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BIOCATALYSIS

Comparative Analysis of the Composition and Properties of Fodder Enzyme Preparations

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Abstract—The composition and properties of a wide variety of domestic and foreign enzyme preparations (EP) used as additives to feeds of farm animals and poultry are analyzed. Content of the main active enzymes – endoglucanases (beta-glucanases), cellobiohydrolases, and xylanases performing biocatalytic destruction of non-starch polysaccharides, which are anti-nutritional factors in feeds causing their incomplete digestion, is determined. It is shown that based on the component composition data and the level of different types of activity the studied enzyme preparations can be classified into three groups: a) with high xylanase and low cellulase (endoglucanase and cellobiohydrolase) content, b) with high cellulase and low xylanase content, c) containing cellobiohydrolases, endoglucanases and xylanases at a different ratio, but without significant prevalence of any of these enzymes. The ability of EP to reduce the viscosity of water-soluble non-starch polysaccharides – xylans and beta-glucans – has been studied. Among the enzyme preparations that have xylanase in their composition and belong to the groups b) and c) a number of preparations have been identified (Econase XT 25, Agroxyl Plus, Agroxyl Premium, Rovabio Max AP, Sunzyme), which reduced the viscosity of aqueous extract of rye containing xylans most effectively when used at the same dosage with regards to xylanase activity. It was shown that xylanase from precisely these EP was not inhibited by the protein inhibitors of rye. The viscosity of water-soluble beta-glucans of barley was most effectively reduced by the EP Xybeten CELL, Cellulase, Agroxyl, Agrocel, Axtra XB 201, Rovabio Max AP, and Vilzim used at the same dosage with regards to beta-glucanase activity. For all the studied EP, no inhibitory effect of the barley extract on beta-glucanase activity was found.

Keywords: Fodder Enzyme Preparations, biocatalist, xylanase, cellulase, non-starch polysaccharides
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Cereal grains (wheat, rye, oats, and barley) comprise the main components of animal and poultry feed. In addition to starch and proteins the cereal grains contain anti-nutritional factors such as non-starch polysaccharides (NSP) – cellulose, β-glucans, xylans (pentosans) [1]. NSP swell, form viscous structures thus obstructing the access of digestive enzymes of animals and poultry to the nutritional components of feed causing incomplete feed conversion and other negative effects [2, 3]. NSP are not digested in the gut of poultry and monogastric animals, as they do not have respective enzymes. Biocatalytic methods are used most often for destruction of NSP: enzyme preparations (EP) with cellulase, beta-gluconase, and xylanase activities are used as feed supplements. The use of EP during fodder production decreases the viscosity of chymus facilitating the access of digestive enzymes to nutritional components and thus increasing the feed conversion rate [4, 5].

Biotechnology progress allowed the domestic and foreign fodder producers to create a wide assortment of EPs that are available on the market of fodder supplements. As a rule, these EPs comprise enzyme complexes with different types of action; their compositions can vary significantly. Hence, the efficiency of the fodder EPs is defined by the level of their biocatalytic activity, the ratio of components in the composition of enzyme preparations, as well as by the specificity and properties of individual enzymes in the complex.

The objective of this work is to determine component composition of the domestic and foreign commercial fodder EP, investigate the activity of these EP towards various types of NSP, as well as investigate the ability of EP to reduce viscosity of NSP and effect of protein inhibitors from cereal grains on this process.

MATERIALS AND METHODS

Enzyme preparations. Dry EPs from different manufacturers were used: Econase XT 25 (AB Enzymes, Germany); Axtra XAP 101 and Axtra XB 201 (Du Pont, United States); Cellolux F (Sibbiofarm, Russian Federation); Xybeten-Cell, Xybeten-Xyl, and Hostazyme C-100 (Biovet-Ferment, Bulgaria); Cellulase (ALBE, China); Roxazyme G2G (DSM, Switzerland), #2585 Agrocel, #2656 Agrocel Plus, #2679 Agroxyl, #2826 Agroxyl Plus, and #2827 Agroxyl Premium (Agroferment, Russian Federation); Rovabio Max AP (Adisseo, France); Vilzim (Enmex, Mexico; Endofeed (Andres Pintaluba, Spain); Sunzyme (Sunhy, China).

