraction of NSP (Smits & Annison, 1996).

However, the method for determining digestibility can significantly affect the results of digestibility. The effects of using exogenous enzymes in diets based on corn and soybeans were determined by three methods. Only the method based on the ileum content revealed a change in digestibility owing to the use of exogenous enzyme, unlike the other two methods (Zanella et al., 1999). However, bearing in mind the nature of digestibility, it is very difficult to choose the method. The researchers who focus on the topic of digestibility of protein and amino acid choose determination on the basis of the ileum content. The process of digestibility of protein is largely completed by the end of the distal part of the ileum, while for digestibility of fiber the important part is going through the lower part of the digestive tract where fibers are a source for microbial fermentation. Moreover, in certain cases, when fiber digestibility was determined in the ileum, for single or all fractions of NSP, some researchers found negative values for digestibility (Jamroz et al., 2002). This can be accounted for by the fact that increased amount of dietary fiber in poultry is connected with increased frequency of antiperistaltic movements as an attempt of the organisms to prolong the exposure of fiber to the process of digestion (Sacranie et al., 2005) causing accumulation of this fraction and receiving negative values of digestibility for fiber fraction or NSP.

Intestinal viscosity

Many polysaccharides dissolved in water produce viscous solutions. The capacity for increasing viscosity in the intestine of an animal depends on many factors, such as the size of molecules, branching, presence of charged groups and of course concentration.

Bedford & Classen (1992) carried out an experiment with rye, demonstrating a direct link between a drop in productivity and an increase in intestinal viscosity. Viscosity of intestinal contents increased exponentially with the concentration of high molecular weight polysaccharides. When β -glucan from barley was added at a concentration of only 10 mg/kg, viscosity was increased three times (White et al., 1983).

The highest increase in intestinal viscosity is in the lower part of the digestive tract. A review study with rye and wheat as the main source of energy feeds examined viscosity of intestinal contents along the digestive tract. Viscosity was increased most in the ileum, although there was a statistical increase also in the upper part of the small intestine (Boros et al., 1998)

Increase in viscosity slows down diffusion and active transport in the digestive system. Active transport of glucose and sodium was decreased with increases of viscosity (Edwards et al., 1988). Intestinal viscosity reduces the contact area between the substrate (e.g. fat) and digestive secretions (lipase) (Smits & Annison, 1996). However, it is known that although there is an increase in intestinal viscosity as a result of consumption feed with elevated level of soluble NSP at the older age of broilers or cocks, the effects on reduced digestibility and performance are much less visible (Almirall et al., 1995). Viscosity is not the only mechanism through which water-soluble NSP have effects on depression of digestibility. Also, the use of antibiotics reduces the negative effect of increased viscosity owing to the use of soluble NSP, indicating a joint effect of microflora and viscosity on digestibility of nutrients (Bedford & Apajalahti, 2000). Increased intestinal viscosity, as an effect of the use of feed with high fiber content, is also associated with prolonged retention of food in the digestive tract of poultry (Van der Klis et al., 1993). Prolonged retention of feed in guts leads to lower consumption (Almirall et al., 1995) and consequently lower production results.

Exogenous enzymes need to cut polysaccharides in a few places, significantly reducing viscosity, and thus also the antinutritive effect. The effects of arabinoxylans and partially depolymerised arabinoxylans on intestinal viscosity were compared. Depolymerised arabinoxylans did not significantly reduce the metabolic energy (Williams et al., 1997). It is interesting to note that addition of exogenous enzymes did not reduce the amount of soluble NSP in digest, and moreover there was a statistically significant increase, without increasing intestinal viscosity. This means that although a larger amount of soluble NSP was dissolved in water, polysaccharide chains were split into smaller fragments which did not contribute to the increase of viscosity (Choct et al., 1995). In some cases, intestinal viscosity can be increased by the use of exogenous enzymes. This can be explained by the fact that the effects of exogenous enzymes depend on the concentration. In a study in vitro, addition of a low concentration of enzymes causes an increase of soluble NSP by solubilization of insoluble NSP, while further degradation of soluble NSP into low molecular weight NSP and sugar was not sufficient (Castañón et al., 1997). In some cases, it can lead to an increase in viscosity (Pettersson & Aman, 1989).

