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THE USE OF PREBIOTICS BASED ON OLIGO- AND DISACCHARIDES IN POULTRY FARMING — a mini review

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Abstract

In meat poultry farming, technologies of chick feeding and growing allow getting a carcass ready for sale for a short period (35-42 days). Such a high growth rate is due not only to proper feeds, but also to various feed additives (E.V. Yaskova et al., 2015). The ban of antibiotics-based growth stimulants in the European Union determines the search for alternative natural substances that provide similar effects. A promising group of such substances is prebiotics (D.S. Uchasov et al., 2014) which provide an increase in the efficiency of nutrient utilization, have a positive effect on the blood morphobiochemistry, poultry natural resistance, productivity, meat quality and economic efficiency (I.V. Chervonova, 2016). This mini review systematizes data on disaccharides as potential modulators of the intestinal microbiome profile and growth stimulants of broiler chickens when antibiotics are rejected. Several groups of substances with a prebiotic effect are widely used as ingredients of premixes and compound feeds. Currently, mono-, oligo-, di- and polysaccharides are being studied as promising prebiotics. The search for new biologically active substances with a multifactorial effect on broiler chickens is relevant. Feed additives used in poultry farming contain components with prebiotic properties. These components are oligo- and disaccharides (maltose, lactose, sucrose, lactulose, fructooligosaccharides, galactooligosaccharides, soy oligosaccharide), polysaccharides (cellulose, pectins, inulin, dextrin, etc.), monosaccharides (xylitol, raffinose), amino acids (arginine, valine, glutamic acid), antioxidants (vitamins A, E, C, carotenoids, selenium salts), organic acids (citric, acetic, propionic), plant and microbial extracts (carrot, corn, rice, garlic, potato, yeast), algae extracts. The prebiotic preparations based on organic acids (lactic, lemon, fumaric, formic) and lactulose are mostly used (E.V. Shatskikh et al., 2008). Natural prebiotics include fructans (fructooligosaccharides, short-chain fructooligosaccharides, oligofructose, inulin), mannooligosaccharides from *Saccharomyces cerevisae*, soy oligosaccharides and galacto- or transgalactooligosaccharides (D. Charalampopoulos et al., 2009). Lactulose, a synthetic structural isomer of lactose (4-O-β-D-galactopyranosyl-D-fructofuranose) consists of fructose and galactose linked by a β-1,4-glycoside bond. It is an odorless white crystalline substance highly soluble in water. Synthetic disaccharides are 1.5 times sweeter than lactose and can crystallize from an alcoholic solution. β-Glycoside bonds in disaccharides are not hydrolyzed by digestive enzymes (H. Rutloff et al., 1967). Therefore, disaccharides pass through the stomach and small intestine without degradation and, being unchanged, reach the large intestine (L.N. Skvortsova, 2010). In addition, lactulose has the highest index of prebiotic activity. It stimulates lacto- and bifidobacteria in the large intestine, promotes the restoration of normal microbial profile, declines pH in the colon, inhibits conditionally pathogenic microbes, improves the absorption of nutrients, and increases immunity (V.S. Buyarov et al., 2012; V.S. Buyarov et al., 2015). Commercial feed additives based on oligo- and disaccharides as a prebiotic component may contain various substances, including trehalose, lactulose, and inulin. All of them have restorative, immunostimulating, therapeutic and prophylactic properties, contribute to the restoration of intestinal microbial community, change the final microbial products, and prevent the occurrence of inflammation and infectious diseases (C. Schumann, 2002; K.M. Tuohy et al., 2002; J.H. Cho et al., 2014).

Keywords: broiler chickens, disaccharides, prebiotics, lactulose

According to some estimates, by 2050 the world's population will reach 9 billion people [1]. As a result, the demand for food, especially for livestock products, is growing and will continue to grow. Therefore, innovative approaches and methods are needed to intensify food production of animal products while simultaneously reducing costs, but maintaining quality and safety, that is, the development of optimization strategies [2].

