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The effects of feed supplemented with *Agaricus bisporus* on health and performance of fattening broilers

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ABSTRACT

Enteric infectious diseases are the most common cause of loss in intensive production of poultry. The possible risk to human health because of the use and/or misuse of antibiotics in food for farm animals has led to an intensive search for alternative strategies in the control and prevention of losses in poultry production. The aim of this study was to compare the bacterial validity of standard poultry feed with feed supplemented with mushroom *Agaricus bisporus*. Furthermore, we monitored the effect of *Agaricus bisporus* on the number of *Escherichia coli, Enterobacteriaceae*, *Salmonella* spp. and *Lactobacillus* spp. in rectal swabs of broilers. The study was performed on ninety broilers, randomly divided into three groups: the control group fed with a standard broiler diet and two groups fed with the standard diet supplemented with *Agaricus bisporus* (10 g/kg or 20 g/kg). The results of this study showed the microbiological suitability of feed supplemented with mushrooms, together with its beneficial effect on production and the health of the animals. The differences in body mass gain were not significant between the three experimental groups, and higher average diarrhoea severity (ADS) was recorded in the control broilers (0.34), whereas the two treated groups had much lower ADS (0 or 0.08). Addition of *Agaricus bisporus* in a concentration of 20 g/kg lowered the total number of *Escherichia coli* and *Enterobacteriaceae* in rectal swabs and significantly increased the number of *Lactobacillus* spp.

Key words: poultry production, *Agaricus bisporus*, animal feeding

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Introduction

Bioactive components of feed, such as nutraceuticals, are effective in protecting gut health, and are harmless for animals and the environment. It has been suggested that these substances could be polysaccharides, such as β-glucans, from different sorts of mushrooms, especially *Shiitake* and *Maitake*, which are reported to have curative properties (AIDA et al., 2009). Thus, the high natural antioxidant activity of the edible mushroom *Agaricus bisporus* has been confirmed in many studies (LIU et al., 2013; TIAN et al., 2012; GIANNENAS et al., 2011; GAN et al., 2013; STOJKOVIĆ et al., 2014). β-glucans have been known for decades as constituents of the cell wall of some pathogenic bacteria, baker's yeast and numerous species of mushrooms and plants. They have been mostly examined in a rodent model (VETVICKA et al., 2007; SOLTANIAN et al., 2009), on invertebrates and vertebrates including humans, in order to determine their possible applications in human medicine (VETVICKA et al., 2002; VETVICKA and YVIN, 2004; CHEN and SEVIOUR, 2007; SOLTANIAN et al., 2009). The effects of β-glucans (isolated from baker's yeast *Saccharomyces cerevisiae*), on pig growth, digestion and immunity is a very important subject in veterinary medicine (LI et al., 2006; PRICE et al., 2010), including their ability to protect pigs from infection by enterotoxic *Escherichia coli* strains (SOLTANIAN et al., 2009). It has been shown that β-glucans isolated from *Agaricus bisporus*, besides their antitumor effect, also act as immunostimulants to the systemic and local (gut) immunity of some species of farm animals (BROWN and GORDON, 2003; SOLTANIAN et al., 2009; BARBISAN et al., 2010; MRŠIĆ et al., 2011; ŠPIRANEC et al., 2013). Based on the percentages of water, protein, fat and ash in the meat of broilers fed with *Agaricus bisporus* supplement, MRŠIĆ et al. (2011) concluded that the meat had a significantly lower fat content. It has been documented that dried white button preparations have a beneficial effect on gut histomorphology and the population of commensal microbiota in fattening broilers (GIANNENAS et al., 2010a) as well as on the production and antioxidative status of their meat (GIANNENAS et al., 2010b). Besides β- glucans, *Agaricus bisporus* contains selenium and vitamin B complex, which also play an important role in modulation of the immune system. VETTER and LELLEY (2004) found that *Agaricus bisporus*, besides its other valuable properties, has a remarkable Se-content and thus consumption of fruit bodies can improve the Se supply to the human organism, thereby decreasing some health risks. It seems that the extract of the whole mushroom has better immunomodulatory effects than its fractions (MRŠIĆ et al., 2011).

