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## **RYE NON-STARCH POLYSACCHARIDES: THEIR IMPACT ON POULTRY INTESTINAL PHYSIOLOGY, NUTRIENTS DIGESTIBILITY AND PERFORMANCE INDICES – A REVIEW\***

Dorota Bederska-Łojewska<sup>1</sup>\*, Sylwester Świątkiewicz<sup>1</sup>, Anna Arczewska-Włosek<sup>1</sup>, Tomasz Schwarz<sup>2</sup>

<sup>1</sup>Department of Animal Nutrition and Feed Science, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

<sup>2</sup>Department of Swine and Small Animal Breeding, Institute of Animal Science, University of Agriculture, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

\*Corresponding author: dorota.bederska@izoo.krakow.pl

### **Abstract**

**A high content of non-starch polysaccharides (NSP), namely arabinoxylans (AX), in rye is a reason for the potential adverse effect of this grain on intestinal functions, gut microflora, absorption of nutrients and performance indices. As such, the use of rye grain in intensively produced poultry diets is limited. However, recently developed new types of hybrid rye are characterised not only by increased yield potential, resistance to fungus and pests and low production costs, but also the content of antinutritive substances may be reduced in these varieties. The aim of this paper is to discuss the mechanisms of NSP effects in the digestive tract, as well as to review the results of recent studies on the use of rye in poultry nutrition. Based on the literature data, it can be concluded that the use of new hybrid rye varieties with decreased NSP concentration and NSP-hydrolysing enzymes may be a way of increasing the share of rye grain in poultry diets.**

**Key words:** poultry, intestinal physiology, feed enzymes, rye, non-starch polysaccharides

Abbreviations: AX, arabinoxylans; BWG, body weight gain; DFM, direct-fed microbial; DM, dry mass; FPD, footpad dermatitis; FCR, feed conversion ratio; NSP, non-starch polysaccharides; SCFA, short chain fatty acids; WEV, water extract viscosity.

Due to the high content of antinutritive factors, mainly non-starch polysaccharides, the use of rye grain as feed material in poultry nutrition is limited and its

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nutritional value is underestimated. The reluctance of poultry producers to use it is primarily due to the fact that rye use in significant amounts as a diet component can decrease performance indices and the absorption of nutrients, as well as increase the viscosity of digesta content and, in this way, cause the excretion of sticky faeces (Boros et al., 1998; Goncharenko et al., 2011). Such a strong undesired effect is not observed when using wheat, maize or triticale, so these cereals have supplanted rye from use in poultry nutrition. However, in recent years new types of hybrid rye have been developed which enable reducing amount of antinutritive compounds, especially AX (Jurgens et al., 2012), whose content in animal diets may be increased without the risk of a reduction in performance indices (Schwarz et al., 2015). However, there is a need for a thorough evaluation of the nutritional efficiency of these new rye hybrids in poultry experiments. These varieties are also characterised by high yield potential, good resistance to fungus and pests and low production costs which, in the light of the rising prices of other cereals, can have great economic importance for farmers (Schwarz et al., 2015). Therefore, this review provides an overview of the characteristics of the new varieties of hybrid rye, the content of NSP and their effects on the digestive tract (including the microbiological status) as compared to other cereals used in poultry nutrition. Methods of preventing the negative effects caused by soluble NSP and the latest scientific research of the use of rye in poultry nutrition are also discussed.

### **The nutritional value of rye hybrids**

Poland, besides Germany, is the biggest rye producer in the European Union (Eurostat, 2015). However, because of the low grain price and the reluctance of farmers to use rye in animal nutrition, the acreage of rye in Poland has been steadily declining for several decades (Wasilewska, 2008; GUS, 2015). This reduction in agricultural production was not halted by the fact that this cereal has many advantages in terms of its great adaptation to climatic and soil conditions in Polish latitude. In 1996, the sown area of rye in Poland was 2,415 thousand ha (Wasilewska, 2008), whereas in 2015 it was only 725 257 ha (GUS, 2015). Rye is characterised by high tolerance to adverse environmental conditions (especially to low temperatures or drought) and high yield, even on less fertile soils of irregular pH. In addition, production costs are reduced in comparison to the other cereals due to lower use of chemicals, calcium and fertilisers.

Over the years, animal producers gradually reduced the proportion of rye in the compound feed, considering them as less valuable and having deteriorating performance indices in comparison to the other cereals. Older varieties were characterised by higher levels of antinutritive substances, alkylresorcinols, and lower resistance to infection caused by ergot and mycotoxins contamination (Schwarz et al., 2015). Over time, breeding programmes have developed hybrid plants without these negative features but which preserve the nutritional value. Rye grain's composition and nutritional value does not differ significantly from the commonly used wheat or triticale (Table 1). The percentage differences in protein content between the different cereal varieties are not large and presented in table below, and it should be remembered that their value can be largely modified by the system of cultivation.