Substrates. Microcrystalline cellulose (MCC/avicel, #123.5 MK-Tsentr, Russian Federation), sodium salt of carboxymethyl cellulose (CMC), birch glucuronoxylan, and barley β-glucan (Megazyme, Ireland) were used as substrates for determination of activities of EPs.

Activity of EP towards MCC (characterizing cellobiohydrolases), CMC (characterizing endoglucanases), barley beta-glucan (beta-glucanases), and birch glucuronoxylan (xylanaes) was determined at concentration of polysaccharide substrates in the reaction mixture of 5 g/L at 50°C and pH 5.0.

Activity of EP has been expressed in international units: 1 unit corresponds to formation of 1 μmol of product per 1 min upon the action of enzymes on the respective substrate. Reducing sugars (RS) were determined as products with the Nelson–Somogyi method [6].

Protein content in EP was determined with the Lowry assay using BSA as a standard [7].

Polyacrylamide gel electrophoresis (PAGE) under denaturing conditions was carried out on a Miniprotean Tetra cell with a Model 300Xi power supply (Bio-Rad Laboratories, United States) according to the manufacturer's manual. Protein in gels was stained with a Coomassie-Brilliant Blue R-250 dye (Ferrak, Germany). A protein mixture #26612 (20–120 kDa) manufactured by Thermo Scientific (United States) was used as a standard.

Qualitative and quantitative composition of EP was determined via analytical fractionation using a high-performance liquid chromatography system AKTA Purifier 100 FPLC System (GE Healthcare, Sweden) with columns and resins from Pharmacia and GE Healthcare (Sweden). An EP aliquot was dissolved in a starting buffer; composition of the starting buffer was different: for the EP based on *Penicillium* (EP #2656 Agrocel Plus, #2826 Agroxyl Plus, and #2827 Agroxyl Premium) – 20 mM Bis-Tris/HCl pH 6.8, for other EPs – 10 mM Bis-Tris/HCl pH 6.0. A low pressure system Econo-System (BioRad, United States) was used for sample preparation as well as for their desalting and buffer exchange. EPs were desalted on a column with acrylex P2 (Reanal, Hungary) in the starting buffer. Next anion exchange chromatography was performed on a column with a Source 15Q resin. Sample was loaded in the respective starting buffer; captured proteins were eluted with a NaCl concentration gradient from 0 to 0.4 M. Avicelase, CMC-ase, beta-glucanase, and xylanase activities were determined in the fractions produced in the course of elution as well as protein content. Quantitative composition of EP was calculated based on the data of the fraction volume, protein concentration in it, enzyme activity, and the data of PAGE. Content of different proteins in the fractions were determined with the help of the GelAnalyzer program.

Identification of enzymes in EPs and chromatographic fractions was performed with the help of mass spectral analysis of the trypsin hydrolyzates of the proteins after electrophoresis followed by the search in the protein database [http://www.matrixscience.com/search_form_select.html].

Preparation of aqueous extracts of rye and barley. Rye or barley was first crashed to a particle size of ≈0.5 mm on a MF 10.1 Basic mill (IKA, Germany). A 0.1 M Na-acetate buffer, pH 5.0 was used for preparation of aqueous extracts at a ratio of 100 mL per 20 g of crashed rye or barley. Extraction was carried out for 1 h at 40°C on an Innova 40 shaker (140 rpm) (New Brunswick Scientific, United States). The produced extracts were centrifuged for 5 min at 5000 rpm on a Universal 320R centrifuge (Hettich, Switzerland). Supernatants were filtered through a polyester cloth producing "native" rye or barley extracts (stored on ice). Half of each "native" extract was incubated in a boiling water bath for 5–7 min followed by centrifugation for 5 min at 5000 rpm. Supernatants represented "heat-treated" extracts of rye or barley with inactivated protein inhibitors (of xylanases), these extracts were stored on ice.

