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## EFFICIENCY OF HYDROLYZED BREWERY YEAST (PROGUT®) IN WEANED PIGLET'S DIET

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### SUMMARY

**Two groups of weaned piglets (N=46), crossbreed (SLxLW) x Pietrain, the same sex proportions, aged 28 days were involved in the investigation. Piglets from all groups were fed mixture for weaned piglets with 22% of crude protein and 13.84 MJ ME/kg until 21<sup>st</sup> day of the trial and with 19% of crude protein and 13.74 MJ ME/kg until 35<sup>th</sup> day of the experiment. The experimental group was added 0.2% Progut® in the feed mixture, during the whole experimental period. Body weight, haematological and some biochemical parameters were determined on the 7<sup>th</sup>, 21<sup>st</sup> and 35<sup>th</sup> day of the trial. No significant differences were determined in average body mass and biochemical parameters (glucose, creatinine, triglycerides, total protein, albumin, globulin, haptoglobin, C-reactive protein) concerned. Total leukocytes count was higher ( $P>0.05$ ) in the experimental group (E) 21<sup>st</sup> and 35<sup>th</sup> day, and significantly higher ( $P<0.01$ ) share of lymphocytes. We found positive and strong correlation between total protein and globulin ( $r=0.86$ ) in the experimental group, and strong positively correlation ( $r=0.84$ ) between total protein and albumin in the control group. Significantly ( $P<0.05$ ) strong negative correlation between triglyceride and total protein concentration ( $r=-0.83$ ), and triglyceride and globulin concentration ( $r=-0.90$ ) were determined in the experimental group of piglets. Dietary addition of hydrolyzed brewery yeast simulated cellular and humoral immune response.**

**Key-words: yeast, immunomodulation, performance, pig, hematological parameters, biochemical parameters**

### INTRODUCTION

Animal feed additives should have nutritive and health effects in order to obtain the final product, the animal product suitable for human food. In the recent years the most important task has been to omit antibiotics from food and to modulate mucosal immune response in order to preserve the animal health. Weaning is a particular challenge in modern pig production. This physiological event which occurs early (21<sup>st</sup> -28<sup>th</sup> day of life) is the trigger for the occurrence of diarrhea that slows down the animal growth and may lead to death. Gastrointestinal disorders are the result of intense changes in the structure and function of the intestinal mucosa depending on the microflora development and the ability of immune response (Bailey et al., 2005). Changes in diet composition of weaned piglets influence the composition of microflora lining the gastrointestinal

tract and their metabolic activity, which can have a desirable effect on the piglet health status.

In the recent years, alternative feed additives have been explored and can be used to inhibit pathogenic bacteria growth without the use of antibiotics. Also there are numerous efforts for rising the immune response. Mannan-oligosaccharides (MOS) are non-digestible carbohydrates extracted from the cell membrane of yeast *Saccharomyces cerevisiae*, well-known yeast in the bakery and brewery industries and its derivative is used exclusively as MOS product in animal nutrition. The basic mode of action is to bind to the cell mem-

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brane proteins of some bacterial species and strains, thus preventing intestinal colonization mechanisms of binding pathogens to the intestinal mucosa epithelial cells (Spring et al., 2000). Nowadays it is known that dietary MOS had an indirect and direct effect on the immune system (Halas and Nochta, 2012). Indirect action is via the inhibition attachment of pathogens and MOS directly enhances lymphocytes proliferation (Davis et al., 2004a), supports establishment of a mature T cells in the gastrointestinal tract (Davis et al., 2004b), increases CD4 and CD8 T cells subpopulation (Šperanda et al., 2008), activates alveolar macrophages to secrete TNF- $\alpha$  and increases IL-10 concentration (Che et al., 2012). Addition of MOS also counts during the virus infections (Franklin et al., 2005) as well as other immune challenge (Halas and Nochta, 2012). Additional methods of treatment of long polysaccharide chains in MOS increases their activity. Therefore we used the formulation of hydrolyzed yeast that is considered together with the cell wall and contains mannan, beta glucan, nucleotides and proteins (Progut®).

The aim of our study was to determine the effect of Progut® formulation on growth, hematological and some biochemical parameters in the blood and the correlation of these parameters in weaned piglets.