Our intention was to use waste products of *Agaricus bisporus* mushroom cultivation, which are ill-suited for human consumption because of their irregular size and shape. Using this kind of extremely nutritious animal feed as an alternative to antibiotic growth promoter (AGP) supplement results in more cost-effective production of healthier broilers, with a reduced quantity of meat fat, which is one of the meat market requirements. Firstly, we analysed the microbial population in the dried mushrooms. Then we evaluated the

microbiological validity of standard broiler feed (free of AGP) before and after adding *Agaricus bisporus* in the form of dried powder, in concentrations of 10 g/kg and 20 g/kg. Finally, we examined the effect of the mushrooms on overall broiler health, with special emphasis on their intestinal health, intestinal microbiology, and thus the potential beneficial effects on their digestive system.

Materials and methods

Mushrooms. The commercial powder of dried *Agaricus bisporus* mushrooms was obtained from a mushroom producer. In 100 g of powder it contained 59.44 % protein, 31.51 % carbohydrates and 6.32 % ash (GEA-com d.o.o., Croatia).

Birds. Ninety 1-day-old healthy broilers (ROSS 308) of both sexes (45 male, 45 female) with body mass of approximately 40 g, were obtained from a commercial rearing farm ("Živković", Kvarte, Perušić, Croatia). All procedures used in this research were in compliance with the European guidelines for the care and use of animals in research (Directive 2010/63/EU).

Study design and procedures. 1-day-old healthy broilers were randomly divided into three groups (C, D1, D2) comprising 30 animals each (15 male, 15 female), kept separately in a floor system (4 square meters per group) with electrically heated units, and they were fed for 38 days. The standard diets were based mainly on maize and soybean meal according to the recipe of the producer of poultry meat and poultry products, the Perutnina Ptuj - Pipo ltd. (Čakovec, Croatia). The control (C) group of broilers were fed with standard diets without antimicrobials or growth promoters: starter diet (1-14 days), grower diet (15-28 days) and finisher diet (29-38 days). The other two groups were given experimental diets based on the standard diets, but containing an addition of 10 g (D1) or 20 g (D2)/kg ground, dried *Agaricus bisporus* mushroom powder. During the experiment access to water and feed was *ad libitum*. The experiment was conducted for a period of 38 days, and the broilers were monitored daily and weighed/sampled at seven day intervals, starting at day 0 before the treatment. On day 38 of the experiment 2 broilers per group were euthanized and sampled for histology.

Microbiological analyses of dried Agaricus bisporus powder and broiler feed. Following the Croatian food legislation (The Food Act, Regulations on Food Hygiene, Regulations on Microbiological Criteria of Foodstuffs, Guideline for Microbiological Criteria of Foodstuffs) the dried *Agaricus bisporus* powder and the standard broiler feed were analysed by standard microbiological methods. The standard methods for microbiological analyses of dried samples of *Agaricus bisporus* mushroom from the Department for Hygiene and Technology of Animal Food Stuffs, Faculty of Veterinary Medicine, University of Zagreb, Croatia, were used in this study: Method of Determining the Count of Aerobic Mesophilic Bacteria according to HRN EN ISO 4833:2003; Method of *Salmonella* spp. detection according to HRN EN ISO 6579:2003; Method

of *Enterobacteriaceae* detection according to HRN EN ISO 7251:2002; Method of detection of sulphate-reducing bacteria in anaerobic conditions (*Clostridia*) according to HRN EN ISO 15213:2004. Microbiological analyses of standard feed for broilers, before and after mixing with dried samples of *Agaricus bisporus* mushrooms, were performed according to the standard procedures of the Poultry Centre of the Croatian Veterinary Institute, Zagreb, Croatia: Horizontal method for enumeration of total saprophytic bacteria - colony count technique at 30 °C (EN ISO 4833:2003); Horizontal method for detection of *Salmonella* spp. (EN ISO 6579:2002); Horizontal method for enumeration of *Clostridium perfringens* - colony count technique (EN ISO 7937:2004); Horizontal method for enumeration of coagulase-positive staphylococci (*Staphylococcus aureus*) (EN ISO 6888-3:2003); Horizontal method for detection of *Escherichia coli* O157 (EN ISO 16654:2001); Horizontal method for detection and enumeration of *Enterobacteriaceae* - colony count technique (EN ISO 21528-2:2004); Enumeration of yeasts and mould (ISO 21527-2:2008).