Investigation of the effect of EP on viscosity of aqueous extracts of rye or barley was carried out with the help of a capillary Ostwald viscometer VPZh-4 (capillary diameter 0.62 mm) placed in a water bath according to the following protocol. Na-acetate buffer (1 ml, 0.1 M, pH 5.0) was added to 4 ml of the extract, the solution was mixed by bubbling air with the help of a rubber blower and incubated at 40°C for 5 min. Next an aliquot (50 μL) of an EP solution in 0.1 M Na-acetate buffer, pH 5.0, containing 1 unit of xylanase or beta-glucanase activity was added followed by measuring at intervals time of the extract flowing through the viscometer (at 40°C). Reaction time was calculated from the moment of addition of EP solution to the extract. The results were presented as a decrease of adjusted viscosity of the diluted extract after 20 min of enzymatic reaction.

$$\text{Decrease } \eta_{\text{пр}}(e) = \left(1 - \eta_{\text{пр}}(e) \Big/ \eta^0_{\text{пр}}(e)\right) \times 100\%,$$

where $\eta_{\text{пр}}(e) \Big/ \eta^0_{\text{пр}}(e)$ – ratio of adjusted viscosity of an extract after addition of EP to adjusted viscosity of initial extract.

Time of enzymatic reaction was calculated as a sum of the time before the start of measuring of the solution flow time and half of the measured flow time.

The buffer flow time was ≈65 s, flow times of the diluted "native" and "heat-inactivated" rye extracts were ≈330 and ≈340 s, respectively, flow time of the 'native" and heat-inactivated" barley extracts were ≈79 and ≈77 s, respectively.

Presence and degree of inhibition of EP by protein inhibitors was determined by comparing the decrease of viscosity of the "native" and "heat-inactivated" extracts under the action of EP. The method is described in detail in [8, 9].

RESULTS AND DISCUSSION

Comparative Analysis of Composition and Activities of Enzyme Preparations

In this work we analyzed the properties and composition of a wide variety of commercial EP used for improving feed conversion for poultry and swine. Characteristics of the investigated EP (protein content, specific activities towards different substrates, as well as content of the main enzymes in their composition) are presented in Table 1.

The EPs were characterized with protein content varying from 53 to 360 mg per 1 g of the preparation (non-protein components of EP contain inert fillers, stabilizers, and other components usually represented by various inorganic salts, poly- or oligosaccharides, wheat bran, and others. The specific activities of EP expressed in units per 1 mg of protein are presented in Table 1; it is our opinion that exactly this method of activity representation allows the for most correct comparison of different EPs as the absolute levels of activities (i.e. units/g of EP) vary significantly. The specific avicelase activity in the investigated EPs varied from 0.02 to 0.35 units/mg, CMC-ase – from 0.7 to 23 units/mg, beta-glucanase – from 0.6 to 28.7 units/mg, and xylanase – from 1 to 53.3 units/mg.

The content of individual enzymes in the EP (in percent of total protein content) was determined with the help of chromatographic techniques (see Materials and Methods section for detailed description). Cellobiohydrolases, endoglucanases, and xylanases were the major components of all the investigated EP, but the ratio of these enzymes in different EP varied noticeably. The enzymes that could be classified as beta-glucanases were not found in the investigated EP as individual enzymes; the activity of EPs towards beta-glucan was mediated by endoglucanases that were capable of hydrolyzing both beta-glucan and CMC thus performing beta-glucanase and CMC-ase activities of the EPs [10].