## MATERIAL AND METHODS

The experiment involved two groups of piglets ( $n = 46$ ), the commercial hybrids (Swedish landrace X Large Yorkshire x Pietrain), originating from seven litters and three boars, weaned the 28<sup>th</sup> day of life. Piglets were placed in two sections at a temperature of  $21 \pm 2^\circ\text{C}$ , and were fed to 21<sup>st</sup> day of the experiment a commercial mixture containing 22% crude protein and 13.84 MJ ME/kg and to 63<sup>rd</sup> day of life with a commercial mixture containing 19% crude protein and 13.74 MJ ME/kg. The commercial mixture was mixed with 0.2% hydrolyzed brewery yeast (Progut®, Suemen Rehu, Finland) in the experimental group of piglets. Body weight was measured and blood samples for hematological and biochemical tests were taken on the 7<sup>th</sup>, 21<sup>st</sup> and 35<sup>th</sup> day of the experiment. A volume of 5 ml blood with anticoagulant (EDTA) for hematology and 5 ml for biochemistry was taken from the *v. cavae cranialis* using Venoject® vacutainer. Number of blood cells (WBC, RBC), hemoglobin concentration and hematocrit were determined by automatic counter Serono Backer 9120. Blood smears were made and stained according to the Pappenheim method. After staining, blood smears were microscoped and examined to determine the proportion of neutrophils and lymphocytes. For biochemistry blood was centrifuged for 10 minutes at 3000 rpm. Concentration of glucose, creatinine, triglycerides, total protein, albumin, globulin and acute phase proteins - haptoglobin and C-reactive protein was determined by automatic analyzer Olympus AU 640. The obtained values of the experimental and control piglet groups were processed with GLM procedure for repeated measures of the statistical software

SAS (Statistical Analysis System) version 9.3 (SAS Institute Inc. 2011). Differences between groups were tested with LSD *post hoc* test. The Spearman coefficients of correlations of the observed measurement on the 35<sup>th</sup> day of the experiment are shown.

## RESULTS AND DISCUSSION

Body weight of weaned piglets fed the yeast hydrolysates additives was not different during the observed period (Figure 1).

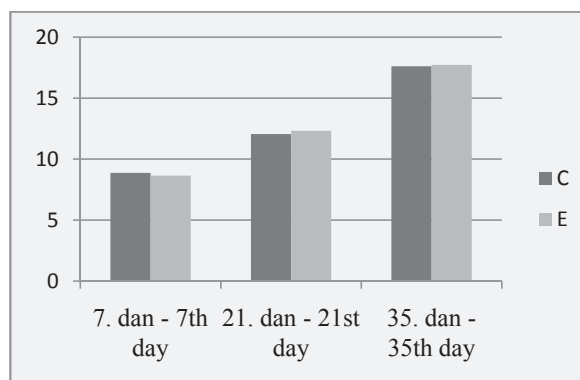


Fig 1. Body weight of weaned piglets fed with Progut® supplementation during 35 days of the trial

Grafikon 1. Tjelesna masa odbite prasadi hranjene uz dodatak pripravka Progut® tijekom 35 dana pokusa

Feed conversion during three weeks was better in the experimental group of piglets (Figure 2), and slightly better at the end of the experiment in the control group of piglets.

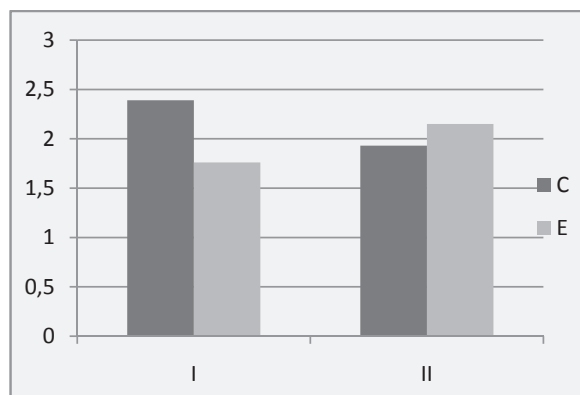


Fig 2. Feed conversion ratio of weaned piglets fed with Progut® supplementation, I-first nutrition period until 21<sup>st</sup> day, II-second nutrition period 21<sup>st</sup>-35<sup>th</sup> day of the trial