Production indicators. The broilers were weighed on days 0, 14, 28 and 38 of the experiment and changes in their body mass were recorded. The changes of body mass in the experimental groups of broilers (D1, D2) were calculated based on the difference between either the body mass at the beginning of the experiment (day 0 equals to 100 % of body mass), or the average group body mass on days 7, 14, 28 and 38 of the experiment, in comparison to the average body mass of the broilers from the control group (C). Feed intake was recorded on a weekly basis, and at the end of the experiment total group feed intake, feed conversion ratio and total group body mass gain were calculated, in relation to day 0.

Clinical observation. The broilers were monitored daily for diarrhoea and/or other clinical signs of health disorders, and the incidence/severity of diarrhoea was recorded. Severity of diarrhoea was scored as follows: 0 = normal faeces, 1 = soft faeces, 2 = fluid faeces and 3 = projectile diarrhoea. Besides morbidity, the mortality was also monitored, and dead broilers were necropsied and examined for gross pathological changes.

Microbiological analyses of rectal swabs. The rectal swabs per group (n = 30) were collected on days 0, 14, 28 and 38 of the experiment and were taken for bacterial analyses within an hour from collection. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. The following procedures were used for microbiological analyses of broiler rectal swabs: a) standard methods applied in the Poultry Centre of the Croatian Veterinary Institute, Zagreb, Croatia: Horizontal method for detection and enumeration of *Enterobacteriaceae* - colony count technique (EN ISO 21528-2:2004); Horizontal method for detection of *Salmonella* spp. in animal faeces (EN ISO 6579:2002/AMD 1:2007); Horizontal method for detection of *Escherichia coli* O157 (EN ISO 16654:2001); b) the procedure of the Department for Hygiene and Technology of Animal Food Stuffs, Faculty of Veterinary Medicine, University of Zagreb, Croatia:

Method on MRS agar (Fluka 80961) in microaerophilic conditions for detection and enumeration of *Lactobacillus* spp.

Histopathological analysis. Immediately following euthanasia of 2 broilers per group (on day 38) the gastrointestinal tract was removed and the small intestine was divided into three parts: the duodenum (from the gizzard outlet to the end of the pancreatic loop), the jejunum (from the pancreatic loop to Meckel's diverticulum) and the ileum (from Meckel's diverticulum to the ileo-caeco-colic junction). Segments one centimetre long were taken from the centre of each part and fixed in 10 % neutral-buffered formalin (pH 7.0-7.6) for 24 hours until used for histopathology analysis under light microscopy. After fixation, the specimens were dehydrated, embedded in paraplast (Sigma, Sherwood Medical Industries, USA), cut into 5 μm thick serial sections and then processed for standard hemalaun (Meyer's solution; Kemika, Zagreb, Croatia) and eosin staining. These sections were examined by a light microscope (DMLB, Leica, Germany) with a photographic device (Pixera Pro 150 ES). The graduation of epithelial damage and changes of thickness in the broilers was determined as follows: 0 no damage/normal thickness; 1 slight damage/slightly thickened; 2 moderate damage/moderately thickened; 3 strong damage/strongly thickened. The graduation of cellular infiltrate in the lamina propria (LP) of the broilers was determined as follows: - no infiltrate; ± slight infiltrate; + medium infiltrate; ++ extensive infiltrate; +++ distinctively extensive infiltrate of mononuclear leukocytes (MNL) and/or globular leukocytes **(**GL). The graduation of solitary lymphatic follicles (SLF) in the LP of mucosa or submucosa of the broilers was determined as follows:: - none; ± low number; + hyperplasia; ++ extensive hyperplasia SLF. The graduation cellularity of the cecal tonsils (CT) of the broilers was determined as follows: - lymphopenia; ± normal cellularity; + slight hyperplasia, ++ moderate hyperplasia; +++ strong hyperplasia.

Statistical analysis. Correlations between days of feeding (0, 14, 28, 38) and the number of *Lactobacillus* spp. bacteria from rectal mucous swabs in the control (C = not treated) chickens and the two groups treated with different concentrations of *Agaricus bisporus* (D1 = 10 g/kg and D2 = 20 g/kg) were calculated using Kendall's t rank correlation as a method of non-parametric statistics, by Statistica 12 (StatSoft Inc.).

Results

The results of the microbiological analyses of the samples of powder of dried *Agaricus bisporus* indicated microbiological suitability (Table 1), and the powdered preparation of *Agaricus bisporus* added to the standard feed for broilers (10 g/kg or 20 g/kg) had no influence on the microbiological composition of the feed. However, at the end of the trial period, 40 % less total bacteria was detected in feed supplemented with *Agaricus bisporus* in the concentration of 10 g/kg when compared to 20 g/kg (Table 2).