Table 1. Cereals chemical composition (% of DM) (Boros and Fraś, 2015)

Item	Protein	Minerals	Lipids	Starch
Winter wheat	12.2	1.6	2.4	63.9
Spring wheat	15.0	1.70	2.70	63.5
Winter rye	10.4	1.8	2.10	59.0
Winter triticale	11.8	2.0	2.2	63.3
Spring triticale	12.6	2.1	2.2	64.7

In the new rye varieties, the amount of antinutritive compounds is limited and a reduction in the feed palatability is no longer observed resulting from the high alkylresorcinols level, that has been significantly reduced from over 1,000 mg/kg (Schwarz et al., 2015) in the old varieties to 815 mg/kg in the new (2010) (Boros and Fraś, 2015), or even to 401 mg/kg according to the results obtained by Schwarz et al. (2015). Also, the inhibitory activity of proteolytic enzymes, such as trypsin and chymotrypsin, has been greatly reduced (Schwarz et al., 2015). Moreover, hybrid rye exhibits a high resistance to ergot contamination, as well as a high resistance to *Fusarium* mycotoxins such as zearalenone, T2 toxin, and nivaleon (Grajewski et al., 2012; Schwarz et al., 2015).

Another advantage that puts rye in a better light than in the past is its price, as it is also currently the cheapest raw feed material. The price per ton of rye (PLN 527; EUR 122.60) at the beginning of July 2016 in Poland (Cereals market, 27/2016) was lower than wheat by PLN 187 (EUR 43.2), than maize – by PLN 335 (EUR 77.39), than triticale – by PLN 79 (EUR 18.25), and barley – by PLN 134 (EUR 30.96). These differences are important taking into consideration the fact that the feed accounts from 50% to up to 70% of the whole cost of livestock production (Boros and Fraś, 2015). Ground grain has a large share in compound feed destined for poultry ranging from 60–80%, providing birds with 60–85% of their daily demand for energy. Grain is also a rich source of protein (Boros and Fraś, 2015). However, it should be noted that the most important issue is the absorption of dietary nutrients from different types of cereals, which determines the nutritional value of a particular component of the compound feed. Hence, it is important to limit in plant products intended for feed material for animals all the factors favouring malabsorption.

In light of the production of improved varieties of hybrid rye, two very important issues need to be analysed in poultry studies: 1) Is the content of antinutritive factors in hybrid rye comparable with other cereals? and 2) Can new rye varieties be successfully used in poultry nutrition with a higher proportion in the diets without any adverse effects on performance and intestinal physiology?

### **The content of non-starch polysaccharides in different cereals and their physicochemical properties**

Cereals such as wheat, triticale, barley, maize, and rye commonly used in animal nutrition are not only a source of valuable nutrients but also of digestibility reducing NSP. Carbohydrates found in plants can be divided into starch and NSP, also called structural carbohydrates, which are responsible for forming the outer covering of seeds, protecting them against damage developing inside the endosperm. The main

proportion of these polysaccharides is constituted by cellulose, AX and  $\beta$ -glucans. Monogastric animals are unable to digest them by themselves and, as such, they are broken down by microbial selective hydrolysis (Boros et al., 1998).

Polysaccharides can be divided into two groups in respect of their ability, or lack, of water solubility (Johansson et al., 2004; Bach Knudsen, 2001). The first group containing AX, glucans and fructans and, in a smaller part, pectins or hemicelluloses, are responsible for reducing the digestibility and absorption of nutrients by the intestinal villi. Depending on their degree of solubility, this comes down to establishing the hydrocolloids of greater or lesser viscosity, which in turn reduces the availability of food molecules present in the intestinal digestive enzymes. The degree of solubility depends on the chemical structure of the compounds.  $\beta$ -glucans present in cereals are mainly composed of cellotrioses – a trisaccharide in which three glucose units are joined with 1,4- $\beta$  linkages, and cellotetraose linked with  $\beta$  1-3 linkages. It is supposed that the ratio of cellotriose/cellotetraose determines the solubility of this polysaccharide (Comino et al., 2014).  $\beta$ -glucans with a lower incidence of bonds 1–3 are prone to form aqueous solutions more easily (Edney et al., 1991; Annisson, 1993). The chemical structure of AX varies widely depending on the various physicochemical properties. They consist mainly of (1,4)- $\beta$ -D-xylopyranose chains which might be substituted to the  $\alpha$ -l-arabinofuranose. The higher content of  $\alpha$ -l-arabinofuranose in the arabinoxylan molecule is associated with improved solubility (Saulnier et al., 2007; Comino et al., 2014).