Disaccharidases and NSP

Digestion of carbohydrates takes place in the lumen of the small intestines, and on the brush borders of the enterocytes. Pancreatic amylase hydrolyzes starch from food to oligosaccharides (mannose, maltotriose) and dextrin. Terminal hydrolysis of these oligosaccharides and disaccharides takes place on the surfaces of brush border of the enterocytes with disaccharidases (diastase, sucrase, isomaltase, maltase, trehalase and lactase).

Maltose is the main product of digestion of complex polysaccharides, such as starch and amylopectin (Caviedes-Vidal et al., 2000). Maltose breaks down two enzyme complexes, maltase-glucoamylase and sucrase-isomaltase. These enzymes belong to glycoproteins that are synthesized on polysomes of enterocytes and are located in/on microvilli and protrude to the outside of the membrane enterocytes (Varljen et al., 2005). They are responsible for the final stage of digestion of carbohydrates. Sucrase-isomaltase is relatively non-specific enzyme complex that breaks down maltose, sucrose and isomaltose. Intestinal enzyme activity is highly correlated with body weights of animals during the physical development of chickens. This highly positive correlation may indicate that the intestinal enzymes play a crucial role in providing substrate for the growth (Uni et al., 1999; Sklan, 2001).

The assumption is that NSP can influence the activity of endogenous enzymes. But there are conflicting results. Ikegami (1990) found that increased soluble NSP in rats leads to an increase of the enzyme activities. However, introduction of soluble NSP in pigs leads to a decline in the activity of some of the endogenous enzymes (Li et al., 2004). Total fiber from legumes in rats, caused by the drop in activity of intestinal disaccharidase, probably makes the matrix of substrate and/or the binding of certain components of the enzyme and its inactivation (Infante et al., 2008). It was observed in some studies that addition of exogenous enzymes which enhance digestion by changing the conditions of digestion in the digestive tract led to an increase in activity of enzymes sucrase or maltase or both disaccharidase in some monogastric animals (Fan et al., 2009; He et al., 2010). Increased disaccharidase activity can be accounted for by an increased amount of substrate. It is possible that the effect of exogenous enzymes on the increased disaccharidase activity depends on the age of broiler chickens, because at a younger age of chickens maltase and sucrase activity does not reach its maximum. Addition of exogenous enzymes and thus increasing the amount of the substrate significantly affects the specific activity of endogenous enzymes. The use of exogenous enzymes was more prominent on the increase in activities of endogenous enzymes in the younger age of chickens

(Pinheiro et al., 2004), while at the older age the effect was much less evident. However, oligosaccharides can also have a significant effect on the activities of the intestinal disaccharides. Diets for turkey made from four different sources of proteins, all originating from soya, with the similar amount of NSP and at the same time different amount of oligosaccharides (2.4, 1.9, 0.9, and 0.1%), affected the activities of disaccharidases. The lowest activities were observed in the diet with only 0.1% oligosaccharides, which was also connected with the smallest body weight (Jankowski et al., 2009). The used exogenous enzymes for NSP cut NSP of feed in smaller polymers, which leads to a significant amount of oligosaccharides produced in site, when the diet is based on the feed ingredients with a significant content of NSP. Maybe it is possible that these oligomers, in addition to affecting the microflora, may increase activities of intestinal enzymes.

CONCLUSION

The effect of NSP soluble and insoluble in water is very complex and depends on many factors. In this paper, we have tried to give an overview of what is happening during the digestion of NSP and what are the determining factors influencing the effect of both NSP and enzymes on which they act. Owing to the complexity of these effects, there are probably great differences among results of the researchers who have studied this issue.