Grafikon 2. Konverzija hrane u odbite prasadi tijekom prvih 21 dan (I) i od 21. do 35. dana pokusa (II)

The data are partially consistent with Miguel et al. (2004) which showed that the slow growing piglets during the first two weeks after weaning respond better to addition of mannanoligosaccharides than piglets growing normally or quickly. Hiss and Sauerwein (2003)

did not find difference in average daily gain during 4 week  $\beta$ -glucan addition. Moreover, the authors agree that MOS are most effective when given immediately after weaning. The results were better under the poor condition, even during infectious challenge (White et al., 2002, Miguel et al., 2004, Xiao et al., 2004). Le Mieux et al. (2010) found improved daily gain and feed efficiency in weaning pigs, but Li et al. (2006) emphasized the

importance of dose and found out that the best results are with the addition of 50 mg/kg beta glucan. The effects of the MOS food addition vary. While some have reported positive results (Davis et al., 2002, Davis et al., 2004b, Peet-Schwering et al., 2007, Vaahtovuori et al., 2007), others have reported that the positive production effect was absent (White et al., 2002).

**Table 1. Hematological indicators of weaned piglets fed with Progut® supplementation during 35 days of the trial**

*Tablica 1. Hematološki pokazatelji odbite prasadi hranjene uz dodatak pripravka Progut® tijekom 35 dana pokusa*

Day - Dan	7 <sup>th</sup> day - 7. dan		21 <sup>st</sup> day - 21. dan		35 <sup>th</sup> day - 35. dan	
	C Mean $\pm$ sd	E Mean $\pm$ sd	C Mean $\pm$ sd	E Mean $\pm$ sd	C Mean $\pm$ sd	E Mean $\pm$ sd
Leukocytes, $10^9 L^{-1}$	21.94 $\pm$ 9.16	22.07 $\pm$ 3.95	25.08 $\pm$ 4.74	26.13 $\pm$ 7.6	25.21 $\pm$ 7.94	26.91 $\pm$ 3.49
Neutrophils, %	55.86 $\pm$ 3.67	45.14 $\pm$ 15.86	22.83 $\pm$ 3.66	40.57* $\pm$ 11.8	48.86 $\pm$ 11.16	45.29 $\pm$ 9.55
Lymphocytes, %	52.29 $\pm$ 8.38	53.57 $\pm$ 1.81	60.14 $\pm$ 1.35	74.86** $\pm$ 1.21	58.14 $\pm$ 7.86	73.00** $\pm$ 4.32
Erythrocytes, $10^{12} L^{-1}$	5.88 $\pm$ 0.56	5.86 $\pm$ 0.46	5.16 $\pm$ 0.6	5.58 $\pm$ 0.66	5.32 $\pm$ 0.96	6.01 $\pm$ 0.45
Hemoglobin, g L <sup>-1</sup>	101.29 $\pm$ 14.53	102.43 $\pm$ 8.16	95.17 $\pm$ 13.14	98.43 $\pm$ 11.18	99.57 $\pm$ 14.27	108.43 $\pm$ 6.27
Hematocrit, L L <sup>-1</sup>	0.32 $\pm$ 0.06	0.31 $\pm$ 0.03	0.29 $\pm$ 0.05	0.3 $\pm$ 0.04	0.3 $\pm$ 0.05	0.33 $\pm$ 0.02

\*P<0.05; \*\*P<0.01

Table 1 shows that the addition of yeast hydrolysates affects some haematological parameters. While the total number of leukocytes did not differ between the groups, the number of lymphocytes was significantly higher (P<0.01) on the 21st and 35th day in the experimental piglets. This is consistent with the research of Davis et al. (2004a), who found a significantly increase of neutrophil and lymphocyte number in the first 14 days of the experiment whereas Nocht et al. (2009) found increased the lymphocyte proliferation only after low dose MOS addition. The opposite effect was noted by Peet-Schwering et al. (2007), who did not record

a change of white blood cell number neither after the addition of yeast culture nor mannanoligosaccharides. Evaluation of biochemical parameters in the use of certain food additives, particularly with a view to modulate health status, is necessary because of the monitoring of the state of growth and weight gain of certain categories of animals. There were no differences in biochemical parameters between groups (Table 2). By calculating the correlation of all the observed features of 35th day of the experiment we found that the addition of yeast hydrolysates affected the immune response in the experimental group.