In poultry production, these NSP are more resistant to degradation, which makes them more problematic than other non-starch polysaccharides. The ratio between  $\beta$ -glucans and AX varies depending on the plant species. Cereals rich in the former polysaccharides are barley and oats, whereas rye, triticale, wheat and maize are rich in AX (Bach Knudsen, 2014). The share of the soluble fraction of NSP in rye grain was investigated by Henry (1985). The result was very high and represented up to 9.7% of the rye dry matter (Table 2). Further analysis of the various types of cereals carried out by Bach Knudsen (2014) showed the highest content of soluble NSP in rye, and later in oat and triticale, however the results were much lower than in the previous years. A recent study by Boros and Fraś (2015) confirms the continued slight decrease in soluble NSP in new varieties of rye, although their percentage is similar to the results obtained by Bach Knudsen (2014) for rye bred in 1997 (Table 2).

Analysis of water extract viscosity (WEV) of different cereals showed that AX present in rye are characterised by high water absorption, which is very advantageous from the standpoint of human health and their baking properties, but in the case of livestock it has a negative effect on the digestibility of nutrients. The literature presents divergent results here. Most often, the different content of the NSP, and thus the viscosity of water solutions, may result from plant varieties (genetics), climatic conditions or harvest date. Also, using different analytical methods for the same feed material results in different data (Bach Knudsen, 2014). NSP content may also increase at a time when, for any reason, the starch present in the grain is not able to bind excess moisture. Pentosanes then take over its function and improve crumb structural and mechanical properties, reducing its stickiness and moisture content (Goncharenko et al., 2011). However, most often these values oscillate between

7–11 mPa·s (Smulikowska and Nguyen, 2001; Petersson et al., 2012). The average value of rye WEV in the study performed by Boros and Fraś (2015) was 10.6 mPa·s, whereas the results obtained by Jurgens et al. (2012) are much lower, and the viscosity rate of the aqueous solutions of this plant was 4.00 mPa·s. Even taking into account one particular grain variety, the differences can be still very high, despite the use of the same methodology. Viscosity of water extract of the Visello variety has been estimated at 12.96 mPa·s in analyses conducted by Boros and Fraś (2015), and at 5.14 mPa·s (Jurgens et al., 2012). The highest average extract viscosity for rye was described by Rodehutschord et al. (2016) – the value was 20.0 mPa × s – but in this case, large differences may arise from methodological differences during sample preparation.

Table 2. Cereal content of total and soluble NSP (% of dry matter) and water extract viscosity (mPa·s)

Cereal	Henry (1985)	Bach Knudsen (2014) harvest year 1997	Boros and Fraś (2015) harvest year 2010	Water extract viscosity *Boros and Fraś (2015), **Jurgens et al. (2012)
Wheat	Arabinoxylans	6.6	Winter varieties	
	β-glucans	1.2	-	Total NSP 8.6 2.0*
Rye	Arabinoxylans	8.5	Total NSP 14.7	Spring varieties
	β-glucans	1.2	Soluble NSP* 25.6 Soluble NSP** 3.76 β-glucans 1.7 Arabinose 3.6	Total NSP 12.1 1.9* Soluble NSP 3.7 10.6* 4.0**
Triticale	Arabinoxylans	7.1	Total NSP 13.1	Winter varieties
	β-glucans	0.7	Soluble NSP* 22.7 Soluble NSP** 2.97 β-glucans 0.7 Arabinose 3.5	Total NSP 9.6 2.5* Soluble NSP 1.8 Spring varieties
Barley	Arabinoxylans	5.7	-	Total NSP 10.6 2.4* Soluble NSP 1.7
	β-glucans	4.4	-	Spring varieties
Maize	-	-	-	Total NSP 14.3 1.4* Soluble NSP 4.7
	-	-	-	Total NSP 4.3 Soluble NSP 0.9
Oat	Arabinoxylans	7.6	Total NSP 23.2	Hulled grain
	β-glucans	3.4	Soluble NSP* 13.3 Soluble NSP** 3.09 β-glucans 2.8 Arabinose 1.8	Total NSP 28.0 1.5* Soluble NSP 3.8 Dehulled grain
				Total NSP 8.1 3.0* Soluble NSP 4.7
				*(as % of total NSP) **(as % of DM)

### Effects of dietary non-starch polysaccharides on intestinal physiology

In nature, NSP are not a problem because the feed collected by wild birds gets into the crop where the pre-digestion process follows and is prolonged because of eating non-ground grain. Digestion occurs due to the presence of hydrolytic enzymes produced by commensal *Lactobacilli* or *Streptococci* strains (Fuller, 2001). In the case of farmed birds receiving finely ground feed, the digestion time is shortened in the crop, which is conducive to the transition of NSP to the distal part of the gastrointestinal tract. This results in the formation of viscous solutions that are mainly responsible for negative effects while feeding animals with cereals rich in NSP.