REFERENCES

- Almirall M., Francesch M., Perez-Vendrell A.M., Brufau J., Esteve-Garcia E. (1995): The differences in intestinal viscosity produced by barley and beta-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *Journal of Nutrition*, 125(4): 947-55.
- Ao T., Cantor A.H., Pescatore A.J., Ford M.J., Pierce J.L., Dawson K.A. (2009): Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poultry science*, 88(1): 111-117.
- Bedford M.R. & Apajalahti J. (2000): Microbial Interactions in the Response to Exogenous Enzyme Utilization. In P.G. Bedford M.R., *Enzymes in Farm Animal Nutrition*, pp. 299-314.
- Bedford M.R. & Classen H.L. (1992): Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is affected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and feed conversion efficiency of broiler chicks. *The Journal of Nutrition*, 122: 560-569.
- Boros D., Marquardt R. R., Guenter W. (1998): Site of exoenzyme action in gastrointestinal tract of broiler chicks. *Canadian Journal of Animal Science*, 78(4): 599-602.
- Buchanan N.P., Hott J.M., Kimbler L.B., Moritz J.S. (2007): Nutrient Composition and Digestibility of Organic Broiler Diets and Pasture Forages. *The Journal of Applied Poultry Research*, 16: 13-21.
- Campbell G.L., Classen H.L., Goldsmith K.A. (1983): Effect of Fat Retention on the Rachitogenic Effect of Rye Fed to Broiler Chickens. *Poultry Science*, 62: 2218-2223.
- Campbell G.L. & Bedford M.R. (1992): Enzyme applications for monogastric feeds: A review. *Canadian Journal of Animal Science*, 72(3): 449-466.
- Carre B. (2004): Causes for variation in digestibility of starch among feedstuffs. *World's poultry science journal*, 60(1): 76-89.
- Castañón J.I.R., Flores M.P., Pettersson D. (1997): Mode of degradation of non-starch polysaccharides. *Animal Feed Science and Technology*, 68(3): 361-365.
- Caviedes-Vidal E., Afik D., Martinez del Rio C., Karasov W.H. (2000): Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. *Comparative Biochemistry and Physiology Part A*, 11-24.
- Choct M., Annison G., Trimble R.P. (1992): Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *Journal of Nutrition*, 122(10-12): 2457-2465.
- Choct M., Hughes R.J., Trimble R.P., Angkanaporn K., Annison G. (1995): Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. *Journal of Nutrition*, 125(3): 485-492.
- Choct M. & Kocher A. (2000): Non starch carbohydrates: digestion and secondary effects in monogastrics. 24th Annual Meeting of the Nutrition Society of Australia.
- Classen H.L., Campbell G.L., Rossmagel B. G., Bhatti R., Reichert R.D. (1985): Studies on the Use of Hulless Barley in Chick Diets. *Canadian Journal of Animal Science*, 65: 725-733.
- Cowieson A.J., Hrubylund M., Pierson E.E.M. (2006): Evolving enzyme technology: impact on commercial poultry nutriti. *Nutrition Research Reviews*, 19: 90-103.
- Edwards C.A., Johnson I.T., Read N.W. (1988): Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? *European Journal of Clinical Nutrition*, 42(4): 307-312.
- Fan C.L., Han X.Y., Xu Z.R., Wang L.J., Shi L.R. (2009): Effects of beta-glucanase and xylanase supplementation on gastrointestinal digestive enzyme activities of weaned piglets fed a barley-based diet. *Journal of Animal Physiology and Animal Nutrition*, 93: 271-276.
- Fuente J.M., Pérez-de Ayala P., Villamide M.J. (1995): Effect of dietary enzyme on the metabolizable energy of diets with increasing levels of barley fed to broilers at different ages. *Animal Feed Science and Technology*, 56(1): 45-53.