**Table 2. Some biochemical parameters of weaned piglets fed with Progut® supplementation during 35 days of the trial**

*Tablica 2. Neki biokemijski pokazatelji u serumu odbite prasadi hranjene uz dodatak pripravka Progut® tijekom 35 dana pokusa*

Day - Dan	7 <sup>th</sup> day - 7. dan		21 <sup>st</sup> day - 21. dan		35 <sup>th</sup> day - 35. dan	
	C Mean $\pm$ sd	E Mean $\pm$ sd	C Mean $\pm$ sd	E Mean $\pm$ sd	C Mean $\pm$ sd	E Mean $\pm$ sd
Glucose, mmol L <sup>-1</sup>	5.34 $\pm$ 1.24	6.08 $\pm$ 0.3	5.94 $\pm$ 1.3	5.5 $\pm$ 0.5	6.48 $\pm$ 0.96	6.17 $\pm$ 0.81
Creatinine $\mu$ mol L <sup>-1</sup>	111.86 $\pm$ 17.26	97.14 $\pm$ 16.36	95.86 $\pm$ 6.04	91.29 $\pm$ 8.83	83 $\pm$ 8.02	95.43 $\pm$ 5
Triglycerides, mmolL <sup>-1</sup>	0.65 $\pm$ 0.19	0.41 $\pm$ 0.15	0.63 $\pm$ 0.29	0.56 $\pm$ 0.19	0.54 $\pm$ 0.18	0.6 $\pm$ 0.18
TP, g L <sup>-1</sup>	54.71 $\pm$ 5.96	51.71 $\pm$ 5.74	55.71 $\pm$ 3.86	52.43 $\pm$ 4.16	56.57 $\pm$ 6.02	51.43 $\pm$ 2.57
Albumin, g L-1	27.63 $\pm$ 4	25.57 $\pm$ 2.89	29.47 $\pm$ 3.57	26.87 $\pm$ 2.4	27.15 $\pm$ 5.49	24.98 $\pm$ 1.73
Globulin, g L-1	27.08 $\pm$ 6.35	26.14 $\pm$ 4.14	26.24 $\pm$ 5.08	25.56 $\pm$ 2.42	29.42 $\pm$ 3.27	26.45 $\pm$ 3.2
Haptoglobin, mg L <sup>-1</sup>	0.38 $\pm$ 0.19	0.27 $\pm$ 0.12	0.31 $\pm$ 0.23	0.24 $\pm$ 0.14	0.23 $\pm$ 0.19	0.26 $\pm$ 0.23
CRP, mg L <sup>-1</sup>	2.07 $\pm$ 1.76	2.36 $\pm$ 3.41	2.8 $\pm$ 1.2	1.42 $\pm$ 0.57	2.88 $\pm$ 1.76	2.07 $\pm$ 1.22

TP=total protein, CRP=C-reactive protein

Statistically significant strong correlation between the number of leukocytes and the concentration of C-reactive protein (r = 0.86) was determined in the experimental group while not in the control one (Table 4). The

proportion of neutrophils on the 35<sup>th</sup> day was negatively correlated with the concentration of albumins (r = -0.86) in E group, while in the control group of piglets this relationship is strong, but not significant (r = -0.50, Table 3).