From our point of view the investigated EPs can be divided into three groups based on the component composition of the enzyme complex and the levels of various activities. The preparations with high content of xylanases and, hence, exhibiting clearly pronounced xylanase activity – Econase XT 25 and Axtra XAP 101 – belong to the first group. In particular, the EP Econase XT 25 contained 52% xylanases of the total protein content, furthermore, 25% of cellobiohydrolases and only 1% of endoglucanasaes was in its composition. The EP Axtra XAP 101 contained 28% xylanses, while no enzymes with cellulolytic function were found in its composition. High level of specific xylanase activity and extremely low levels of CMC-ase and beta-glucanase activities (characteristic for endoglucanases) and specific avicelase activity (characteristic for cellobiohydrolases) corresponded to the component composition of the abovementioned EPs.

The EPs with high total content of cellulases (cellobiohydrolases and endoglucanases) and low level of xylanases belong to the second group. Cellolux F, Xybeten-Cell, Cellulase, Hostazyme C100, and Agrocel Plus are in this group. The total content of cellobiohydrolases and endoglucanases in these EP varied from 61 to 92%, while the content of xylanases was only 1–6%. The distinctive feature of the Cellulase and Agrocel Plus EPs was the content of endoglucanases, which was 1.8–3-fold higher than the content of cellobiohydrolases in these preparations, while the other EP in this group were characterized with largest content of cellobiohydrolases. This component composition of EPs corresponded to the high level of CMC-ase, beta-glucanase, and avicelase specific activities, and very low level specific xylanase activity. Note that the EP Cellolux F contained maximal amount of cellobiohydrolase among the investigated preparations (72%) and exhibited maximal specific avicelase activity. The highest content of endoglucanases between the all analyzed EP was detected in the Agrocel Plus preparation (78%), which correlated well with the maximal specific CMC-ase activity and second best specific beta-glucanase activity.

The third most numerous group consists of EPs containing in their composition cellobiohydrolases, endoglucanases, and xylanases at various ratios, but without significant prevalence of one of the enzymes. Roxazyme G2G, Agroxyl, Argoxyl Plus, Agroxyl premium, Agrocel, Axtra XB 201, Xybeten-Xyl, Rovabio Max AP, Vilzim, Endofeed, and Sunzyme are in this group. The total cellulase content (cellobiohydrolases and endoglucanases) varied from 39% (Agroxyl Premium) to 82% (Sunzyme). Noticeable content of cellobiohydrolases (more than 50%) was detected in the EPs Roxazyme G2G, Agrocel, and Agroxyl; Rovabio Max AP and Sunzyme were characterized with high endoglucanase content (above 30%); Agroxyl Plus, Agroxyl Premium, and Vilzim had relatively high content of xylanases (above 20%). The value of specific avicelase activity in general corresponded to the level of cellobiohydrolase content, and the value of specific xylanase – to the level of xylanse content (with Sunzyme with low xylanase content but high xylanase activity being an exception, which likely could be

Table 1. Specific activity of enzyme preparations towards various substrates (units/mg), protein content and component composition (% of total protein content)

