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professional paper

Multienzymatic Preparation for Animal Nutrition

Multienzimski pripravak za hranidbu životinja

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Summary

Hydrolysis of food components in animal intestinal tracts may be advanced by adding suitable microbial enzymes. Besides usual food hydrolysis, the addition of microbial enzymes showed also other improvements: degradation of the indigestible parts of meal (e.g. use of less valuable feed), better organoleptic food characteristics, favorable influence on fecal consistency and ecological conditions in inhabitancies.

The efficacy of the multienzymatic preparation added to feed was investigated in chicks fattening during 50 days and in fattening of pigs from 9.5 kg until they reached 25 kg weight. The chicks, that consumed multienzymatic preparation showed the weight gain increase in the range of 0.75–5.33 % and improved feed conversion between 1.92 and 8.34 %. In comparison with control group without enzyme addition, 1.16 % lower weight gain was detected after 28 days of pigs nutrition. However, the feed consumption decreased by 9.83 % and feed conversion increased by 8.46 %.

The presented results confirmed the economic justifiability of meat production using the multienzymatic preparation in feed.

Sažetak

Hidroliza sastojaka hrane u probavnom traktu životinja može se poboljšati dodavanjem odgovarajućih enzima iz mikroorganizama. Dodatkom tih enzima, uz uobičajenu hidrolizu hrane, primijećena su i druga poboljšanja, kao što su: razgradnja neprobavljivih dijelova obroka (tj. primjena manje vrijednih krmiva), bolja organoleptička svojstva hrane, povoljan utjecaj na konzistenciju fekalija te ekološki uvjeti u nastambama.

Djelotvornost dodanog multienzimskog pripravka za hranidbu životinja istražena je u tovu pilića tijekom 50 dana, kao i u tovu prasadi od 9,5 kg do postignute mase od 25 kg. Pokusne skupine pilića, kojima je u hranu umiješan multienzimski pripravak, u usporedbi s kontrolnom skupinom (bez dodatka enzima) pokazale su tjelesni prirast mase od 0,75 do 5,33 % i povoljniju konverziju hrane 1,92–8,34 %. Uspoređujući rezultate dobivene za pokusnu skupinu prasadi s rezultatima kontrolne skupine (kojoj nisu dodani enzimi) vidi se da je prirast tjelesne mase prasadi nakon 28 dana bio 1,16 % niži, utrošak se hrane smanjio za 9,83 %, a konverzija hrane povećala za 8,46 %.

Postignuti rezultati potvrdili su ekonomsku opravdanost primjene multienzimskog pripravka u proizvodnji mesa.

Introduction

Industrial feed production requires at the same time as high as possible profitability and the production of »healthy meat«. Limited sources of proteins and energy stimulate the use of different untraditional feedstuffs with lower nutritive value. Portions based on such feedstuffs should be enriched with additives that improve digestibility. The feed additives may be nutritive and medical. The enzymes, which are nutritive additives can be mixed into the feedstuffs to improve: animal digestive capacity, the degradation of specific polysaccharides, plant cell membranes, and proteins. They also degrade so-called antinutritive factors and nutritive stress factors. The enzymes are natural catalysts and their use in feed has no negative influence on ecology and they could be considered as »healthy food«.

The feedstuffs usually contain maize, barley, wheat, oat, soya- and sunflower grits. Salts, fat, aminoacids, vitamins, and minerals are added in lower quantities. The