Table 1. Microbiological criteria for powder of dry *Agaricus bisporus*

Indicator / Boundary values (m*, M**)	Sample powder of dry *Agaricus bisporus* (25 g)
Aerobic mesophilic bacteria / m = 104 CFU /g ; M = 105 CFU /g	$<10^4$/ satisfying
Salmonella spp. / n.d. in 25 g	0/ satisfying
Enterobacteriaceae / m = 102 CFU g ; M = 103 CFU /g	$0<10^2$/ satisfying
Sulphate-reducing clostridia / m = 10 CFU /g ; M = 102 CFU /g	<10/ satisfying

n.d. - not detected; m* - accepted boundary value under which all the results are considered satisfying; M* - accepted boundary value above which all the results are considered satisfying; CFU - colony-forming unit

The results of the least significant difference test showed that on the 14th, 28th and 38th days of feeding the differences in body masss of the broilers were not significant between the control group of broilers and the two treated groups ($P \geq 0.05$). Also, the values of the Spearman Rank Orders Correlations, between the days of feeding (independent variable) and body mass of broilers are the same for the control group of broilers as for both treated groups ($r_s = 0.97$; $P = 0.027$; 0.026; and 0.031, respectively) (Fig. 1).

Regarding the incidence and severity of diarrhoea, much lower total diarrhoea severity score (DSS) was recorded in broilers in the D1 group (0 or - 100 %) than in the control broilers (Table 3). A higher average diarrhoea severity (ADS) was recorded in the control broilers (0.34), whereas the broilers in the D1 group had much lower ADS (0 or - 100 %) after 38 days of the experiment. None of the broilers treated with the experimental diet died during the experimental period, whereas a rather high mortality rate (10 %) was recorded in the control non-treated broilers.

Only non-pathogenic isolates of *Enterobacteriaceae* at physiological levels were found during the whole experiment in all experimental groups. Furthermore, *Salmonella* spp. was not isolated in any group during the trial. The addition of *Agaricus bisporus* mushroom to the commercial feed in the concentration of 20 g/kg (D2) lowered the total number of *Escherichia coli* in rectal swabs, starting with day 14. Moreover, the population of *Escherichia coli* decreased steadily during the remaining experimental period. The same was observed for *Enterobacteriaceae.* In group D2 the lowest total number of bacteria was observed when compared to groups C and D1 (Table 4). At day 38 of the experiment, in spite of a very high Kendall t rank correlation coefficient (Table 5), no significant difference was found in the number of *Lactobacillus* spp. between

Table 2. Microbiological criteria of standard/experimental broiler feed

Food		Indicators						
Sort	Day of experiment	Saproph. bact./CFU of bact. in 1 g of feed	*Salmonella* spp./CFU of bact. in 50 g of feed	*Clostridium* spp./CFU of bact. in 50 g of feed	*Staphylococcus* spp./ CFU of bact. in 50 g of feed	*E. coli* 0157 CFU of bact. in 50 g of feed	Other pathogenic bact./ CFU of bact. in 50 g of feed	Yeasts and moulds/ CFU in g of feed
Starter diet /w.s.	0 th	6.5×10^2	neg.	neg.	neg.	neg.	neg.	1.5×10^3
	38 th	5.5×10^2	neg.	neg.	neg.	neg.	neg.	2.5×10^3
Grower diet /w.s.	0 th	4.5×10^2	neg.	neg.	neg.	neg.	neg.	2.0×10^3
	38 th	4.0×10^2	neg.	neg.	neg.	neg.	neg.	1.5×10^3
Finisher diet /w.s.	0 th	5.5×10^2	neg.	neg.	neg.	neg.	neg.	1.0×10^2
	38 th	5.0×10^2	neg.	neg.	neg.	neg.	neg.	1.5×10^3
Starter diet / with *	0 th	6.5×10^2	neg.	neg.	neg.	neg.	neg.	2.5×10^3
	38 th	4.5×10^2	neg.	neg.	neg.	neg.	neg.	neg.
Grower diet /with *	0 th	4.5×10^2	neg.	neg.	neg.	neg.	neg.	1.5×10^3
	38 th	3.5×10^2	neg.	neg.	neg.	neg.	neg.	1.5×10^3
Finisher diet /with *	0 th	5.5×10^2	neg.	neg.	neg.	neg.	neg.	2.0×10^3
	38 th	5.0×10^2	neg.	neg.	neg.	neg.	neg.	1.0×10^3
Starter diet/ with **	0 th	6.5×10^2	neg.	neg.	neg.	neg.	neg.	1.2×10^3
	38 th	3.5×10^2	neg.	neg.	neg.	neg.	neg.	neg.
Grower diet /with **	0 th	4.5×10^2	neg.	neg.	neg.	neg.	neg.	1.0×10^3
	38 th	3.0×10^2	neg.	neg.	neg.	neg.	neg.	0.5×10^3
Finisher diet /with**	0 th	5.5×10^2	neg.	neg.	neg.	neg.	neg.	0.5×10^3
	38 th	4.5×10^2	neg.	neg.	neg.	neg.	neg.	neg.