Group	Preparation	Protein content, mg/g	Specific activity, units/mg				Content, %			Inhibition of xylanases by protein inhibitors
			avicelase	CMC-ase	β-Glucanase	xylanase	cellobio-hydrolases	endoglucanases	xylanases	
Xylanases	Econase XT 25	95 ± 4	0.09 ± 0.01	0.7 ± 0.3	0.6 ± 0.02	**22.1 ± 0.9**	25 ± 2	1 ± 0.1	**52** ± 4	No
	Axtra XAP 101	100 ± 4	<0.01	<0.1	<0.1	**12.8 ± 0.5**	0	0	**28** ± 2	Yes
Cellulases	Cellolux F	270 ± 10	**0.35 ± 0.01**	13.0 ± 0.5	12.3 ± 0.5	5.3 ± 0.2	**72** ± 5	17 ± 1	6 ± 0.4	No
	Xybeten-Cell	350 ± 14	**0.23 ± 0.01**	11.4 ± 0.5	8.9 ± 0.4	1.7 ± 0.1	**71** ± 5	13 ± 1	1 ± 0.1	Yes
	Cellulase	360 ± 14	**0.26 ± 0.01**	**16.3 ± 0.7**	**20.8 ± 0.8**	1.4 ± 0.1	27 ± 2	**49** ± 3	2 ± 0.1	No
	Hostazyme C100	100 ± 4	0.17 ± 0.01	5.1 ± 0.2	3.7 ± 0.2	1.0 ± 0.04	**46** ± 3	15 ± 1	2 ± 0.1	No
	Agrocel Plus	100 ± 4	0.15 ± 0.01	**23.0 ± 0.9**	**21.0 ± 0.8**	4.5 ± 0.2	14 ± 1	**78** ± 6	3 ± 0.2	No
Cellulases + xylanases	Roxazyme G2G	53 ± 2	**0.29 ± 0.01**	6.6 ± 0.3	6.0 ± 0.2	13.6 ± 0.5	**55** ± 4	21 ± 2	18 ± 1	Yes
	Agroxyl	130 ± 5	**0.27 ± 0.01**	6.3 ± 0.3	5.2 ± 0.2	23.1 ± 0.9	**53** ± 4	17 ± 1	18 ± 1	Yes
	Agroxyl Plus	175 ± 7	**0.21 ± 0.01**	6.5 ± 0.3	7.6 ± 0.3	**23.4** ± 1	**41** ± 3	3 ± 0.2	**31** ± 2	No
	Agroxyl Premium	125 ± 5	**0.20 ± 0.01**	**12.2 ± 0.5**	13.8 ± 0.6	**21.6** ± 0.8	23 ± 2	16 ± 1	**47** ± 3	No
	Agrocel	90 ± 3	**0.20 ± 0.01**	8.7 ± 0.3	7.6 ± 0.3	6.8 ± 0.3	**60** ± 4	16 ± 1	15 ± 1	Yes
	Axtra XB 201	150 ± 6	**0.20 ± 0.01**	3.4 ± 0.1	3.6 ± 0.1	6.7 ± 0.3	**42** ± 3	19 ± 1	19 ± 1	Yes
	Xybeten-Xyl	180 ± 7	0.20 ± 0.01	8.9 ± 0.4	7.2 ± 0.3	22.2 ± 0.9	41 ± 3	13 ± 1	21 ± 2	Yes
	Rovabio Max AP	232 ± 9	0.17 ± 0.01	6.4 ± 0.3	13.7 ± 0.6	11.7 ± 0.5	27 ± 2	**36** ± 3	11 ± 1	No
	Vilzim	307 ± 12	0.16 ± 0.01	7.1 ± 0.3	12.9 ± 0.5	**35.9** ± 1.4	41 ± 3	29 ± 2	**21** ± 2	Yes
	Endofeed	80 ± 3	0.16 ± 0.01	7.3 ± 0.3	17.5 ± 0.7	12.5 ± 0.5	22 ± 2	27 ± 2	14 ± 1	No
	Sunzyme	77 ± 3	0.12 ± 0.01	**18.8 ± 0.8**	**28.7 ± 1.1**	**53.3** ± 2	40 ± 3	**42** ± 3	8 ± 0.6	No