Tellez et al. (2014) evaluated the intestinal viscosity in broiler chickens fed on a rye- and maize-based diet. The birds fed with maize had much better growth performance than those from the control group. Mathlouthi et al. (2002) conducted a study where the effect of a maize-, wheat- and barley-based diet was investigated. Birds fed with wheat- and barley-based diet showed a reduced body weight gain (BWG), feed intake, an increased feed conversion ratio (FCR) and higher viscosity level compared to the maize-based diet. Such findings confirm that NSP are responsible for a higher ileal viscosity level. Hydrocolloids formation leads to reduced BWG and a higher FCR due to the decreased interaction between endogenous enzymes and digested feed (Cardoso et al., 2014). The undigested nutrients could not be used by the birds as a source of energy or building material for their growth. The increase in viscosity in the gastrointestinal tract mainly impairs the absorption of fats in broiler chickens. A negative correlation between viscosity level and BWG in broiler chickens was reported by Choct and Annison (1992) with a wheat-based diet and by Bedford et al. (1991) in birds fed a mixed wheat- and rye-based diet. The lower body weight gain may lead to increase of the feed intake in order to compensate poorer nutrient availability. Unfortunately, this may result in significantly raised cost of production (Boros et al., 1998; Goncharenko et al., 2011; Cardoso et al., 2014). Higher viscosity slows down the gastric passage rate, which negatively affects feed intake and microbiological status (Slominski, 2011). The prolonged transit time of the intestinal contents creates the possibility for the proliferation of pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens* (Józefiak et al., 2006; Hashemipour et al., 2016). Moreover, elevated bacteria translocation, which consists of microorganisms passing through the epithelium of the intestinal mucosa into the portal circulation, was observed while feeding a diet rich in NSP (Latorre et al., 2015 a). The most serious complications of bacterial translocation is systemic infection that can lead to death. These findings are in full agreement with the results reported by Tellez et al. (2015), who found out that a rye diet also negatively influences intestinal viscosity and bacterial translocation.

In addition, soluble NSP causes the formation of sticky faeces that increase litter humidity. This promotes the development of pathogens and a deterioration of animal welfare (Bach Knudsen, 2014). Wet litter on which the poultry live can lead to the occurrence of footpad dermatitis (FPD) that affects the welfare of the birds, causing severe pain (Meluzzi et al., 2008). What is more, a negative effect on bone quality was observed while feeding broilers a rye-based diet (Latorre et al., 2015 a). Also, other research conducted by Campbell et al. (1983) and Tellez et al. (2014) showed

that feed rich in soluble NSP causes malabsorption of minerals and fat-soluble vitamins. It is likely due to a reduction of micelle formation that is responsible for the transferring of fat soluble vitamins and minerals to the brush border of epithelial cells, where their absorption occurs (Latorre et al., 2015 a).

### **Impact of non-starch polysaccharides on intestinal microflora**

Microflora is an important factor influencing the health status of animals; it affects intestinal development, digestion, absorption of nutrients and immunity system (Matin et al., 2012). A lot of effort is made to maintain a balance between beneficial bacteria (*Lactobacillus*, *Enterococcus*, *Bifidobacterium*) and potentially pathogenic species (*Clostridium perfringens*, *Escherichia coli* or *Salmonella* sp.). Homeostasis can easily be disturbed by factors such as diet, age, pH, gastric passage rate, mucosal secretion or any disorders in the immunity system (Matin et al., 2012). Some papers report the positive effect of using soluble fibre on the microbial population, while the results of other authors are completely different.

As AX cannot be degraded by non-ruminant enzymes, they mainly reach the colon where they stimulate growth of residing bacteria such as *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, and *Eubacterium* (Rivière et al., 2014). The high activity of the fermentation of dietary fibre is also observed in caeca, which is characterised by the highest microbial biodiversity where 200 different species of bacteria were determined by Józefiak et al. (2004). However, in birds, as was mentioned previously, a part of the NSP is also used in the crop due to the presence of bacteria of the *Lactobacilli*, *Streptococci* and *Coliform* genus (Fuller, 2001). Commensal bacteria of the gastrointestinal tract are capable of fermentation of nutrients supplied with the diet that are not digested by animal endogenous enzymes. This applies to both resistant starch, NSP and oligosaccharides or proteins. As a result, lactic acid and short chain fatty acids (SCFA) – mainly acetate, propionate and butyrate – are formed. These compounds play an important role as energy sources for both ruminant and monogastric animals. The fermentation process is heterogeneous and varies between different substrates. It is important to highlight that the ability of the broilers gut microflora to carry out the fermentation process of dietary fibre is less effective than in other non-ruminant species (Józefiak et al., 2004). What is more, SCFA reduce the number of pathogenic bacteria by lowering the pH value of the gastrointestinal tract, which creates unfavourable conditions for their proliferation, while such an environment favours the development of commensal anaerobic population.