- Glamočić D., Polovinski-Horvatić M., Ivković M., Beuković D., Bjedov S. (2011): Efekat egzogenih enzima u ishrani brojlera na svarljivost i metaboličnost u smešama sa različitim nivoom metaboličke energije. *Biotechnology in Animal Husbandry*, 27(3): 583-590.
- Glamočić D., Polovinski-Horvatić M., Beuković D., Ivković M., Bjedov S. (2012): Enzyme supplementation of a broiler diets containing different level of sunflower meal. *Proceedings. 47th Croatian and 7th International Symposium on Agriculture*. Opatija, Croatia, 684-687.
- He J., Liu C., Fu C., Li J. (2010): Effects of extrusion and supplementation of exogenous enzymes to diets containing Chinese storage brown rice on the carbohydrase activity in the digestive tract of piglets. *Journal of Animal Physiology and Animal Nutrition*, 94: 146-153.
- Hetland H., Svihus B., Krogdahl A. (2003): Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science*, 44(2): 275-282.
- Hetland H., Choct M., Svihus B. (2004): Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poultry Science Journal*, 60: 415-422.
- Ikegami S., Tsuchihashi F., Harada H., Tsuchihashi N., Nishide E., Innami S. (1990): Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. *Journal of Nutrition*, 120(4): 353-360.
- Infante R.B., Garcia O.E., Carmona A., Rivera C.J. (2008): Effect of legume dietary fiber on rat disaccharidases activity *in vitro*. *Nutrition and Food Science*, 38(4): 316-324.
- Jamroz D., Jakobsen K., Knudsen K.E.B., Wiliczekiewicz A., Orda J. (2002): Digestibility and energy value of non-starch polysaccharides in young chickens, ducks and geese, fed diets containing high amounts of barley. *Comparative Biochemistry and Physiology Part A*, 131: 657-668.
- Jankowski J., Juskiewicz J., Gulewicz K., Lecewicz A., Slominski B.A., Zdunczyk Z. (2009): The effect of diets containing soybean meal, soybean protein concentrate, and soybean protein isolate of different oligosaccharide content on growth performance and gut function of young turkeys. *Poultry Science*, 88: 2132-2140.
- Jozefiak D., Rutkowski A., Jensen B.B., Engberg R.M. (2007): Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation on growth performance of broiler chickens and fermentation in the gastrointestinal tract. *Animal Feed Science and Technology*, 132: 79-93.
- Li W.F., Feng J., Xu Z.R., Yang C.M. (2004): Effects of non-starch polysaccharides enzymes on pancreatic and small intestinal digestive enzyme activities in piglet fed diets containing high amounts of barley. *World Journal of Gastroenterology*, 10(6): 856-859.
- Meng X. & Slominski B.A. (2005): Nutritive Values of Corn, Soybean Meal, Canola Meal, and Peas for Broiler Chickens as Affected by a Multicarbohydrase Preparation of Cell Wall Degrading Enzymes. *Poultry Science*, 84: 1242-1251.
- Nian F., Guo M., Ru1 Y.J., Li F.D., Péron A. (2011): Effect of Exogenous Xylanase Supplementation on the Performance, Net Energy and Gut Microflora of Broiler Chickens Fed Wheat-based Diets. *Asian - Australasian Journal of Animal Sciences*, 24(3): 400-406.
- Pettersson D. & Åman P. (1989). Enzyme supplementation of a poultry diet containing rye and wheat. *British Journal of Nutrition*, 62(1): 139-149.
- Pinheiro D.F., Cruz V.C., Sartori J.R., Vicentini Paulino M.L.M. (2004): Effect of Early Feed Restriction and Enzyme Supplementation on Digestive Enzyme Activities in Broilers. *Poultry Science*, 83: 1544-1550.
- Polovinski-Horvatić M., Glamočić D., Žikić D., Hadnadev T.D. (2015): Performance and some intestinal functions of broilers fed diets with different inclusion levels of sunflower meal and supplemented or not with enzymes. *Revista Brasileira de Ciencia Avicola*, 17: 25-30.
- Redig P. (1989): The avian ceca: obligate combustion chambers or facultative afterburners? The conditioning influence of diet. *Journal of Experimental Zoology*, 3: 66-69.
- Sacranie A., Svihus B., Denstadli V., Moen B., Iji P.A., Choct M. (2012): The Effect of Insoluble Fibre and Intermittent Feeding on Gizzard Development, Gut Motility, and Performance in Broiler Chickens. *Poultry Science*, 91(3): 693-700.
- Scott T.A., Silversides F.G., Classen H. L., Swift M.L., Bedford M.R. (1998): Effect of cultivar and environment on the feeding value of Western Canadian wheat and barley samples with and without enzyme supplementation. *Canadian Journal of Animal Science*, 78(4): 649-656.
- Sklan D. (2001): Development of the digestive tract of poultry. *World's Poultry Science Journal*, 57: 415-428.
- Smits, C.H.M. & Annison G. (1996): Non-starch plant polysaccharides in broiler nutrition - towards a physiologically valid approach to their determination. *World's Poultry Science Journal*, 52(2): 203-222.
- Sredanović S., Lević J., Đuragić O. (2005): Enzyme Enhancement of the Nutritional Value of Sunflower Meal. *Biotechnology in Animal Husbandry*, 21(5-6-2): 197-202.
- Sacranie A., Iji P.A., Choct M. (2005): Reflux of digesta and its implications for nutrient digestion and bird health. *Australian Poultry Science Symposium*, pp. 171-175.
- Torok B.A., Ophel-Keller K., Loo M., Hughes R.J. (2000): Application of Methods for Identifying Broiler Chicken Gut Bacterial Species Linked with Increased Energy Metabolism. *Applied and Environmental Microbiology*, 74(3): 783-791.
- Uni Y., Noy Y., Sklan D. (1999): Posthatch Development of Small Intestinal Function in the Poult. *Poultry Science*, 78: 215-222.
- Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science*, 74(10): 3583-3597.
- Van der Klis J.D., Van Voorst A., Van Cruyningen C. (1993): Effect of a soluble polysaccharide (carboxymethyl cellulose) on the physico-chemical conditions in the gastrointestinal tract of broilers. *British Poultry Science*, 34: 971-983.