In poultry farming, birds reach maturity in a short time, which corresponds to the growing needs for animal protein. Global poultry meat production doubled from 2009 to 2021, especially in developing countries [3]. Due to modern feeding and housing technologies in poultry farming, it takes 35-42 days to produce carcasses ready for sale. Such a high growth rate is ensured both by complete feeds and feed additives of various types [4]. The ban on antibiotic-based growth promoters in the European Union since January 1, 2006 has spurred the search for alternative natural substances that provide similar effects. In addition, the intensification and optimization of poultry farming technologies should not adversely affect the quality and safety of poultry products.

Prebiotics are substances that have a positive effect on the host by selectively stimulating metabolic activity and the growth of beneficial intestinal microbiota [5]. It is believed that prebiotic drugs will be in demand due to the lack of negative impact on product quality and human and animal health unlike antibiotics [6].

There is a massive data on prebiotics in the scientific literature. Published results indicate that such drugs in the poultry diets provide an increase in the feed nutrient utilization and improve blood morpho-biochemical parameters, natural resistance, productivity, quality of products and economic indicators [7]. However, in most cases, information on different groups of substances is not systematized and is fragmented.

Several groups of substances have a prebiotic effect [8]. These are oligosaccharides (soy oligosaccharides, fructooligosaccharides, galactooligosaccharides), monosaccharides (xylitol, raffinose, sorbitol, xylobiose), disaccharides (lactulose), polysaccharides (cellulose, hemicellulose, pectins, gums, mucilage, inulin), peptides (soybean, milk), enzymes (saccharomyces proteases, β-galactosidases of microbial origin), amino acids (valine, arginine, glutamic acid); antioxidants (vitamins A, C, E, carotenoids, glutathione, Q10, selenium), fatty acids (eicosapentaenoic acid), organic acids (acetic, citric), plant and microbial extracts (carrot, potato, corn, rice, pumpkin, garlic, yeast) and other products (lecithin, para-aminobenzoic acid, lysozyme, lactoferrin, lectins, extracts of various algae).

Some substances with prebiotic properties (enzymes, amino acids, vitamins) are already widely used in premixes and mixed feed formulations. Mono-, oligo-, di- and polysaccharides are considered as potential prebiotics. These compounds constitute an indigestible component of the diet that can be utilized by the intestinal microflora followed by a beneficial effect on the host [9]. The search for new bioactive substances that can have multiple effects remains relevant for modern broiler poultry farming. The use of compounds that have prebiotic effects is a way to improve gut health and animal performance without antibiotic growth promoters. This group of substances includes oligosaccharides, in particular lactulose. Lactulose is one of the promising disaccharides for prebiotic use. It is a synthetic structural isomer of the milk sugar lactose. Lactulose has the highest index of prebiotic activity, it stimulates the growth of lacto- and bifidobacteria in the large intestine, inhibits the opportunistic microbiota, helps restore normal microbiota, reduce the pH of the colon contents, improve utilization of nutrients, and increase immunity [10].

The purpose of this review was to systematize data on the effectiveness of prebiotics by the example of disaccharides (lactulose) as potential regulators of the gut microbiome composition and growth stimulants for broiler chickens when avoiding the use of antibiotics.

Structure, properties, and classification of disaccharides.

According to the chemical structure, most prebiotics are carbohydrates, non-fermentable poly- and disaccharides. In the large intestine, due to activity of the microorganisms that utilize prebiotics, organic acids (acetic, propionic, butyric, lactic) and hydrogen are produced. These acids are important for the macroorganism, they ensure the constancy of positive microbiota and pH in the intestinal lumen, the absorption of water and calcium, sodium, chlorine, magnesium ions, have a bactericidal and fungicidal effect, serve as natural metabolites and are completely assimilated in the animal's body, supplying it with additional energy [11, 12].