w.s. - without supplement; * - 10 g/kg of *Agaricus bisporus* dried powder; ** - 20 g/kg of *Agaricus bisporus* dried powder; CFU - colony-forming unit

Table 3. Incidence and severity of diarrhea, and mortality in broilers during 38 days of the experiment

Group *	No. of diarrheic broilers/ total no. of broilers (%)**	Diarrhea severity score (DSS)		Average diarrhea severity (ADS)		No. of dead broilers/total no. of broilers (%)
		Sum of DSS***	% difference *vs.* control	ADS ratio****	% difference *vs.* control	
C (commercial feed)	7/30 (23)	13	/	0.34	/	3/30 (10)
D1 (10 g/kg of *Agaricus bisporus*)	0/30 (0)	0	- 100	/	- 100	0/30 (0)
D2 (20 g/kg of *Agaricus bisporus*)	2/30 (7)	3	- 77.0	0.08	- 77.0	0/30 (0)

* - each group comprised 30 broilers.** During 38 day of the experiment.*** diarrhea severity score (DSS):0 = normal feces, 1 = soft feces, 2 = fluid feces or 3 = projectile diarrhea as summarized during 38 days of the experiment.****Sum of DSS/38 days.

Table 4. Bacterial isolates from rectal mucous swabs taken from experimental broilers on days 0, 14, 28 and 38 of the experiment

Group (n = 30)	Isolate (r.m.s.)	No. of bacteria (CFU/mL) on days of experiment			
		0	14	28	38
C	*Enterobacteriaceae*	$>10^3$	$>10^3$	$>10^3$	$>10^3$
	Salmonella spp.	-	-	-	-
	Escherichia coli	$>10^5$	$>10^5$	$>10^5$	$>10^4$
D1	*Enterobacteriaceae*	$>10^3$	$>10^3$	$>10^2$	$>10^2$
	Salmonella spp.	-	-	-	-
	Escherichia coli	$>10^5$	$>10^5$	$>10^5$	$>10^4$
D2	*Enterobacteriaceae*	$>10^3$	$>10^3$	$>10^3$	$>10^2$
	Salmonella spp.	-	-	-	-
	Escherichia coli	$>10^5$	$>10^4$	$>10^4$	$>10^3$

r.m.s. - rectal mucous swab

Table 5. Number of *Lactobacillus* spp. from rectal mucous swabs taken from experimental broilers on days 0, 14, 28 and 38 of the experiment

Group (n = 30)	No. of *Lactobacillus* spp. bacteria (CFU/mL) on days of experiment				Kendall t rank correlation coefficient
	0	14	28	38	t
C	3.9×10^7	3.9×10^7	4.4×10^7	4.7×10^7	0.91
D1	4.1×10^7	4.1×10^7	4.7×10^7	5.2×10^7	0.91
D2	4.2×10^7	4.9×10^7	5.0×10^7	5.8×10^7	0.99*

* $P<0.05$

Table 6. Histopathological changes in duodenal, jejunal and ileum mucosa of broilers during 38 days of the experiment

Samples	Histological parameters	GROUP*		
		C	D1	D2
Duodenal	Damage**	1	1	1
	Thickness**	1	1	1
	Cellular infiltrate in LP***MNL / GL	+/±	++/+	+++/+++
	SLF****	±	+	++
Jejunal	Damage**	1	1	1
	Thickness**	2	1	0
	Cellular infiltrate in LP*** MNL / GL	+/±	++/+	+++/++
	SLF***	±	+	-
Ileum	Damage**	1	1	1
	Thickness**	1	1	0
	Cellular infiltrate in LP***	+	++	+++
	SLF****	±	±	+
	CT*****	++	++	+++