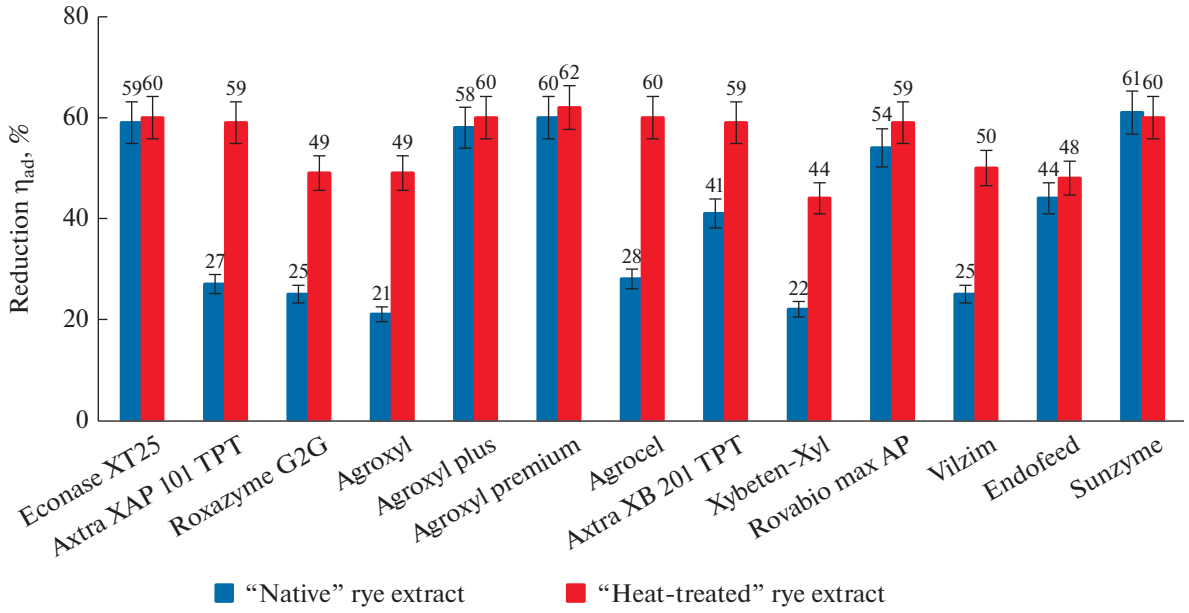

Fig. 1. Decrease of adjusted viscosity of the "native" and "heat-treated" rye extracts under the action of EPs. EP dose corresponds to 1 unit of xylanse activity in the reaction mixture (40°C, pH 5.0, reaction time – 20 min).

explained by high molecular activity of the xylanases in its composition). No clear correlation between the content of endoglucanases and the specific CMC-ase and beta-glucanase activities was observed, which likely could also be explained by the differences in molecular activities of individual endoglucanases in the composition of EPs.

Ability of EPs to Reduce the Viscosity of Aqueous Extracts of Rye and Barley and Inhibition of EPs with Protein Inhibitors from Cereal Grains

The ability of EPs to reduce viscosity of water-soluble NSP (xylans and beta-glucans), which affect negatively on assimilation of nutrients from feed, is an important factor defining the efficiency of biocatalytic action of EPs used as supplements to fodder of farm animals and poultry. It is our opinion that in addition to the activity levels of the EP towards various specific substrates (CMC, xylan, beta-glucan) determined from the rate of RS formation, the ability to reduce viscosity of xylan and betal-glucan is another important characteristic of the EPs used as feed supplements. Hence, we examined the ability of the investigated EPs to reduce viscosity of aqueous extracts of rye (viscosity of which is due to the presence of water-soluble arabinoxylan) and barley (aqueous extracts of which are enriched with beta-glucan) [11].

It must be also taken into consideration that the resistance of xylanases to the action of protein inhibitors (TAXI- and XIP-like inhibitors affecting xylanases, but not cellulases and beta-glucanases) is also an important factor defining the efficiency of xylanase action on the xylans from cereal grains [12]. Hence, the presence or absence of xylanase inhibition by the protein inhibitors was examined for the investigated EPs by estimating the degree of viscosity reduction of the aqueous rye extract before and after heat-treatment of the extract (heat-treatment resulted in denaturation of the rye protein inhibitors, but did not affect the viscosity of the aqueous rye extract [9]).