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- Varljen J., Detel D., Batičić L., Eraković V., Štrbo N., Ćuk M., Milin Č. (2005): Age Dependent Activity of Brush-border Enzymes in BALB/c Mice. *Croatia Cemica Acta*, 78(3): 379-384.
- Vukić Vranješ M., Pfirter H.P., Wenk C. (1994): Influence of processing treatment and type of cereal on the effect of dietary enzymes in broiler diets. *Animal Feed Science and Technology*, 46: 261-270.
- Wang Z.R., Qiao S.Y., Lu W.Q., Li D.F. (2005): Effects of Enzyme Supplementation on Performance, Nutrient Digestibility, Gastrointestinal Morphology, and Volatile Fatty Acid Profiles in the Hindgut of Broilers Fed Wheat-based Diets. *Poultry Science*, 84: 875-881.
- Weurding R.E. Veldman A., Veen W.A.G., Van der Aar P.J., Verstegen M.W.A. (2001): *In Vitro* Starch Digestion Correlates Well with Rate and Extent of Starch Digestion in Broiler Chickens. *Journal of nutrition*, 131: 2336-2342.
- White W.B., Bird H.R., Sunde M.L., Marlett J.A. (1983): Viscosity of β -D-Glucan as a Factor in the Enzymatic Improvement of Barley for Chicks. *Poultry Science*, 62: 853-862.
- Williams P.E.V., Geraert P.A., Uzu G., Annison G. (1997): Factors affecting nonstarch polysaccharide digestibility in poultry. *Options Mediterraneennes*, Atenas, 26: 125-134.
- Zanella I., Sakomuran. K., Silversides F.G., Figueirido A., Pack M. (1999): Effect of Enzyme Supplementation of Broiler Diets Based on Corn and Soybeans. *Poultry Science*, 78: 561-568.
- Zhang B., Yunhe C., Yiqun C., Yihang L., Shiyan Q., Yongxi M. (2010): Effects of [alpha]-galactosidase supplementation on performance and energy metabolism for broilers fed corn-non-dehulled soybean meal diets. *Asian - Australasian Journal of Animal Sciences*, 23(10): 1340-1347.
- Zhou Y., Jiang Z., Lv D., Wang T. (2009): Improved energy-utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolizable energy levels. *Poultry Science*, 88: 316-322.

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