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main constituents of feed are carbohydrates, while proteins, fats and other compounds are in lower concentrations. The most frequent carbohydrate in feed is starch, a polysaccharide in which glucose units are connected by α-glucosidic linkages. Starch is degraded with the enzymes called amylases. The important group of polysaccharides also are β-glucans, the constituents of plant stems and grains providing the rigidity of the cells. The most spreaded β-glucan in nature is cellulose in which glucose units are joined by β-1,4-glucosidic linkages. Enzymes which can degrade this polysaccharides are cellulases. Linear polysaccharide in which β-D-glucopyranosidic units are joined by β-1,3- and β-1,4-glucosidic linkages in ratio 1:2 is very interesting from nutritional point of wiev. This polysaccharide is present in the endosperm cell wall of barley, wheat and oat grains. The barley endosperm cell wall contains 75 % of β-glucan or 2.8 – 5.5 % of total grain weight. Although this content of β-glucan in grains is rather low, it causes more undesirable effects in animal feeding. Namely, β-glucan increases the viscosity of the content of animal intestinal tract, restricting the mixing with intestinal enzymes, that results with speeding up of the food passage. This is the reason for lower appearance of simple sugars and aminoacids that are necessary for biosynthesis of the building compounds in organism. Consequently, β-glucan in the feedstuffs gives rise to lower production results and increased fecal moisture. Undesirable effects of β-1,3;1,4—glucan could be overcome by its hydrolysis with the enzyme β-glucanase *(1)*.

The research into addition of microbial enzymes to feedstuffs started more than 30 years ago. The intestinal enzymes differ from commercial microbial enzymes, prepared in the fermentation processes, in molecular weight, amino acid sequences and in optimal activity conditions (pH, temperature, substrate concentration) *(2)*. However, these differences have no influence on their elementary role in food components degradation.

The microbial enzymes have the main use in nutrition of young animals in that the digestive enzymes are insufficient for optimal food utility. In adult animals, the addition of selected microbial enzymes can also increase digestive efficacy in degradation of indigestible or insufficiently digestible food contents.

The efficiency of microbial enzyme addition to cereal—based mixtures in broiler fattening has been described by several authors *(3-5)*. Burnett *(6)* investigated the effect of β-glucan on the viscosity of the intestinal content in poultry and he concluded that high viscosity and shortage of β-glucanase in fact hindered the degradation of carbohydrates and proteins. This was also confirmed by several other authors *(7-9)*. Matošić-Čajavec and co-authors *(10)* reported an improved weight gain and feed conversion when β-glucanase was added in broiler fattening based on barley. Also, better production was achieved when feed mixtures contained combined acid proteinase, nutritive antibiotic and β-glucanase *(11)*.

Alkaline proteinase from *Bacillus licheniformis* and acidic proteinase from *Aspergillus awamori* showed significant improvement in pig productivity during first 10 days after wean. In addition, preliminary experiments showed that using alkaline proteinase either alone or in combination with β-glucanase both had stimulative effects in continued pig fattening *(12)*.

Based on wide research, the procedure for multienzymatic preparation production containing α-amylase, β-glucanase and alkaline proteinase with marked xylanase and cellulase activity for animal feeding was developed. In this report the effect of multienzymatic preparation on the production in broiler fattening during 50 days and pigs nutrition from 9.5 kg until 25 kg weight was evaluated.

Materials and Methods

The bioactive components of multienzymatic preparation for animal fattening were produced by PLIVA using selected strains of *Aspergillus niger* and *Bacillus* sp. The preparation contained enzymes: α-amylase 10 × units/g, β-glucanase 10 U/g and alkaline proteinase 40,000 DU/g with accompanying xylanase and cellulase activity *(13–15)*.

The efficiency of multienzymatic preparation in chicks fattening was estimated in 4 trials *(16)*. The trials 1 and 2 were carried out in the cages with 598 chicks. The ingredients and nutritional values of the starter mixture (0–30 days of fattening) and of the finisher mixture (30–50 days of fattening) (Table 1), were the same in both trials and they differed only in relation to added enzymatic preparation (0.1 %). The trials 3 and 4 were carried out on deeply spread floor with 612 chicks. The ingredients and nutritional values for starter and finisher mixtures

Table 1. Ingredients and nutritional values of starter and finisher mixtures in chicks fattening in trials 1 and 2
Tablica 1. Sastav i hranidbena vrijednost smjesa na početku i kraju tova pilića u pokusima 1 i 2