**Table 3. Correlation of production, hematological and some biochemical indicators of weaned piglets in control group on 35<sup>th</sup> day of trial**

Tablica 3. Povezanost proizvodnih svojstava, hematoloških i nekih biokemijskih pokazatelja u odbite prasadi kontrolne skupine 35. dan pokusa

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	-0.78	1.00													
3	-0.39	0.56	1.00												
4	0.44	-0.49	<b>-0.98</b>	1.00											
5	-0.54	0.49	0.43	-0.46	1.00										
6	-0.42	0.45	0.40	-0.40	<b>0.99</b>	1.00									
7	-0.55	0.70	0.60	-0.57	<b>0.93</b>	<b>0.94</b>	1.00								
8	-0.77	<b>0.88</b>	<b>0.82</b>	-0.76	0.53	0.48	0.71	1.00							
9	-0.19	-0.26	-0.03	-0.04	0.49	0.46	0.22	0.04	1.00						
10	<b>0.84</b>	-0.35	-0.15	0.24	-0.36	-0.23	-0.22	-0.49	-0.57	1.00					
11	-0.27	0.40	-0.21	0.29	0.61	0.67	0.61	0.17	0.22	-0.08	1.00				
12	-0.42	0.28	-0.50	0.48	0.45	0.44	0.33	-0.02	0.25	-0.35	<b>0.84</b>	1.00			
13	0.20	0.28	0.44	-0.27	0.38	0.50	0.57	0.35	-0.02	0.44	0.43	-0.13	1.00		
14	-0.48	0.50	-0.01	0.14	0.25	0.26	0.32	0.49	0.30	-0.46	0.63	0.50	0.31	1.00	
15	0.40	-0.15	0.13	-0.19	0.14	0.18	0.15	-0.28	-0.41	0.65	-0.13	-0.22	0.14	-0.80	1.00

Bold printed values were statistically significant (P < 0.05); 1=body weight, 2=leukocytes, 3=neutrophils, 4=lymphocytes, 5=hemoglobin, 6=hematocrit, 7=erythrocytes, 8=triglycerides, 9=glucose, 10=creatinine, 11=total protein, 12=albumin, 13=globulin, 14=haptoglobin, 15=C-reactive protein

Significantly (P<0.05) strong connection between concentrations of total serum protein and globulin was found in the experimental group of piglets (r = 0.86), whereas the relationship in the control group of piglets was strong and positive between total protein and albumin (r = 0.84). These data clearly indicate the effectiveness of the preparation in raising the immune response, particularly humoral, probably due to raising the antibody repertoire that a 63 days old piglet managed to create. Total protein concentration is in a strong (P<0.05) negative relation to the concentration of triglycerides (r=-0.83) in the experimental group, even more negatively associated are globulins to plasma triglycerides (r=-0.90). In the control group of piglets the relation between these properties is positive and weak. Although the haptoglobine concentration did not statistically differ between the groups (Table 2), correlation between eritrocytes and haptoglobin was positive and strong in the experimental group. Correlation between body weight and haptoglobin was positive and moderate (r=0.41) in the experimental group, but

negative in the control group (r=-0.48), which is in relation with Hiss and Sauewein (2003) and confirm the hypothesis that MOS improve immune response. Maes et al (1993) found higher haptoglobin concentration in animals during infection. Li et al. (2006) confirmed that β-glucan to weaned pig diets offered some benefits on growth performance and immune response to an lipopolysaccharide challenge. We can conclude that the piglets in the experimental group mobilized energy to synthesize proteins, especially globulins, at the expense of fatty substances, while the piglets of the control group showed normal ratio of protein and fat. It is known that activation of the immune response results in changes in metabolic activity and a shift from investments of proteins and fats in animal growth to creating elements. Moreover, Davis et al. (2002) found that mannanoligosaccharides had no influence on the function of lymphocytes, while Podzorski et al. (1990) reported on the inhibitory effect on the lymphocyte functions, but in our investigation there was no detrimental effect for piglets growth.

**Table 4. Correlation of production, hematological and some biochemical indicators of weaned piglets in the experimental group feed with hydrolyzed yeast additive on 35<sup>th</sup> day of trial**

Tablica 4. Povezanost proizvodnih svojstava, hematoloških i nekih biokemijskih pokazatelja u odbite prasadi pokusne skupine hranjene uz dodatak hidroliziranoga kvasca 35. dan pokusa