A disaccharide is two monosaccharide units joined by an acetal or ketal bond [13]. The glycosidic bond connects two monosaccharides and can be either α-glycosidic in case of α configuration of the anomeric hydroxyl group in the sugar, or β-glycosidic for the β configuration [14]. The three most common disaccharides are maltose, lactose, and sucrose [15] (Fig. 1). Maltose is a reducing sugar derived from starch hydrolysis by α-amylase [16]. Lactose is also a reducing sugar that consists of a D-glucosyl unit and an α-D-galactopyranosyl unit linked by a β-(1,4)-glycosidic bond. Lactose is a constituent of milk and dairy products, such as skim milk and whey [17]. Sucrose consists of glucose and fructose linked by an α-(1,2)-glycosidic bond (see Fig. 1). Maltose, lactose and sucrose are hydrolyzed by maltase, lactase and sucrase into their constituent monosaccharide units. The α-glucosidase complexes maltase-glucoamylase and sucrase-isomaltase, present in the brush border of the small intestine, cleave glycosidic bonds in maltose and sucrose, respectively, with most of the maltase activity occurring in the sucrase-isomaltase complex [18].

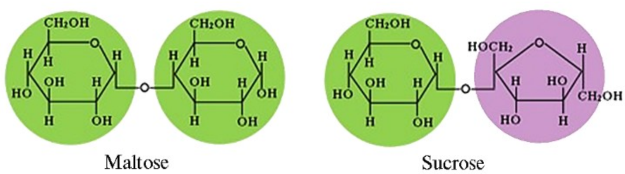

Fig. 1. Chemical structure of disaccharides (18).

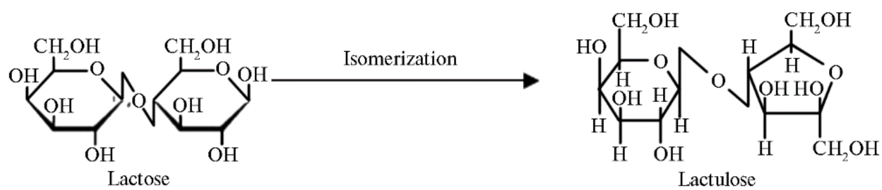

Fig. 2. Scheme of the isomerization for production of lactulose (20).

The synthetic disaccharide lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose), consisting of two sugar molecules fructose and galactose linked together by a β-1,4-glycosidic bond, also belongs to the class of oligosaccharides, a subclass of disaccharides (Fig. 2). It is a white crystalline substance, odorless, highly soluble in water. Synthetic disaccharides are 1.5 times sweeter than lactose and can crystallize from an alcohol solution. The β-glycosidic bond of the disaccharide is not hydrolyzed by digestive enzymes [19]. Once in the body, this disaccharide passes through the stomach and small intestine without degradation, that is, the peculiarity of lactulose is that it can reach the large intestine unchanged [20].

The production of lactulose by chemical and enzymatic methods has been reported. The disadvantage of the chemical method is the need to use high temperatures and strong acids to purify the product, which can lead to environmental pollution [21]). On an industrial scale, lactulose is produced by chemical isomerization of lactose in an alkaline environment [22]. Since the 1950s, lactulose has been recognized as a bifidogenic factor when added to the diet [23].

1. Chemical and physiological characteristics of sugars and other glycemic carbohydrates (24)

Carbohydrate	Type	Digestive enzyme	In the intestinal lumen	In blood	Glycemic index	Possible metabolization options
Sucrose	Disaccharide: glucose-fructose, α-1,2 bond	Sucrase	Glucose, fructose	Glucose, lactate, fructose	65	Used as a source of energy, stored as glycogen and/or converted to other metabolites. Partially converted to lactic acid and glucose, which are used as energy sources or stored as glycogen, and fatty acids are used as energy sources or triacylglycerol, stored as lipids
Isomaltulose	Disaccharide: glucose-fructose, α-1.6 bond	Isomaltulase	Glucose, fructose	Absent	32	
Lactose	Disaccharide: glucose-galactose, α-1-4 bond	Lactase	Glucose and galactose	Glucose and galactose	45	Used as energy sources, stored as glycogen and/or converted to other metabolites
Maltose	Disaccharide: glucose-glucose, α-1,4-glycosidic linkage	Maltase	Glucose	Glucose	105	
Trehalose	Disaccharide: glucose-glucose, α-1,1-glycosidic linkage	Trehalase	Glucose	Glucose	70	

