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Chemical evaluation of the quality of meat of broilers fed with the supplement from button mushroom, *Agaricus bisporus*

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short communication

Summary
The effect of added supplement from button mushroom, Agaricus bisporus of the quality of broilers meet was researched in this paper. Nutritional content of an animal feed is influenced not only by nutrient content but also by many other aspects such as, feed presentation, hygiene, digestibility, and effect on health. Usage of antibiotic growth promotors is abandoned in poultry production as well, it is neccesery to find alternative strategies for control and prevention of infections. Broilers buttlock and white meat from seven broilers from each experimental group were taken in order to examine the effect of addition of the supplement of white button mushroom to the controlled food intended for broilers on chemical quality of broilers meat using standard chemical methodes. Based on the percentages of water, protein, fat and ash in meat of broilers fed with food additive made of Agaricus bisporus we conclude that it is characterized by a low energy value, and as such can be considered as favorable dietary product, so called "light meat" intended for human consumption.
Key words: *broiler, Agaricus bisporus, light meat, antibiotic growth promotors*

Introduction

Danger of use of antibiotic growth promotors in food for animals and/or misuse of antibiotic growth promotors, led to ban of their use in European Union (Regulation EC No. 1831/2003). Consequences of the prohibition of the antibiotic growth promotors are lower usability of food, reduction of production feature and higher mortality and morbidity rate of animals. Therefore it is necessery to find alternative and sustainable methods of control of stress factors on animal health with apropriate feeding systems. So, today, when the usage of antibiotic growth promotors is abandoned in poultry production as well, it is neccesery to find alternative strategies for control and prevention of infections. However, alternative strategies could have a big impact on well being of animals raised for human consumption and therefore increase the risk of ailment of animals and people from various diseases. Connecting food with possible cause of diseases, developed so called "functional food" concept. Food is considered functional if its composition contains substances which in a positive way affect normal functioning of organism. Such substances are called food supplements, medicinal food or food for special medical purposes. Food for animals must ensure sufficient quantities of digestible nutrients without pathogens, protect animals from oxidative stress, minimize diseases outbreaks and maintain an effective immune system. Having that in mind the wider scientific community researched numerous types of mushrooms and proved their beneficial effects (Aida et al., 2009). Torus, mycelium and spores of mushrooms accumulate a series of bioactive metabolites with immunomodulatory, antiinflammatory, anticancer, antioxidant and antimicrobial effect (Hu et al., 2004, Špoljarić et al., 2011). In accordance with article 17. of regulation (EU) No. 1831/2003 on animal nutrition supplements, Commission founded Register of animal nutrition supplements, whereby recommending natural animal nutrition supple-

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Figure 1 **Commercial production of white button mushroom, *Agaricus bisporus*, GEA-COM d.o.o., Zagreb.**

Figure 2 **White button mushroom *Agaricus bisporus:* a) sample of fresh biomass, b) sample of dry biomass, c) sample of dry biomass powder.**

ment, *Agaricus bisporus* and its extract CoE 543. Positive effect of *Agaricus bisporus* manifests in its nutritional value as well. *Agaricus bisporus* is rich in water, minerals, proteins, fibers and carbohydrates with low calorific values due to the small share of fat (Wani et al., 2010). Guo et al. (2003) described the positive effect of mushrooms, or supplements isolated from mushrooms on broilers. They described exceptional antioxidant, antibacterial and immunostimulatory effect of mushrooms, and their impact on the reduced occurrence of stress in broilers. Furthermore, Petek et al. (2013), also on the model of broilers, found that a natural mixture of white button mushroom, *Agaricus bisporus,* has the characteristics of nutraceuticals. In contemporary culture conditions, supplement made of white button mushroom, *Agaricus bisporus,* as applicable replacement for antibiotic growth promotors in broilers food, shows not only severe nutritional value, but positive effects on production traits and health status of the animals and antimicrobial and antiparasitic activity as well (Mršić, 2011). Therefore, the purpose of this study was to show the chemical evaluation of the quality of meat of broilers fed with the supplement from button mushroom, *Agaricus bisporus.*

Material and methods

The research was conducted within the project: 053-0532265-2255 and 2012-11-17. The study was carried out in 38 days on the farm „Živković", Kvarte, Perušić on 90 broilers (breed ROSS 308, 45 male, 45 female). Broilers were divided into 3 groups, containing 30 animals each. Groups were kept apart, but in the same facility. C group of broilers during the experiment was fed with basal food intended for broilers (starter 0-14 days of age; finisher I. 14-28 days of age; finisher II. 28-38 days of age). Broilers groups A and B during the whole experiment were fed with basal food intended for broilers with addition of powder supplement of white button mushroom, *Agaricus bisporus* in concetration of 10 g/kg (group A) and 20 g/kg (group B). During the whole experiment food and water were available to broilers *ad libitum.* As a supplement to the basal