*Samples were taken from two euthanized broilers from each group on day 38 of the experiment. **Gradation of epithelial damage and changes of thickness: 0 = no damage/normal thickness; 1 = slight damage/slightly thicken; 2 = moderate damage/moderately thicken; 3 = strong damage/strongly thicken. ***Gradation of cellular infiltrate in LP: - = no infiltrate; ± = slight infiltrate; + = medium infiltrate; ++ = extensive infiltrate; +++ = distinctively extensive infiltrate mononuclear leukocytes (MNL) and/or globular leukocytes (GL). **** Gradation of solitary lymphatic follicles (SLF) in LP of mucosa or submucosa: - = none; ± = low number; + = hyperplasia; ++ = xtensive hyperplasia SLF. *****Gradation of cellularity of cecal tonsils (CT) is graded as follows: - = lymphopenia; ± = normal cellularity; + = slight hyperplasia, ++ = moderate hyperplasia; +++ = strong hyperplasia.

broilers from the control group and group D1. However, the value of the Kendall t rank correlation coefficient was significant for the D2 group ($P<0.05$) in comparison to the control group. In other words, *Agaricus bisporus* significantly increased the number of *Lactobacillus* spp. in animals fed with commercial feed supplemented with 20 g/kg of *Agaricus bisporus*.

Animals in all groups had normal intestinal structure. Dietary supplementation with mushrooms did not affect the duodenum, jejunum or ileum villous height or crypt depth in comparison to the untreated control group (Table 6).

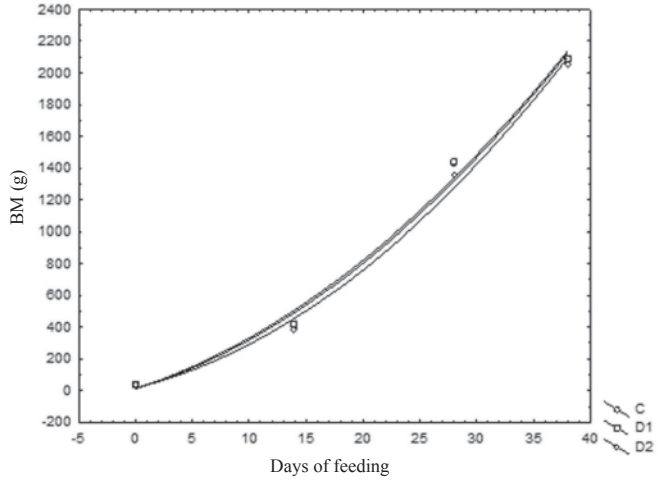

Fig. 1. Increasing of body mass of broilers in grams (C, D1 and D2) during the 38 days of feeding (LSD test in 14th, 28th and 38th days of feeding was not significant; $P\geq0.05$, at the same time Spearman Rank Order Correlations r_s for all 3 groups were 0.97 with P = 0.027; 0.026; and 0.031, respectively)

Discussion

Clinical and subclinical doses of antibiotics have been used for decades as treatment of infectious diseases, as well as growth promoters in intensive poultry production. However, the frequent use of AGP has gradually led to the loss of their efficiency. The scientific community, searching for new therapeutic alternatives, has studied many kinds of mushrooms, and has found variable therapeutic activities, such as anticarcinogenic, anti-inflammatory, immuno-suppressor and antibiotic, among others.

ZHANG et al. (2014) indicated that *Agaricus bisporus* polysaccharide possesses strong immunostimulatory and anti-tumour bioactivity *in vivo* and *in vitro*. Thus, our results are in agreement with reports from numerous authors who used bioactive polysaccharides from mushrooms and plants as additives to feed for fattening broilers, and showed their