2. Feed additives based on oligo- and disaccharides

Feed addiive	Manufacture	Dosage	Reference
Trehalose	100ING.RU — online distributor of ingredients and raw materials for food and other industries	2 g/kg feed	https://100ing.ru/category/tregaloza/
Trehalose (Tre)	Hayashibara Co., Ltd, Japan	0.25, 0.50 and 0.75 % of diet	[35]
Inulin (chicory extract dry)	SENSUS BV, the Netherlands	1 g/kg feed	[36]
Inulin (powder)	Beneo, China	1 g/200 ml water	https://100ing.ru/product/inulin-poroshok-500-gr/10878/
	Jarrow Formulas, Inc., USA	1 g/200 ml water	https://100ing.ru/product/inulin-beneo-gr-orafti-1-kg/7421/
Lactulose	VTF, Russia	1 g/kg feed	https://vtf.ru/goods/stm/
Lactovit	North Caucasus Research Institute of Animal Husbandry, Russia	1 ml/100 g bodyweight	[37]
Lactoflex	Povolzhsky Research Institute of Production and Processing Meat and Dairy Products RAAS, Russia	0,1-0,3 g/kg bodyweight	http://volniti.ucoz.ru/
Ecofiltrum	OAO ABBA RUS, Russia	0,4-1,6 kg/g feed	[10, 38, 39]
Lactumin	Lactoprot Deutschland GmbH, Германия	200 mg/kg bodyweight	[40]
Todikam-Lact	Povolzhsky Research Institute of Production and Processing Meat and Dairy Products RAAS, Russia	200 mg/kg bodyweight	http://volniti.ucoz.ru/

In contrast to chemical methods, the production of lactulose using enzymes such as α-galactosidase or cellobiose-2-epimerase has several advantages. This technology is specific, safe and environmentally friendly. However, the production of lactulose using α-galactosidase is not cost-effective because it requires fructose and lactose and the reaction only occurs at high substrate concentrations. The enzymatic production of lactulose using cellobiose-2-epimerase provides high yield of lactulose from a single lactose substrate [23].

The dietary fiber present in disaccharides may vary (α- or β-glycosidic linkage) that affects the rate of digestion and absorption. Table 1 shows the chemical and molecular parameters, the digestion, absorption, distribution, and metabolization of some carbohydrates [24].

Mechanism of action (pharmacokinetics) of lactulose. The metabolism of indigestible sugars with the participation of intestinal microbiota, provides the macroorganism with several advantages [25]. Intestinal condition improves, which is associated with an increase in the abundance of bifidobacteria (bifidogenicity) and suppression of putrefactive processes. Absorption of minerals increases, in particular calcium, magnesium and iron, which affects the state of the skeletal system and hematopoiesis (reduction of anemia) [26] and immunomodulation occurs.

Let us consider the mechanism of action of disaccharides using lactulose as an example. Compared to lactose, it has superior sweetness and high solubility, meaning it is a sugar that can be functionally useful and used in various food industries.

The therapeutic and prophylactic properties of lactulose include stimulating the growth of beneficial microflora, inhibiting the development of pathogenic bacteria, and protecting against intestinal infections. It also promotes the synthesis of vitamins and the absorption of minerals, reduces cholesterol in the blood, prevents the formation of liver stones, and is effective in the treatment of liver and kidney diseases [27, 28].