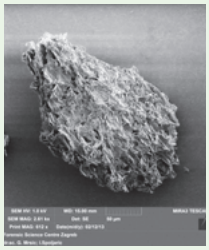

Figure 3. **Visualized ultrastructure of dry biomass powder of white button mushroom, using electornic microscope SEM Tescan Mira3 FEG (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia).**

chromatography - mass spectrometry (GC-MS, Perkin Elmer, SAD) (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia) and by the electronic microscope SEM Philips XL 30 with EDX detector using the software package Genesis version 6.02 (Edax), and by EDX active surface detector 10 mm^2 (Edax, model 135-10 PV9760/68) (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia). Then, dried white button mushroom in powder were mixed with basal food intended for broilers in concentration of 10 g/kg and 20 g/kg (Figure 2, Figure 3).

At the end of the experiment, 38th day, in abattoir, broiler buttlock and white meat from seven broilers from each experimental group were taken in order to examine the effect of addition of the supplement of white button mushroom to the basal food intended for broilers on chemical quality of broiler meat. On Department of Hygiene and Technology of Foodstuffs of Faculty of Veterinary Medicine, University of Zagreb, shares of: water (Method according to ISO 1442 standard), fat (Method according to ISO 1443 standard), proteins (Method according to ISO 937 standard) and ash (Method according to

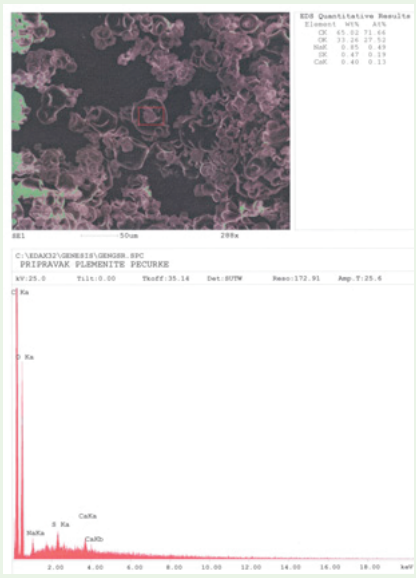

Figure 4 **Histogram display of GC-MS qualitative analysis of the white button mushroom preparations used in the experiment (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia).**

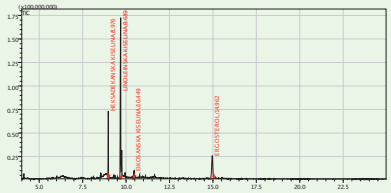

Figure 5 **Elemental chemical analysis of the sample of the white button mushroom preparation for the presence of heavy metals, performed on electron microscope SEM Philips XL 30 with EDX detector (EDAX), with 10mm^2 of active surface (Forensic Science Centre „Ivan Vučetić", Zagreb, Croatia).**

ISO 936 standard) were determined. Statistical analyses of experimental data in content of fats and protein in meat of control (untreated) group of animals and treated groups (A and B group) of broilers, were provided using the t-test for dependent samples.

Discussion and results

A number of factors including: genetics, method of cultivation and bearing, frequency of exposure to pathogens; affect productivity and health of domestic animals. Certainly, feeding is one of the most important part in the modulation of the susceptibility of domestic animals to infectious diseases. There is a dual interaction between nutrition and infectious diseases. Firstly, nutritional needs can be significantly altered due to the presence of clinical, and also of nonclinical disease. Secondly, nutritional status can affect immunocompetence of domestic animals, and consequently its resistance to pathogens. In contemporary production of domestic animals it is necessary to compile recipes which will promote growth and be economically feasible at the same time. Such meals, need not only to meet nutritional requirements of animals depending on species and breeding category, but also need to be effective in modulating the selection of species/strains and development of the microbiota, and in that way stabilizing commesal microorganisms, enhancing the function of the immune system and enhancing resistance to infectious diseases. Nowdays, research increasingly recognize the role of nutricines in creating and maintaining good health in humans and animals through various effects on metabolism. Some of the beneficial effects include; improved food intake, reduction of oxidative stress, prevention of growth of microorganisms in food, modulation of the immune system, better digestion and absorption of nutrients and modification of the micropopulation of digestive system. Individual nutricines

are considered as molecules that are able to prevent and even cure certain diseases and they are known as nutraceuticals (Popović et al., 2008; Popović et al., 2010).