Their ability to easily penetrate the lipid membrane is considered as one of the antimicrobial mechanisms of action of SCFA. Inside the bacterial cell, where the neutral pH of the cytoplasm is observed, they dissociate into anions and protons, destroying bacterial cells due to the lack of the ability to maintain the desired pH. Commensal bacteria probably are protected by the high intracellular concentration of potassium but the full bacteriostatic mechanism still remains unknown (Ricke, 2003). *In vitro* studies have confirmed a reduction of the population of *Salmonella typhimurium* under the influence of the bacteriostatic effect of SCFA (Van der Wielen et al., 2000; Józefiak et al., 2004). In addition, an experiment conducted by

Guyard-Nicodème et al. (2015) showed that SCFA are able to decrease the number of *Campylobacter jejuni* in broilers. The Roberts et al. (2013) study investigated the ability of plantain NSP to inhibit epithelial cell adhesion and the invasion of different bacterial pathogens. Used soluble dietary fibre was able to inhibit epithelial adherence of *C. difficile*, enterotoxigenic *E. coli*, *Salmonella*, both *in vitro* and *ex vivo*, probably through its action on the epithelium. At present, increased epithelial Cl<sup>-</sup> secretion is considered as a main mechanism of action. Also of interest is the fact that *Bacteroides* are the major fermenters of this kind of NSP fraction, whereas *Ruminococci* or *Bifidobacteria* are unable to use it in their metabolic processes. A similar situation was observed by these authors in their previous experiment with broccoli fibre. However, the situation was different in the case of leeks and apples that did not have such a positive effect (Roberts et al., 2010).

There is also no doubt that a higher ileal viscosity level, which slows down the gastric passage rate and leads to deterioration of digestion, has a negative effect on intestinal microbiota as undigested nutrients become a breeding ground for pathogenic bacteria. In conjunction with the slow passage rate of gastric contents, it gives a great opportunity for the proliferation of undesirable bacteria (Bedford and Cowieson, 2012). An important observation was reported by Tellez et al. (2015) who have shown that that rye (37.2%) significantly increased viscosity level and *Clostridium perfringens* proliferation in comparison with maize in an *in vitro* digestive model. In the same experiment the authors found that supplementing the maize- and rye-based diets with selected direct-fed microbial (DFM), which produce protease, phytase, lipase, xylanase and cellulases, improved viscosity and reduced the *Clostridium perfringens* population. Similar results with using DFM in a rye-based diet to reduce the growth of pathogenic *Clostridium perfringens* bacteria and viscosity in comparison to the control group have been observed by Latorre et al. (2015 b). According to Montagne et al. (2003) enzymes are also able to decrease susceptibility to infections caused by *Salmonella*, *Campylobacter jejuni* and *Brachyspira intermedia*. It is supposed that the produced oligosaccharides may stimulate the growth of commensal bacteria. Mathlouthi et al. (2002) reported that wheat and barley consumption, increases bacteria in the caeca – both commensal (from *Lactobacillus* strains) and pathogenic (*E. coli*).

### Effects of rye grain on poultry performance

The effects of rye inclusion in grower diets on health and rearing parameters of broiler chickens was recently investigated by Van Krimpen et al. (2015). The birds received a diet with 0.5 or 10% of rye between 14 and 28 d of age. The results showed lower BWG, poorer FCR and a decreased population of commensal bacteria such as *Lactobacillus reuteri*, *Staphylococcus saprophyticus* and *Aerococcaceae* in the group fed with the 10% rye-based diet. Of note is that the increase in jejunal villus height and crypt depth was reported in the experimental groups at day 21. This suggests that soluble NSP may have a positive effect on the development of the intestinal mucosa. It is supposed that in poultry receiving a viscous diet, the villi length will increase in order to expand the absorption area. In this experiment feed intake, number and size of jejunal goblet cells and spleen weight were not affected

by the rye inclusion. In another study, Teirlynck et al. (2009) did not observe growth depression in the broilers receiving a diet with 5% of rye between 1–42 d of age. Furthermore, it was shown by Langhout (1998) that a 25% rye-based diet decreased BWG by 15% and FCR was 13% higher between 1 and 21 d of age. The basal diet of the broiler chicken in Santos et al. (2013) experiment contained 60% of rye and was formulated to meet the nutrient requirements. Although the experiment was designed to estimate the possibility of using xylanase supplementation only in the earliest periods of the life of the broiler, the effect of such a high dose of the rye in the diet was also investigated. At 21 d of age the birds' body weight (without enzyme supplementation) was very low, and much lower with respect to the control group. Similar findings were reported by Mourão and Pinheiro (2009) whose experiment also confirmed that a high dose of rye results in lower BWG in broiler chickens (Table 3).