The diagram is presented in Fig. 1 depicting the decrease of adjusted viscosity of the aqueous rye extract under the action of the investigated EPs, which demonstrates that the higher reduction of the adjusted viscosity corresponds to the higher efficiency of the particular EP toward the cleavage of water-soluble xylans (the experiments on the investigation of the effect of EP on the viscosity of the rye aqueous extracts were conducted with the same dose of EP in the reaction mixture with regards to xylanase activity, see Materials and Methods section). Note that the EPs from the second group (see previous section and Table 1) with low xylanase content (1–6% of total protein content – Xybeten-Cell, Hostazyme C100, Cellulase, Agrocel Plus, and Cellulux F) were excluded from consideration in this section.

Econase XT 25, Agroxyl Plus, Agroxyl Premium, Rovabio Max AP, and Sunzyme reduced the viscosity of water-soluble xylans most efficiently. Notably, xylanases from these particular EPs were not inhibited by the rye protein inhibitors: the difference in the reduction of adjusted viscosity by the "native" and "heat-treated" rye extracts for these EPs did not exceed 5% (see Fig. 1). The xylanase from Endofeed EP was not inhibited by the rye protein inhibitors, but treatment with this preparation caused less significant

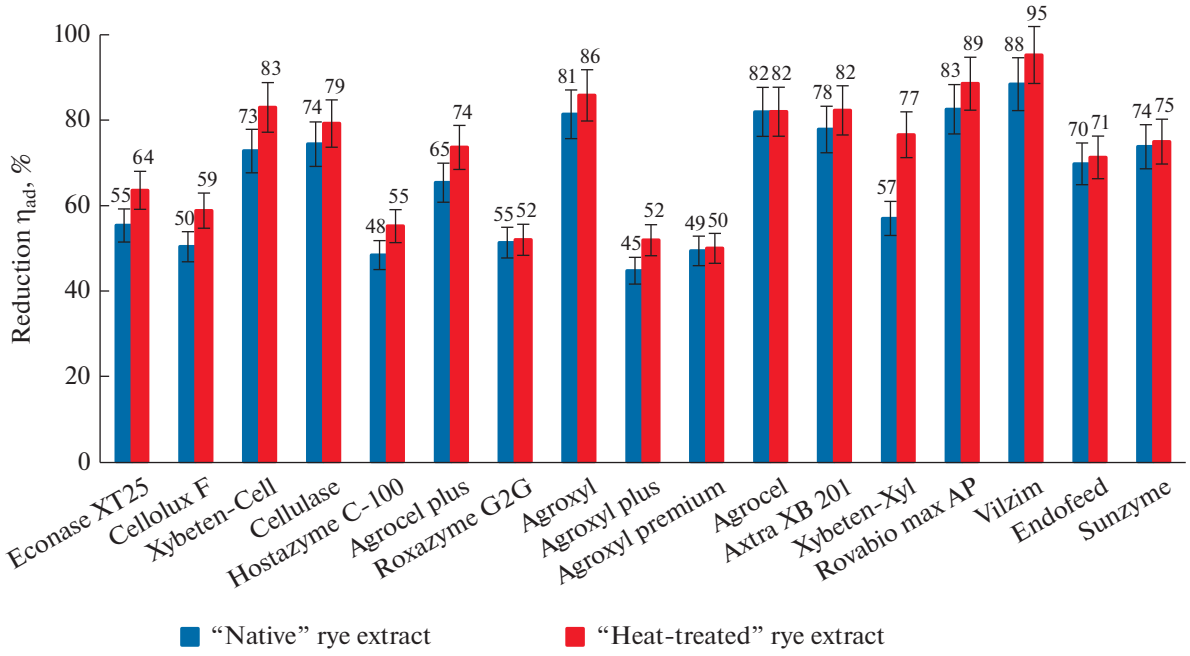

Fig. 2. Decrease of adjusted viscosity of the "native" and "heat-treated" barley extracts under the action of EPs. EP dose corresponds to 1 unit of beta-glucanase activity in the reaction mixture (40°C, pH 5.0, reaction time – 20 min).