Lactulose is metabolized by colon bacteria to monosaccharides and then to volatile fatty acids, hydrogen and methane. It reduces the production and absorption of ammonia in the intestines in three ways. First, the metabolism of sugars causes a laxative effect by increasing gas production and osmolality, which leads to a decrease in the transit time of the contents through the intestines and a decrease in pH in the intestinal lumen. Second, lactulose promotes a higher ammonia uptake by colon bacteria, which use ammonia as a source of nitrogen for protein synthesis. Third, lowering intestinal pH facilitates the conversion of ammonia (NH_3) produced by intestinal bacteria to ammonium (NH_4^+) [28], an ionized form that cannot cross biological membranes. Finally, lactulose causes a decrease in ammonia production in the intestine. An acidic environment destroys bacteria that produce urease and participate in the formation of ammonia. The unabsorbed disaccharide also inhibits glutaminase activity, which blocks the intestinal absorption of glutamine and its metabolism to ammonia. There is also an improvement in lipid metabolism, a decrease in renal nitrogen excretion (similar to the effect of dietary fiber), activation of hormone production, an effect on the central nervous system and gut-associated lymphoid tissue [29].

Thus, resistance to the effects of gastric juice and digestive tract enzymes is the main feature of disaccharides, which determines their physiological function.

Feed additives based on prebiotic oligo- and disaccharides. In poultry farming, feed additives for various purposes are used. Their components can be prebiotics, e.g., oligo- and disaccharides (lactulose, fructooligosaccharides, galactooligosaccharides, soy oligosaccharide), polysaccharides (cellulose, pectins, inulin, dextrin), monosaccharides (xylitol, raffinose), amino acids (arginine , valine, glutamic acid), antioxidants (vitamins A, E, C, carotenoids, selenium salts), organic acids (citric, acetic, propionic), plant and microbial extracts (carrot, corn, rice, garlic, potato, yeast), extracts of various algae. The most popular prebiotics are based on organic acids (lactic, citric, fumaric, formic) and lactulose [30]. Natural prebiotics include fructans (e.g., fructooligosaccharides, short-chain fructooligosaccharides, oligofructose, inulin), followed by mannooligosaccharides (derived from *Saccharomyces cerevisae*), soy oligosaccharides, and galacto- or transgalactooligosaccharides [31].

Commercially available feed additives based on oligo- and disaccharides may contain various substances as a prebiotic component, including trehalose, lactulose, and inulin (Table 2). All of them have general strengthening, immunostimulating, therapeutic and prophylactic properties, help restore intestinal microflora, and prevent the occurrence of inflammations and infectious diseases [32-34].

Lactulose. Interest in this type of oligosaccharide may be due to the numerous beneficial properties that lactulose exhibits [41]. Acting as a prebiotic, lactulose promotes growth, improves digestion and strengthens the bird's immunity [42].

Lactulose was approved by the Food and Drug Administration (FDA, USFDA) in the USA in 1977 [32] and is currently used in both medicine and the food industry [43], including including as a functional food. Based on oligosugars, food products and their components have been created that have a pronounced positive functional effect on the human and animal body in general and on the intestinal microbiome in particular [41].

The main function of lactulose as a prebiotic is to improve intestinal microflora. Under the influence of lactulose, the number of bifidobacteria and lactobacilli in the gastrointestinal tract increases, and clostridia, salmonella and Escherichia coli decrease [44].

An increase in the number of goblet cells when taking lactulose may be associated with the growth of bacteria that determine the dynamics of mucin. The results of histomorphological studies provide new insight into the potential prebiotic effects of lactulose in broilers [45]. It was shown that in birds fed 0.2% dietary lactulose, the number of lactobacilli increased on day 28 and *E. coli* decreased compared to the control birds [34].

Prebiotics remove potentially pathogenic bacteria from the intestines or reduce their number by enriching the microbial population with beneficial strains. This improves the state of the intestinal tract and can have a positive effect both on metabolism in general and on organ-specific biochemical processes [46]. If necessary, lactose can be replaced with lactulose (4-O-β-D-galactopyranosyl-D-fructose) [47, 48]. It has been shown that lactulose in combination with tetra-ammonium bromide salt improves the main indicators of livestock products while simultaneously increasing resistance to internal and external infectious factors [49].