Table 3. Body weight of broiler chickens fed different doses of rye-based diets

% Rye	Body weight (g)				Rye effect compared to the control treatment (maize-based diet)	References
	14 d	21 d	28 d	35 d		
60%	334	652	919		(no control diets in this study)	Santos et al. (2013)
53%		812		1571	BW decreased by 7.9% at 35 d	Mourão and Pinheiro (2009)
10%	541	1096	1816	2552	BW decrease by 1.5% at 21 d; no significant effect at 14, 28 and 35 d	Van Krimpen et al. (2015)
5%	551	1127	1877	2567	No significant effect	Van Krimpen et al. (2015)

The situation is different in the case of laying hens. It is possible that cereal rich in soluble fibre, including rye and barley, can successfully replace maize in the diets. According to Lazaro et al. (2003) no significant differences in egg performance and FCR were observed while feeding layers with maize, wheat, barley or rye. Similar results were reported by Brufau et al. (1994) and Pan et al. (1998) who found no difference in egg production in the hens fed with a rye-based diet and a maize-based diet. However, Patel and McGinnis (1980) reported that a high dietary level of rye negatively affected egg production in comparison with the control, maize-based diet.

#### Methods of lowering the content of non-starch polysaccharides in feed materials

Of monogastric animals, young chickens are particularly susceptible to viscous components of the diet so the recommended doses of cereals in the diets. The only grain that can be used without limitation in the ration is maize. A major cereal in broiler nutrition is wheat because of its high protein and starch content but, due to the presence of soluble polysaccharides, unlike maize its utilisation is limited (Hashemi-pour et al., 2016). In the case of young birds, a maximal dietary level of wheat should not exceed 20%; at the time of application of the enzyme, the share of this grain can be 40% (Smulikowska and Rutkowski, 2005). Rye, due to the high content of AX, should be introduced into the feed for young birds only with the NSPases, excluding laying hens, which can receive 10% of this cereal. Its maximum content, when

using exogenous enzymes, can be 10–20% in 4-wk-old birds and 10–40% in laying hens (Smulikowska and Rutkowski, 2005). Unfortunately, the level of this grain proposed in Polish ‘Standards of Poultry Nutrition’ (Smulikowska and Rutkowski, 2005) is very low, however it is dictated by the fear of deterioration of the production indicators. Barley grain without additives should not exceed 10% in young birds; the exception is laying hens where the share of barley may be increased up to 40%. The last often-used cereal is triticale. Its content in the feed should be in the range of 10–20% in young birds and 40% in laying hens. When using enzyme preparations, the permitted proportion increases from 20 to 40% (Table 4).

Table 4. The maximum recommended dose of individual cereals in the diet of broiler chickens

Cereal	Addition of feed enzymes	Recommended maximal level in the diet	References	
Wheat	–	0–4 wk (young)	20%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	40%	
		4–20 wk (laying hen)	40%	
		laying hen – without limits		
Wheat	+	0–4 wk (young)	40%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)		
		4–20 wk (laying hen)		
		laying hen – without limits		
Triticale	–	0–4 wk (young)	10%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	20%	
		4–20 wk (laying hen)	20%	
		laying hen	40%	
Triticale	+	0–4 wk (young)	30%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	40%	
		4–20 wk (laying hen)	40%	
		laying hen	40%	
Barley	–	0–4 wk (young)	10%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	10%	
		4–20 wk (laying hen)	10%	
		laying hen	40%	
Barley	+	0–4 wk (young)	20%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	40%	
		4–20 wk (laying hen)	40%	
		laying hen	40%	
Rye	–	0–4 wk (young)	0%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	0%	
		4–20 wk (laying hen)	0%	
		laying hen	10%	
Rye	+	0–4 wk (young)	0%	Smulikowska and Rutkowski (2005)
		4–18 wk (slaughter)	20%	
		4–20 wk (laying hen)	10%	
		laying hen	10%–0%	

As previously mentioned, the fraction of NSP in different kinds of cereals is not fixed and fluctuates depending on the variety and climatic conditions. One such paper, Gebruers et al. (2010), did suggest that the content of water-extractable AX in

wheat is fifty percent determined by its genotype. It shows how important the interaction is between genotype and environment (temperature and its variation, precipitation, altitude and soil properties) for estimating the level of soluble NSP in particular cereals. The genetically determined contents of polysaccharides were investigated by Cyran and Lapinski (2006), who reported that the content of AX is almost halved in tetraploid triticale cultivars compared to hexaploid cultivars. The environmental impact on grain quality is visible during temperature fluctuations which are known to affect the amounts and properties of starch and NSP (Andersson et al., 1993; Coles et al., 1997). Studies carried out by Toole et al. (2007) show that the AX structure in the wheat endosperm cell walls changes from an easily soluble highly branched form to a water insoluble less branched form depending on the weather conditions. The whole process is faster when the cereal is grown at a higher temperature with restricted water availability.

The potential applied viscosity in triticale or wheat was influenced mainly by daily mean temperature in the Philippe et al. (2006), Toole et al. (2007) and Häner et al. (2013) experiments. Water availability was less important in the modification of grain composition (Toole et al., 2007; Häner et al., 2013). Furthermore, it was shown that there is an influence of time storage of the grain on its quality features. These changes occur the most intensively after harvest and are the result of post-harvest maturation of grain. A higher content of NSP is observed in freshly collected material – this is the reason why a month is required before the grain can be used as a feed mixture (Fuente et al., 1998). The conducted experiments report that wheat just after harvest often causes nutritional problems in broilers. During prolonged storage, the germination process helps to decrease polysaccharides content thanks to the presence of the endogenous enzymes (Williams et al., 1997; Jacob and Pescatore, 2012).