Ingredients	Starter mixture *w*/%		Finisher mixture w/%	
	Control	Trial	Control	Trial
Multienzymatic preparation	–	0.10	–	0.10
Maize	32.40	32.30	32.90	32.80
Barley	24.00	24.00	24.00	24.00
Soy-bean meal	30.00	30.00	27.50	27.50
Feed yeast	5.00	5.00	5.00	5.00
Fat animal	5.00	5.00	5.00	5.00
Alfalfa meal dehyd.	–	–	2.00	2.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Limestone	1.20	1.20	1.20	1.20
Premix PT	0.50	0.50	–	–
VAM PT	–	–	0.50	0.50
Salt	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10
	100.00 %			
Nutritional values				
Crude protein	21.38	21.38	20.57	20.57
Crude fat	7.25	7.25	7.29	7.29
Crude fibers	3.95	3.95	4.01	4.01
Ash	5.69	5.69	5.81	5.81
Methionine	0.41	0.41	0.40	0.40
Lysine	1.17	1.17	1.11	1.11
Calcium	0.87	0.87	0.94	0.94
Total phosphorous	0.68	0.68	0.62	0.62
ME / (MJ/kg)	12.18	12.18	12.07	12.07

are presented in Table 2. All the trials were performed during 50 days. Food and water were given *ad libitum* during the whole period of time. The portions were prepared in an appropriate mixing room.

The stimulative effect of the multienzymatic preparation in feed mixtures was also investigated in fattening of 54 pigs whose average weight was 9.5 kg during 28 days or until 25 kg body weight *(17)*. Pigs were fed with a standard starter feed mixture all the time during the trial; multienzymatic preparation was added in the concentration of 0.1 %. The ingredients and nutritional values of used starter feed mixtures are presented in Table 3.

Table 2. Ingredients and nutritional values of starter and finisher mixtures in chicks fattening in trials 3 and 4
Tablica 2. Sastav i hranidbena vrijednost smjesa na početku i kraju tova pilića u pokusima 3 i 4

Ingredients	Starter mixture *w*/%		Finisher mixture *w*/%	
	Control	Trial	Control	Trial
Multienzymatic preparation	–	0.10	–	0.10
Maize	18.85	18.75	21.85	21.75
Wheat	20.00	20.00	20.00	20.00
Barley	20.00	20.00	20.00	20.00
Soy-bean meal	20.00	20.00	18.00	18.00
Feed yeast	10.00	10.00	9.00	9.00
Fat animal	5.00	5.00	5.00	5.00
Alfalfa meal dehyd.	2.50	2.50	2.50	2.50
Dicalcium phosphate	1.50	1.50	1.50	1.50
Limestone	1.20	1.20	1.20	1.20
Premix PT	0.50	0.50	–	–
VAM PT	–	–	0.50	0.50
Salt	0.30	0.30	0.30	0.30
Methionine	0.15	0.15	0.15	0.15
	100.00 %			
Nutritional values				
Crude protein	20.20	20.20	19.06	19.06
Crude fat	7.03	7.03	7.10	7.10
Crude fibers	3.95	3.95	3.69	3.69
Ash	5.75	5.75	5.60	5.60
Methionine	0.43	0.43	0.42	0.42
Lysine	1.04	1.04	0.97	0.97
Calcium	0.90	0.90	0.89	0.89
Total phosphorous	0.71	0.71	0.69	0.69
ME / (MJ/kg)	11.96	11.96	12.09	12.09

Results and Discussion

The addition of different enzymatic preparations containing β-glucanase into mixtures for chicks fattening based on barley, oat and rye showed improved weight gain in the range between 4.4 and 69.1 % and better feed conversion from 1.9–59.4 % *(7)*. A wide-range effectiveness of added enzymes is a result of different contents of β-glucan polysaccharide complex in individual cereal varieties. Microbial β-glucanase is commonly more efficient when added to the various mixtures with higher β-glucan content. Which content of barley or rye will be used in preparation of poultry mixtures depends on the cost of individual cereals and also on capability of the enzymes to degrade their specific carbohydrates *(18)*.