Agaricus bisporus known as white button mushroom, is one of the most widely cultivated mushrooms in the world and it contains 5,52% of dry matter containing 59,44% of proteins, 31,51% of carbohydrates and 6,32% of ash (Novak, 1997). Mushrooms, as eukaryotic organisms, have cells shrouded with the cell wall whose primary role is to ensure the solidity of cells. The cell walls of fungi are composed of polysaccharides, whose share reaches 89% of dry matter, proteins, whose share in the dry matter is between 3% -20%, and in a smaller proportions lipids, minerals and pigments (Mohaček-Grošev, 2001) also. Polysaccharide part is made of fibrillar components and amorphous, or matrix components. Major fibrillar component is chitin, while matrix components are made of other polysaccharides of which the most common are manans and α- and ß- (1 to 3)- glucans (Cheung, 1997; Hu et al., 2004). Good nutritional characteristics of white button mushroom, low in fats and rich in proteins and carbohydrates, among which the most common are dietary fiber, makes them preferred food not only for humans but also for domestic animals intended for human consumption, such as poultry. In this study by using gas associated method of mass chromatography-spectrometry (GC-MS) in dry preparation of white button mushroom, mixed in a standard poultry food, the presence of toxic chemicals harmful to the health of poultry has not been established (Table 1, Figure 4). So, white button mushroom can serve as a good and cost-effective source of antioxidants in diet. Extracts of white button mushroom possess significant antioxidant activity, which is largely attributed to the presence of polyphenolic compounds, but to α- and ß- tocopherol, carotenoids, ascorbic acid and ergothioneine (Dubost et al., 2007; Elmastas et al., 2007; Barros et al., 2008) as well. Also, elemental chemical analysis of the sample preparation for the presence of heavy metals, done on the electron microscope SEM Philips XL 30 with EDX detector (EDAX), with 10mm^2 of active surface, in this study has not shown their presence, while sodium, sulfur and calcium were found only in traces (Figure 5).

Table 1 **GC-MS qualitative analysis of experimental preparations**

GC-MS qualitative analysis of the white button mushroom supplement
palmitic acid
linoleic acid
eicosanic acid
ergosterol

Table 2 **Chemical composition of meat of broiler fed with the addition of: 10 g/kg (group A) and 20 g/kg (group C) of supplement of the white button mushroom during the 38 days experiment (n=7 for each group)**

Experimental group	Sliced broiler meat	Water, %	Ash, %
C	Buttock a*	72,64	1,01
	Buttock b**	72,73	0,95
	White meat	70,58	1,14
A	Buttock a	73,24	1,14
	Buttock b	73,76	1,08
	White meat	72,85	1,13
B	Buttock a	73,85	1,03
	Buttock b	73,36	1,09
	White meat	72,70	1,13

* buttock from left side of broiler body
** buttock from right side of broiler body

Table 3 **Differences in content of fat (%) in meat of broiler left buttock (n=7 per each group).**

	C	A	B
Mean	8.00	6.92	6.45
Stand. Error	0.038	0.096	0.151
Comparisons	C vs. A	C vs. B	A vs. B
Diff.	1.08***	1.55***	0.47*
Stand. Dev. Diff.	0.18	0.38	0.41
t	16.25	10.86	2.97
p	0.000003	0.000036	0.02

C=Control, A=treatment with 10 g/kg *Agaricus bisporicus*, B=treatment with 20 g/kg *Agaricus bisporicus*,***: p<0.001; *: p<0.05

Today the preparations of the white button mushroom are important components of the alternative strategies of nonclinical use of antibiotics, especially in the production of animals and animal products intended for human consumption and especially as an alternative prophylaxis / treatment for the growing number of microbes resistant to antibiotics (Gallois et al., 2009). So, for example, it was found that the preparation of dried white button mushroom is beneficial to intestinal histomorphology and population of commensal microbiota in broilers (Giannenas et al., 2010a), as well as the production features and antioxidant status of their meat (Giannenas et al., 2010b). During the

Table 4 **Differences in content of proteins (%) in meat of broiler left buttock (N=7 per each group).**

	C	A	B
Mean	18.07	17.13	17.65
Stand. Error	0.033	0.054	0.030
Comparisons	C vs. A	C vs. B	B vs. A
Diff.	0.94***	0.42***	0.52***
Stand. Dev. Diff.	0.095	0.138907	0.153
t	26.10	7.891	9.024
p	p<0.00001	0.0002	0.000104

C=Control, A=treatment with 10 g/kg *Agaricus bisporicus*, B=treatment with 20 g/kg *Agaricus bisporicus*,***: p<0.001; *: p<0.05; ns: not significant