### **Methods of preventing the negative effects caused by non-starch polysaccharides**

The NSP antinutritive effect may be reduced by adding an insoluble fibre fraction to the feed, principally as components of the lignin and cellulose. It has the ability to bind water, thereby limiting the increase in viscosity of the gastric contents and also accelerates the gastric passage rate. A positive effect of feeding young broiler chickens with coarsely ground oat hulls was reported by Hetland and Svihus (2001) who investigated its effects and their interaction with soluble fibre. There was a tendency for a faster flow of content through the digestive tract in comparison to the control group, although BWG remained unaffected. Insoluble wheat bran and cellulose elevate faecal weight and bulk and shorten the intestinal transit time in non-ruminant animals. A similar effect occurs with the administration of whole grains to the birds, which results in a longer pre-digestion in the crop and better preparation of gastric contents, thus quickening the transit time in the gut (McNab and Boorman, 2002).

More detail was measured in an extended study conducted by Hetland et al. (2005). They reported that the retention time of insoluble fibre is longer in comparison with other nutrients, and that whole grains are able to stimulate the activity of the gizzard which regulates the motility of the gastrointestinal tract (Taylor and Jones, 2004; Hetland et al., 2005). Other studies have reported that fibre is able to lower giz-

zard pH through increased HCL secretion (Jiménez-Moreno et al., 2009). A reduction of the intestinal pH content helps maintain homeostasis of the gastrointestinal microflora, because the acidic environment does not support the growth of pathogenic bacteria (Engberg et al., 2004). Moreover, stimulation of the proventriculus to produce more HCL and bile salts was observed while feeding animals with whole grains (Hetland et al., 2005). Another study showed that a diet rich in insoluble fibre favours better development of intestinal villi, which increase the surface absorption of nutrients (Sarikhani et al., 2010).

The final way which helps to avoid problems resulting from too high a concentration of NSP in the diet is the use of enzymes, mainly xylanase (which break down AX) and  $\beta$ -glucanase (hydrolysing  $\beta$ -glucans), as well as cellulases, pectinases and hemicellulases. These are proteins produced mainly by fungi and bacteria and have been in use since the late 1970s. NSP-degrading enzymes are safe feed additives designed to improve BWG and FCR thanks to being able to partially hydrolyse polysaccharides present in the cell wall that soften the so-called 'cage effect' (Ravn et al., 2016). This allows pancreatic enzymes for digestion of nutrients e.g. starch, to be closed inside the cell. Furthermore, it enables oligosaccharides and monosaccharides to be absorbed by intestinal villi or degraded by commensal bacteria, increasing the amount of digestible energy for the animal and enhancing the nutritional value of the cereals (Masey O'Neil et al., 2014). The released oligosaccharides are able to reach the colon where they selectively stimulate the growth and activity of beneficial intestinal bacteria such as *Bifidobacterium* and *Lactobacillus* (Thammarutwasik et al., 2009). Another mechanism of NSPases action is preventing increased viscosity in the digestive tract and associated problems (Santos et al., 2013; Munyaka et al., 2015; Tellez et al., 2015).

Lazaro et al. (2003) investigated the influence of a fungal beta-glucanase/xylanase enzyme complex addition in different doses to different cereal-based diets for laying hens. The birds fed with wheat as the main grain in the diet had viscosity of digesta 11.1 mPa·s. Supplementation with  $\beta$ -glucanase (1375 IU/kg) and xylanase (1550 IU/kg) lowers this value to 5.0 mPa·s. As for barley and rye, the control groups had viscosity of digesta of 33.2 mPa·s and 111.9 mPa·s, respectively. NSPases decreased it to 4.4 mPa·s for barley and 30.8 mPa·s for rye. The majority of the experiments with poultry confirmed that a high dietary level of pentosans reduces the digestibility of starch, protein and fat, and that the addition of NSP degradative enzymes can soften this effect by reducing the viscosity of the intestinal content, irrespective of what kind of grain is used (Simon, 2000; Francesch et al., 2012; Mendes et al., 2012; El-Wafa et al., 2013; Latorre et al., 2015 b).