Trehalose. Trehalose (Tre) feed additives have a wide range of properties, including increasing growth rate (possibly by improving innate immune responses, such as suppressing Toll-like receptors and inflammatory cytokines in the chick duodenum [50]. Tre is a glucose-glucose disaccharide with an α,α-1,1-glycosidic bond and is found in a variety of organisms, including bacteria, yeast, fungi, and invertebrates [35].

Interestingly, despite numerous reports on the ability of trehalose to stabilize proteins upon cooling and heating, the mechanism of interaction of trehalose with proteins has not yet been studied [51]. To explain the nature of the interaction of the disaccharide with protein molecules, various hypotheses have been put forward. However, none of them has been confirmed so far. It has been established that in the presence of water, trehalose forms pastes and exhibits tropism towards protein molecules, but does not form hydrogen bonds with them [52].

Inulin. Inulin is a prebiotic found in many plants. It reaches the large intestine unchanged, where it is fermented by beneficial bacteria. Inulin also inhibits the growth of pathogenic bacteria. Inulin consumption by chickens increases yield at slaughter, but little is known about the effects of inulin on poultry meat [53].

If its dietary intake is insufficient, inulin-containing supplements can be recommended. Regular use of the functional additive inulin modifies the composition of microbial associations in the intestinal tract, improves the functioning of the digestive system and immune system, inulin reduces the severity of inflammatory and infectious processes, and is effective in metabolic syndrome [54].

A study of the effects of inulin (alone and in combination with isomaltooligosaccharide and fructooligosaccharides) showed that the abundance of lactobacilli in the intestines of birds fed supplemented diets was higher than those fed the same diets without prebiotics [55, 56].

Imbalance in the intestinal microbial assiiations can affect the functions of the liver, adipose tissue, kidneys and pancreas. In chickens fed at an early age a combination of *Lactococcus lactis* with inulin and the prebiotic galactooligosaccharide, the overall activity of pancreatic enzymes significantly increased followed by an increase in bodyweight. Moreover, both synbiotics have been shown to have a positive effect on the activity of two liver enzyme markers (alanine aminotransferase and aspartate aminotransferase) [57]. Increased activity of pancreatic amylase, lipase, and trypsin may be due to the production of additional enzymes by gut bacteria, which contributes to improved nutrient absorption and weight gain [58].

So, early maturity and high productivity of poultry are ensured both by complete feeds and feed additives. Prebiotics can serve as an alternative to antibiotic-based growth promoters banned in the European Union. Several groups of substances have a prebiotic effect, and many probiotics have already found wide practical use. Oligo- and disaccharides, polysaccharides, monosaccharides, amino acids, antioxidants, organic acids, plant and microbial extracts, algae extracts can be prebiotic components of feed additives for poultry farming. Prebiotics based on organic acids and lactulose are often used. The most common natural prebiotics are fructans (fructooligosaccharides, short-chain fructooligosaccharides, oligofructose, inulin), mannooligosaccharides (derived from *Saccharomyces cerevisae*), soy oligosaccharides, and galacto- or transgalactooligosaccharides. Here, we consisder the benefits of lactulose, a synthetic structural isomer consisting of two sugar molecules (fructose and galactose) linked by a β-1,4-glycosidic bond. Synthetic disaccharides are 1.5 times sweeter than lactose. The β-glycosidic bond of the disaccharide is not hydrolyzed by digestive enzymes, so lactulose enters the large intestine unchanged. Lactulose has the highest index of prebiotic activity. It stimulates the growth of lacto- and bifidobacteria in the large intestine, helps restore normal microflora, reduces pH, inhibits opportunistic microflora, improves the absorption of nutrients, and increases immunity. Commercially available feed additives based on oligo- and disaccharides may contain trehalose, lactulose, and inulin as a prebiotic component. All such drugs have restorative, immunostimulating and therapeutic and prophylactic properties.

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