Table 5 **Differences in content of fat (%) in meat of broiler right buttock (n=7 per each group).**

	C	A	B
Mean	8.34	6.72	7.00
Stand. Error	0.038	0.113	0.039
Comparisons	C vs. A	C vs. B	B vs. A
Diff.	1.62***	1.34***	0.28 ns
Stand. Dev. Diff.	0.404	0.137	0.344
t	10.577	25.73	2.196
p	0.000042	<0.00001	0.070

C=Control, A=treatment with 10 g/kg *Agaricus bisporicus*, B=treatment with 20 g/kg *Agaricus bisporicus*,***: p<0.001; *: p<0.05; ns: not significant

Table 6 **Differences in content of proteins (%) in meat of broiler right buttock (N=7 per each group).**

	C	A	B
Mean	17.49	16.88	17.33
Stand. Error	0.097	0.094	0.028
Comparisons	C vs. A	C vs. B	B vs. A
Diff.	0.61*	0.16 ns	0.45**
Stand. Dev. Diff.	0.47	0.29	0.26
t	3.47	1.46	4.62
p	0.013	0.193	0.003

C=Control, A=treatment with 10 g/kg *Agaricus bisporicus*, B=treatment with 20 g/kg *Agaricus bisporicus*,***: p<0.001; *: p<0.05; ns: not significant

research described in this paper, Mršić (2011) established antioxidant activity of methanol extracts of the sample in this study of dry mixture of the white button mushroom before mixing with food for broilers. Furthermore, according to the Jeong et al. (2010) healing properties of the white button mushroom, *Agaricus bisporus* are reflected in the effects on lowering cholesterol and blood glucose levels as well. Mršić (2011), also recorded that in broilers fed with food additive made of white button mushroom the cholesterol levels are 10% lower on average and that serum glucose concentration is 18% lower compared to broilers fed with commercial food for broilers. Accordingly, meat of broilers fed with the addition of mixture of the white button mushroom did not have altered chemical composition with respect to the shares of water and ash in white and dark meat (broiler buttocks) in relation to meat of 38 days old broilers fed with commercial food (Table 2).

However, the meat of broilers fed with the supplement of the white button mushroom had statistically lower content of fat and protein (Table 3). It is obvious in Table 3 that total fat content in left broiler buttock is significantly higher in control group compared with both treated groups of animals (p<0.0001). However, content of total fat is significantly higher in A group of treated animals in the comparison with B group (p<0.05).

Content of total proteins in left broiler buttock is significantly higher in control group, compared with both treated groups of animals (p<0.0001). Content of total proteins in B group of treated animals is significantly higher in the comparison with A group (Table 4.)

It is obvious in Table 5 that total fat content in left broiler buttock is significantly higher in control group of broilers comparing the both treated groups of animals (p<0.0001). However, significant differences in content of total fats between A and B group of treated animals were not obtained (D=0.28; p=0.07 to p=0.05).

Content of proteins in right buttock of broilers is significantly lower in A group of treated animals in the comparison with control and B group of treated animals. At the same time, the difference in content of proteins, between control and B group of animals, was not significant (Table 6).

Content of total fats in white meat of broilers is significantly higher in control group of animals in the comparison with A and B group, but the differences in content were not obtained between A and B group of treated animals (Table 7).

Content of proteins in white meat

of broilers is significantly higher in control compared with A and B group of treated animals. However significant difference in protein content between A and B group was not obtained (Table 8).

Nowdays food industry, among others, are engaged in the development of specific food products in order to improve health or reduce the risk of developing the diseases. Poultry meat and eggs can be used for the production of "functional foods". In this sense, the recent declarations on fresh meat is increasingly encountered the term "light" meat, that is now tied up with most dairy products. As consumers are becoming more aware of eating healthy and taking care of the quality of the food we consume, food producers must adapt to new products and create demand for them. Consumers term "light" usually associated with a small amount of fat in foods and different opinions, and consumers do not trust these labels (as much as 28% of respondents), especially the younger age groups are skeptical towards these descriptions (Cerjak et al. , 2007). People who consume "light" products have a positive attitude towards this type of product and intend to continue to consume them. Study which were conducted by authors, suggests that producers of "light" products must adapt their marketing practices to better inform consumers what means mark "light" on their products, and indicate that the legislator should specifically stipulate which products must be labeled "light". Although many food products, called "light" in Croatian law there is no definition of this kind of products. Food and Drug Administration (FDA) has set the rules and define the terms to food producers. Thus, the "light" foods (or "lite") contains 50% fewer calories from fat and contains 50% less fat than the normal chemical composition of the same food. If the food contains fewer calories derived from fat, number of calories must be reduced to less than one-third of average values. Also, the percentage of fat can be reduced by 50% or more compared to normal composition. The success of market of these products depends on the consumers. Assumptions for the increase in production, as well as consumation products made from poultry meat are numerous, but we must emphasize that improve the quality of dietary products plays a significant role.