beneficial effects on production and health indicators (GUO et al., 2003; WILLIS et al., 2007; WALLACE et al., 2010; GIANNENAS et al., 2010a,b; ŠPOLJARIĆ et al., 2011). Namely, at the end of the experimental period, the broilers fed with the addition of dried *Agaricus bisporus* in a concentration of 10 g/kg gained 2 % higher body mass in comparison to the control group (Fig.1). It is evident that the higher body mass contributes to shortening the production cycle by two days, and also represents a scientifically based recommendation for the safe and effective introduction of an economically competitive alternative for farm animal feed, regardless of the potential impact of increasing the price of animal feed due to the addition of selected *Agaricus bisporus*. Furthermore, there was almost no occurrence diarrhoea in broilers fed with *Agaricus bisporus* (Table 3). After bacterial analysis of the rectal mucous of the broilers fed with a mushroom supplement in concentrations of 10 g/kg and 20 g/kg, we did not find any pathogenic strains of *Escherichia coli* or *Salmonella* spp. (Table 4). This is a remarkable result considering that 48 % *Escherichia coli* serotypes are pathogenic for broilers at the age of up to three weeks, and may cause pericarditis and death. OZTÜRK et al. (2011) described the strong antibacterial effect of *Agaricus* mushroom preparations on gram positive bacteria, especially on *Micrococcus luteus*, *Micrococcus flavus* and *Bacillus subtilis*. In our study, the number of *Enterobacteriaceae* decreased in the rectal mucous swabs of 14 day old broilers fed with *Agaricus bisporus* in the concentration of 10 g/kg. Furthermore, our results are in agreement with those of other authors who showed that *Agaricus bisporus,* added to commercial feed in a concentration of 20 g/kg or 30 g/kg, raised the number of *Lactobacillus* spp. in the broilers' cecum (GIANNENAS et al., 2010b; KAVYANI et al., 2014). Proper nutrition, which provides for production needs, is important for animal health. This form of nonspecific prevention could avoid the nutritional stringency and disorders affecting the normal function of the organism. Thus, in this study, the histopathological changes of intestinal mucosa in broilers fed with the addition of *Agaricus bisporus* were not significantly different from the broilers fed with the commercial feed. The results obtained are in concordance with those published by GIENNENAS et al. (2010a). Furthermore, on the basis of water, protein, fat and ash content in broiler meat fed with the addition of *Agaricus bisporus,* in their research MRŠIĆ et al. (2011) noted that it is characterized by low energy value and as such can be considered a favourable dietary product, so-called "light meat", intended for human consumption. Finally, using mushroom waste products, extremely nutritious animal feed as an alternative to antibiotic growth promoter (AGP) supplement, results in more cost-effective production of healthier broilers, with a reduced quantity of meat fat, which is one of the meat market requirements.

Conclusion

The data provided by this study illustrate the antimicrobial potential of the white button mushroom *Agaricus bisporus,* as well as better production results in broilers

treated with *Agaricus bisporus* supplement, and support the recommendation of *Agaricus bisporus* as a dietary component for fattening broilers.

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SAŽETAK

Crijevne zarazne bolesti najčešći su uzrok gubitka u intenzivnoj proizvodnji peradi. Mogući rizik za ljudsko zdravlje zbog uporabe i/ili zlouporabe antibiotika u hrani za životinje namijenjenih prehrani ljudi, doveo je do intenzivnog traženja alternativnih strategija u kontroli i prevenciji gubitaka u peradarstvu. Cilj ovog istraživanja bio je usporediti bakterijsku valjanost standardne hrane za perad s onima s dodatkom gljive *Agaricus bisporus*. Nadalje, promatrali smo učinak *Agaricus bisporus* na brojnost bakterija *Escherichia coli*, *Salmonella* spp., *Enterobacteriaceae* i *Lactobacillus* spp. iz rektalnih obrisaka tovnih pilića. Istraživanje je provedeno na devedeset tovnih pilića nasumično podijeljenih u tri skupine: kontrolnu skupinu hranjenu standardnom hranom za tovne piliće i dvije skupine hranjene standardnom hranom uz dodatak *Agaricus bisporus* (10 g/kg ili 20 g/kg). Rezultati ovog istraživanja pokazali su mikrobiološku prikladnost hrane obogaćene gljivama koja ujedno ima blagotvorni učinak na proizvodnju i zdravlje životinja. Razlike u tjelesnoj masi tovnih pilića nisu bile statistički značajne između tri pokusne skupine i veći postotak životinja koje su imale proljev (ADS-average diarrhea severity) zabilježen je kod kontrolne skupine (0,34), dok su dvije tretirane skupine imale znatno niži ADS (0 ili 0,08). Dodatkom *Agaricus bisporus* u koncentraciji od 20 g/kg spušten je ukupan broj bakterija *Escherichia coli* i enterobakterija u rektalnim obriscima i znatno povećan broj *Lactobacillus* spp.

Ključne riječi: tovni pilići, *Agaricus bisporus,* hranidba