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.30	1.00													
3	-0.38	0.24	1.00												
4	0.39	-0.17	<b>-0.99</b>	1.00											
5	-0.59	0.32	0.56	-0.45	1.00										
6	-0.57	0.46	0.58	-0.47	<b>0.98</b>	1.00									
7	-0.20	0.70	0.46	-0.32	<b>0.88</b>	<b>0.91</b>	1.00								
8	0.28	-0.62	-0.64	0.59	-0.56	-0.67	-0.68	1.00							
9	-0.44	0.23	0.00	0.07	0.47	0.53	0.48	-0.66	1.00						
10	-0.09	0.10	0.72	-0.63	0.55	0.49	0.51	-0.54	0.17	1.00					
11	-0.36	0.58	0.27	-0.26	0.34	0.50	0.46	<b>-0.83</b>	0.75	0.00	1.00				
12	0.53	0.25	<b>-0.86</b>	<b>0.86</b>	-0.49	-0.42	-0.18	0.24	0.19	-0.65	0.12	1.00			
13	-0.61	0.41	0.69	-0.68	0.57	0.67	0.52	<b>-0.90</b>	0.60	0.34	<b>0.86</b>	-0.40	1.00		
14	0.41	0.68	0.43	-0.31	0.43	0.44	0.72	-0.57	0.15	0.71	0.16	-0.10	0.20	1.00	
15	0.28	<b>0.86</b>	-0.11	0.17	0.25	0.35	0.57	-0.21	0.05	-0.29	0.35	0.44	0.09	0.38	1.00

1=body weight, 2=leukocytes, 3=neutrophils, 4=lymphocytes, 5=hemoglobin, 6=hematocrit, 7=erythrocytes, 8=triglycerides, 9=glucose, 10=creatinine, 11=total protein, 12=albumin, 13=globulin, 14=haptoglobin, 15=C-reactive protein

## CONCLUSION

Hydrolyzed yeast supplement did not improve the growth of weaned piglets, but has strongly induced cellular immune response via the proliferation of immunocompetent cells (lymphocytes). Significantly strong correlation between the concentration of total protein in serum and globulin in experimental animals indicates that Progut® changed the humoral immune response with no negative effect on general health status of the experimental animals, as determined by hematological and biochemical tests.

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## UČINKOVITOST DODATKA HIDROLIZIRANOGA PIVSKOGA KVASCA (PROGUT®) U HRANIDBI PRASADI

### SAŽETAK

Istraživanje je provedeno na dvije skupine odbite prasadi (N=46), križancima švedskoga landrasa, velikoga jorkšira i pietrena ((ŠLxVJ) x Pietren), ujednačenih spolova. Prasad je do 21. dana pokusa hranjena krmnom smjesom za odbitu prasad s 22% sirovih proteina i 13,84 MJ ME/kg, a do 35. dana pokusa smjesom s 19% sirovih proteina i 13,74 MJ ME/kg. Pokusnoj skupini životinja (P) umiješano je u hranu 0,2% hidroliziranoga pivskoga kvasca (Progut®, Suomen Rehu, Finska) u krmnu smjesu tijekom cijeloga pokusnoga razdoblja. Tjelesna masa, hematološki i neki biokemijski pokazatelji mjereni su 7., 21. i 35. dana pokusa. Nisu utvrđene značajne razlike u prosječnoj tjelesnoj masi, niti u promatranim biokemijskim pokazateljima (glukoza, kreatinin, triacilgliceroli, ukupni proteini, albumin, globulini, haptoglobin, C reaktivni protein). Pokusna skupina životinja imala je veći broj ukupnih leukocita ( $P > 0,05$ ) 21. i 35. dana pokusa i značajno veći ( $P < 0,01$ ) udio limfocita. U pokusnoj je skupini utvrđena pozitivna i jaka korelacija između ukupnih proteina i globulina ( $r = 0,86$ ), a u kontrolnoj skupini pozitivna jaka korelacija između ukupnih proteina i albumina ( $r = 0,84$ ). Značajno jaka ( $P < 0,05$ ) i negativna korelacija utvrđena je između koncentracija triacilglicerola i ukupnih proteina ( $r = -0,83$ ) i koncentracije triacilglicerola i globulina ( $r = -0,90$ ) u pokusnoj skupini prasadi. Dodatak hidroliziranoga pivskoga kvasca u hrani prasadi potaknuo je stanični i humoralni imuni odgovor.

**Ključne riječi:** kvasac, imunomodulacija, proizvodni pokazatelji, svinja, hematološki pokazatelji, biokemijski pokazatelji

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