Table 7 **Differences in content of fat (%) in white meat of broiler (n=7 per each group).**

	C	A	B
Mean	6.02	5.45	5.27
Stand. Error	0.009	0.090	0.023
Comparisons	C vs. A	C vs. B	A vs. B
Diff.	0.57***	0.75***	0.18 ns
Stand. Dev. Diff.	0.228	0.073	0.277
t	6.712	26.963	1.645
p	0.00053	<0.00001	0.15

C=Control, A=treatment with 10 g/kg *Agaricus bisporicus*, B=treatment with 20 g/kg *Agaricus bisporicus*,***: p<0.001; *: p<0.05; ns: not significant

Table 8 **Differences in content of proteins (%) in white meat of broiler (N=7 per each group).**

	C	A	B
Mean	20.26	19.64	19.78
Stand. Error	0.081	0.175	0.100
Comparisons	C vs. A	C vs. B	B vs. A
Diff.	0.62*	0.48**	0.14 ns
Stand. Dev. Diff.	0.59	0.27	0.48
t	2.715	4.61	0.77
p	0.034	0.0036	0.468

C=Control, A=treatment with 10 g/kg *Agaricus bisporicus*, B=treatment with 20 g/kg *Agaricus bisporicus*,***: p<0.001; *: p<0.05; ns: not significant

Conclusion

Based on the percentages of water, protein, fat and ash in chicken meat of broilers fed with food additive made of *Agaricus bisporus* we conclude that it is characterized by a low energy value, and as such can be considered as favorable dietary product, so called "light meat" intended for human consumption. Further research should confirm or refute for now established effects of supplement of *Agaricus bisporus* which will be indicators of justification of mixing the supplement with food in intensive farming of broilers without the use of antibiotic growth promotors.

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Chemische Bewertung der Hühnerfleischqualität stammend von Masthühnern gefüttert mit Zutaten von Edelegerling *Agaricus bisporus*

Zusammenfassung

Die Gefahr vom Gebrauch der antibiotischen Wachstumanreger im Viehfutter auf menschliche Gesundheit und/oder Missbrauch der antibiotischen Wachstumanreger führte zum Verbot für deren Gebrauch in der Europäischen Union (Regulation EC No. 1831/2003). Im Einklang mit Art. 17. der Verordnung (EZ-a) Nr. 1831/2003 über die Zutaten, die bei der Fütterung der Tiere gebraucht werden, bestimmte die zuständige Komission das Register der Zutaten im Viehfutter, womit natürliche Zutaten im Viehfutter empfohlen werden, Agaricus bisporus und dessen Extrakt CoE 543. Wir können deshalb in dieser Arbeit zu dem Schluss kommen, auf Grund der erzielten Resultate über den Anteil von Eiweißstoffen und Fetten im Fleisch der Hühner, gefüttert mit Futter mit Zutaten des Edelegerlings Agaricus bisporus, dass das analysierte Fleisch einen niedrigen energetischen Wert hat, und somit dasselbe als günstiges Diäterzeugnis in der menschlichen Nahrung darstellt.

Schlüsselwörter: *Zutaten von Edelegerling Agaricus bisporus, Fleischqualität*

Valutazione chimica della qualità della carne di pollo proveniente dai polli da ingrasso alimentati con l'aggiunta del fungo coltivato *Agaricus bisporus*

Sommario

I rischi dell'uso degli stimolatori antibiotici della crescita negli alimenti per animali per la salute umana e/o l'abuso di stimolatori antibiotici della crescita, hanno portato alla proibizione del loro uso nell'Unione Europea (Regolamento CE n. 1831/2003). In conformità all'articolo 17 del Regolamento (UE) n. 1831/2003 sugli additivi che si usano nell'alimentazione degli animali, la Commissione ha stabilito il Registro degli additivi destinati all'alimentazione animale, secondo il quale raccomanda l'additivo naturale Agaricus bisporus nell'alimentazione animale e il suo estratto CoE 543. Dunque, sulla base dei risultati ottenuti della percentuale di proteine e grassi nella carne dei polli alimentati con il supplemento del fungo Agaricus bisporus, possiamo concludere che la carne analizzata si distingue per un basso valore energetico e come tale è da considerarsi come un prodotto dietetico favorevole destinato all'alimentazione umana.

Parole chiave: *aggiunta del fungo coltivato Agaricus bisporus, qualità della carne*