The results of chicks fattening during 50 days for all 4 trials are presented in Table 4. The weight gain in trials

Table 3. Ingredients and nutritional values of starter mixes in pigs fattening
Tablica 3. Sastav i hranidbena vrijednost početne smjese u tovu prasadi

Ingredients	Starter mixture *w*/%	
	Control	Trial
Multienzymatic preparation	–	0.10
Maize	46.05	45.95
Barley	22.00	22.00
Soy-bean meal	19.00	19.00
Feed yeast	6.67	6.67
Alfalfa meal dehyd.	1.50	1.50
Fat animal	1.20	1.20
Dicalcium phosphate	1.00	1.00
Limestone	1.15	1.15
Salt	0.30	0.30
VAM-SP without antibiotics	1.00	1.00
DL-Methionine	0.13	0.13
	100.00 %	
Nutritional values		
Crude protein	18.59	18.59
Crude fat	7.44	7.44
Crude fibers	3.48	3.48
Methionine	0.43	0.43
Lysine	1.03	1.03
Calcium	0.76	0.76
Total phosphorous	0.64	0.64
ME / (MJ/kg)	13.28	13.28

Table 4. Results of chicks fattening during 50 days
Tablica 4. Rezultati tova pilića tijekom 50 dana

Trials	1		2		3		4		Improvements in relation to control groups Index/%
Groups	Control	Trial	Control	Trial	Control	Trial	Control	Trial	
w (Multienzymatic preparation)/%	–	0.1	–	0.1	–	0.1	–	0.1	
Number of chicks-start	95	100	105	104	102	102	102	102	
Number of chicks-finish	93	91	99	99	91	97	101	96	
Total average weight gain/g	2095	2139	2137	2153	2030	2104	1877	1977*	
Index/%	100.00	102.10	100.00	100.75	100.00	103.74	100.00	105.33	2.98
Total feed consumption/g	4548	4477	4789	4422	4843	4779	4405	4551	
Index/%	100.00	98.44	100.00	92.83	100.00	98.68	100.00	104.45	1.92
Feed conversion	2.17	2.09	2.24	2.05	2.39	2.27	2.35	2.30	
Index/%	100.00	96.41	100.00	91.66	100.00	95.10	100.00	98.08	4.69

*P<0.01

1-4, when the chicks were fattened using the multienzymatic preparation, was between 0.75 and 5.33 % (average 2.98 %) higher than in controls. At the same time, the average feed consumption was 1.92 % lower than in controls. As it can be also seen from Table 4, feed conversion was 1.92–8.34 % (average 4.69 %) better than in the control without enzyme addition. These favorable effects of enzyme addition in chicks fattening are in agreement with previously published results *(3,6,7)*.

The results presented in Table 5 also confirmed the assumption about the effect of multienzymatic preparation in pig fattening. Although the addition of 0.1 % of multienzymatic preparation to feed during 28 days caused 1.16 % lower weight gain on the average, daily, that is statistically negligible. However, the feed consumption was 9.83 % lower, hence feed conversion raised 8.46 %. These results are in agreement with numerous earlier published reports *(12)*.

Table 5. Results of pigs fattening during 28 days
Tablica 5. Rezultati tova prasadi tijekom 28 dana

Group	Control	Trial
w (Multienzymatic preparation)/%	–	0.10
Average daily weight gain/g	516	510
Index/%	100.00	98.84
Average daily feed consumption/g	1038	936
Index/%	100.00	90.17
Feed conversion	2.01	1.84
Index/%	100.00	91.54

Presented results and those reported earlier suggest possible use of hydrolytic enzymes (β-glucanase, α-amylase, proteinase) to increase the utilization of nutritional components and to enable a higher economy of meat production. Furthermore, the use of enzymes facilitates higher flexibility in formulation of feed mixtures (possible use of unconventional feed) that could be very important in economizing the production.

Conclusion

The addition of 0.1 % of the multienzymatic preparation (β-glucanase, α-amylase, proteinase, xylanase) to the feed mixtures for chicks and pigs fattening resulted in higher weight gain, lower feed consumption and more favorable feed conversion in comparison with control feed mixtures without enzyme addition. Higher utilization of nutritional components provides more flexibility in feed mixtures formulation. In addition, this makes possible the use of cheaper raw materials in meat production.

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Erratum

Effects of Water Activity on Biooksidation Kinetics,
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Page 11, Fig. 3
The destination of the abscissa on Fig. 3.b
(γ_S, γ_{WO}) / (g/L)
Shoud be replaced by
(γ_{WT}, γ_{WO}) / (g/L)