NSP hydrolysing enzyme activities had a beneficial effect on the growth performance of young chickens fed a barley-based diet in the Jeroch (1998) experiment, which reported improved BWG even up to 9% and FCR up to 5%. The positive impact of combined  $\beta$ -glucanase and xylanase on growth performance was also noted by Munyaka et al. (2015). Birds fed a diet supplemented with enzymes had higher BWG (4%) and FCR (7%) than birds on non-supplemented diets. Similar results were later obtained by several other authors in chickens fed diets containing NSP-rich cereals (rye, triticale and barley) (Nahas and Lefrançois, 2001; Silva and Smi-

thard, 2002; Lazaro et al., 2003; Mendes et al., 2012). Similar findings were also obtained in another study with broiler chickens by Kalantar et al. (2015). The birds from the group supplemented with enzymes on a barley based-diet had significantly higher BWG and lower FCR than those from the control group. Williams et al. (2014) have shown that a reduced-energy broiler diet supplementation with  $\beta$ -mannanase or NSPase (cocktail carbohydrase – xylanase,  $\beta$ -glucanase, and  $\alpha$ -galactosidase) improved growth performance and reduced mortality to a level comparable to the control. In this experiment all the diets were maize- and soybean meal-based. Similarly, Meng and Slominski (2005) reported that broilers fed a reduced-energy diet (containing 69% maize) with the addition of a xylanase, glucanase, pectinase, cellulase, mannanase, and galactanase showed better FCR. Santos et al. (2013) highlighted that the action of exogenous NSP-hydrolysing enzymes may be restricted to the first 21 d of the chicken's lifecycle without causing growth depression or worsening FCR while feeding a rye-based diet. However, studies carried out by Mourão and Pinheiro (2009) reported that xylanase addition to rye- (53%) or wheat- (53%) based diets did not improve FCR in comparison to the control group on a maize-based diet (53%).

Furthermore, high digesta viscosity promotes the proliferation of anaerobic microbes in the upper parts of the gastrointestinal tract (Józefiak et al., 2007). It was also shown that in a rye-based diet the type of fat is able to influence the digesta pH and effectiveness of NSP-degrading enzymes – probably due to microbial activity (Dänicke et al., 1997 a). Researchers have stated that the negative effect induced by soluble NSP, is a slower rate of passage that in birds also results in reduced feed intake and promotes changes in the intestine such as an enlargement of the gastrointestinal organs (Montagne et al., 2003; Santos et al., 2013; Cardoso et al., 2014). The results obtained by Almirall and Esteve-Garcia (1994) on broilers fed with a barley-based diet, and by Dänicke et al. (1997 b) on poultry with inclusion of rye, indicate that the addition of the beta-glucanase (in the former experiment) and xylanase (in the latter) to an animal's diet fastens the ileal passage rate.

On the other hand, some reports highlight that the hydrolysis of all cell wall components might not be so beneficial. The degree of NSP degradation could be crucial and should be considered for establishing the optimal efficiency of the enzymes, as it does not seem to grow linearly (O'Neill et al., 2014). Similar findings were reported by Mendes et al. (2012), who added 1,170 U/kg, 1,560 U/kg and 3,125 U/kg of the xylanase recommended dose to the basal diet containing 60% triticale. Enzyme supplementation improved the BWG of broilers fed triticale-based diets in comparison to the control group, but only when supplementation was 75%. The BWG of birds fed the diets with higher doses of the xylanase did not differ from the birds from the unsupplemented group. This evidence may suggest that enzyme addition improves performance indices only to a certain level. Similarly, Francesch et al. (2012) reported that FCR improved significantly in broilers fed a wheat-based diet supplemented with endoxylanase. A positive response was observed at 400 FXU/kg and did not differ when the enzyme dose was 4,000 FXU/kg. Furthermore, enzyme addition reduced the viscosity of the ileal digesta and increased the digestibility of dry matter, protein and lipids. The results from the groups in which the birds were fed with the

highest concentration of the enzyme were comparable with those that received much lower doses (Francesch et al., 2012).

Summing up, the spectrum of action of NSPases is multidirectional. They limit the increase of digesta viscosity through the hydrolysis of plant soluble polysaccharides, promote the proliferation of beneficial microflora populations by providing substrates for fermentation, increase nutrient availability, thus improving the nutrients value of NSP-rich cereals, increasing poultry performance and reducing the cost of production. Although enzymes have been widely used in broiler feed to overcome the antinutritional effects of NSP, their effectiveness varies depending on the study. Different results indicate that factors such as the dose and type of NSPases used, the type and variety of the cereals (Mendes et al., 2012), the birds' age (Cardoso et al., 2014) and even other components of the diet (i.e. fats; Dänicke, 1997 a) are very important for taking into consideration which enzyme should be used. One additional important factor might be choosing enzymes with a broad pH and temperature adaptability for obtaining better growth performance.

### Conclusions

Although rye is a valuable feed grain it still remains the most problematic cereal for broiler nutrition. It is clear that with a growing share of this cereal in diets, growth performance begins to deteriorate. However, in the case of modern rye hybrids this negative influence can be significantly reduced. The supplementation of rye-based diets with NSP-degrading enzymes can also ameliorate the adverse effect of rye on BWG, FCR, ileal viscosity and gastric passage rate in broiler chickens. However, to date more research is still required when considering use of a rye-based diet in broiler chicken production. The content of rye grain can be higher in the case of laying hens, where the risk of the adverse effects of rye on performance indices is much lower.

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