reduction of the adjusted viscosity than the above-mentioned preparations. The EPs Axtra XAP 101, Axtra XAP 201, and Agrocel produced significant reduction of the adjusted viscosity of the "native" rye extract, but xylanases of these EPs were inhibited by the rye protein inhibitors, which was manifested by the noticeable difference in the reduction of the adjusted viscosity of the "native" and "heat-treated" rye extracts. And finally, the EPs Roxazyme G2G, Agrosyl, Xybeteb-Xyl, and Vilzim exhibited lower efficiency in the reduction of adjusted viscosity of the "native" rye extract in comparison with the other EPs, and, moreover, were inhibited by the rye protein inhibitors.

The diagram presented in Fig. 2 demonstrates the decrease of adjusted viscosity of the barley aqueous extract under the action of investigated EPs (the experiments on investigation of the effect of EP on viscosity of the barley aqueous extracts were carried out at the same dose of EP with regards to beta-glucanase activity in the reaction mixture). The barley aqueous extracts contain beta-glucans, hence, the degree of viscosity reduction of these extracts under the action of EPs allows estimating the efficiency of these preparations as feed supplements. Note that the EP Axtra XAP 101 was excluded from consideration in this section as the EP without endoglucanases in its composition, and the EP not exhibiting beta-glucanase activity (see Table 1).

Xybeten Cell, Cellulase, Agroxyl, Agrocel, Axtra XB 201, Rovabio Max AP, and Vilzim decreased the viscosity of barley water-soluble beta-glucans most efficiently. Other EPs affected the viscosity of aqueous barley extract to a lesser extent. Note that the reduction of adjusted viscosity of the "native" barley extract was practically the same for all investigated EPs as in the case of "heat-treated" extracts, which indicated the absence of protein inhibitors of beta-glucanases in the extracts.

CONCLUSIONS

Hence, in this work we analyzed composition and properties of the 18 EPs used as supplements in farm animal and poultry fodder. The content of main acting enzymes in the preparations causing destruction of NSP– endoglucanases (beta-glucanases), cellobiohydrolases, and xylanases – was determined. The investigated EPs can be classified into three groups based on the data on component composition and the levels of various specific activities: a) preparations with high xylanase content and low cellulase content (endoglucanases and cellobiohydrolases) content, two EPs are in this group; b) preparations with high content of cellulases and low content of xylanases, five EPs are assigned to this group; c) preparations containing in their composition cellobiohydrolases, endoglucanases, and xylanases at different ratios, but without significant prevalence of the content of one of these enzymes, this group includes eleven EPs.

The ability of investigated EPs to reduce viscosity of water-soluble NSP (xylans and beta-glucans) that affect negatively assimilation of nutrients from fodder was investigated. Six preparations belonging to the groups "b" and "c" were identified that had xylanses

in their composition and reduced the viscosity of aqueous rye extract containing xylans most efficiently when used at a dose with equal xylanase activity (Econase XT 25, Agroxyl Plus, Agroxyl Premium, Rovabio Max AP, Endofeed, and Sunzyme). It was established that xylanases from precisely these EPs were not inhibited by the cereal protein inhibitors.

Xybeten Cell, Cellulase, Agroxyl, Agrocel, Axtra XB 201, Rovabio Max AP, and Vilzim were demonstrated to be most efficient in reduction of the viscosity of water-soluble barley beta-glucans, when used at the dose containing the same beta-glucanase activity. The barley extract did not inhibit beta-glucanase activity of the EPs investigated in this work.

The data characterizing activity and composition of EPs in combination with the data on ability for destruction (viscosity reduction) of aqueous rye extracts facilitate the selection of the EP for practical application as a fodder supplement.

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