

Immunological and anti-*Eimeria* Effects of Hot Water and Methanolic Extracts of *Pleurotus sajor-caju* in Broiler

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Abstract

Present study reports the immunomodulatory and anti-*Eimeria* effects of hot water and methanolic extracts of *Pleurotus (P.) sajor-caju* (a locally grown oyster mushroom). Mushrooms were processed to obtain hot water and methanolic extracts followed by lyophilization. The lyophilized extracts were subjected to proximate analysis followed by their evaluation for immunomodulatory and anti-*Eimeria* activities. Immunomodulatory evaluation of these extracts in industrial broilers revealed significantly higher ($P<0.05$) cell mediated immunity through lymphoproliferative response to phytohemagglutinin-P as compared to control. These extracts also showed higher humoral immune response through elicited total Ig, IgG, and IgM titers at day 7th and 14th after primary and secondary intramuscular injections of sheep red blood cells. Further, all the groups were orally inoculated with infective dose of sporulated oocysts of *Eimeria (E.)* species (including *E. tenella*, *E. maxima*, *E. acervulina* and *E. necatrix*) followed by monitoring of per cent mortality, oocysts per gram of feces (OPG) and intestinal lesion scoring. OPG values in birds of experimental groups administered with mushroom extracts were significantly lower ($P<0.05$) as compared to those of control group. Control group showed higher mortality ratio and lesions scores as compared to groups administered with extracts. In conclusion, hot water and methanolic extracts of *P. sajor-caju* showed significant immune boosting activity in broilers and subsequent protective efficacy against *Eimeria* infection.

Keywords: Mushroom extracts, *Pleurotus sajor-caju*, *Eimeria*, Immunological, Broiler

Etlik Piliçlerde *Pleurotus sajor-caju* Sıcak Su ve Metanolik Ekstraktının İmmunolojik ve Anti-*Eimeria* Etkileri

Öz

Bu çalışmada *Pleurotus (P.) sajor-caju* (yerel yetişen bir istiridye mantarı) sıcak su ve metanolik ekstraktının bağışıklık düzenleyici ve anti-*Eimeria* etkileri araştırılmıştır. Mantarlardan liyofilizasyonla sıcak su ve metanolik ekstraktı elde edildi. Liyofilize edilen ekstraktlar bağışıklık düzenleyici ve anti-*Eimeria* aktivitelerini test etmek amacıyla analiz edildi. Ticari broiler tavuklarda bu ekstraktların bağışıklık düzenleyici etkisinin değerlendirilmesi sonucunda kontrol ile karşılaştırıldığında fitohemaglutinine karşı lenfoproliferatif cevap ile karakterize hücre aracılı bağışıklığın anlamlı derecede yüksek olduğu belirlendi ($P<0.05$). Bu ekstraktlar, koyun kırmızı kan hücrelerinin primer ve sekonder kas içi enjeksiyonu sonrası 7. ve 14. günlerde total Ig, IgG ve IgM titreleri ile karakterize humoral bağışıklık cevabın da yüksek olmasına neden oldu. Tüm gruplara *Eimeria (E.)* türleri (*E. tenella*, *E. maxima*, *E. acervulina* ve *E. necatrix*) enfektif dozda oral yolla inokule edildi ve sonrasında mortalite yüzdesi, her bir gram dışkıda oosit miktarı ve barsak lezyonları incelendi. Kontrol ile karşılaştırıldığında mantar ekstraktı verilen hayvanların yer aldığı deney gruplarında her bir gram dışkıda oosit miktarı anlamlı derecede düşüktü ($P<0.05$). Kontrol grubunda mortalite oranı ve lezyon skorları ekstrakt verilen gruplarla karşılaştırıldığında daha yüksekti. Sonuç olarak, *P. sajor-caju* sıcak su ve metanolik ekstraktı etlik piliçlerde bağışıklığı artırıcı ve *Eimeria* enfeksiyonuna karşı koruyucu etki gösterdi.

Anahtar sözcükler: Mantar ekstraktı, *Pleurotus sajor-caju*, *Eimeria*, Bağışıklık, Broiler



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INTRODUCTION

Pleurotus (*P.*) species are one of the 25 commonly consumed species of mushrooms worldwide and are becoming popular now a day because of excellent nutritional and medicinal properties^[1]. In the world, button mushroom i.e. *Agaricus bisporus* is among the leading specie as far as cultivation is concerned, followed by shiitake (*Lentinus edodes*) and oyster (*P. ostreatus*)^[2]. Commercially, mushroom production is dramatically increased in the world with mainly China and Europe contributing in its production to be increased up to almost 8 million tonnes, annually^[3].

Among the oyster species, *P. sajor-caju* is gaining its due share because of its reported immunomodulatory, anti-inflammatory and anti-tumor activities^[4,5]. There are several important nutrients including carbohydrates, proteins, dietary fibers, minerals and vitamins present in the mushrooms due to which they are considered as essential in the dietary composition of human beings^[6,7]. For the last many years, research has been focused to explore the utility of mushroom compounds as immunomodulators in different animal models^[8,9]. In this regard, several types of extracts from various mushroom species were evaluated for immune modulation in different animal models^[10,11]. Moreover, various biologically active metabolites including glycoproteins, hydrolytic and oxidative compounds, phenolics and lipids had also been isolated from crude extracts of *Pleurotus* spp. to determine their biological activities^[12]. These studies revealed immunomodulatory effects of these active substances through enhancing mitogenicity and activation of immune effector cells including macrophages, natural killer cells and lymphocytes. These immune cells further increase the production of cytokines including tumor necrosis factor (TNF- α), interleukins (ILs) and interferons (IFNs)^[13].

In this regard, water soluble proteoglycans fractions from *P. ostreatus* had been reported for enhanced cytotoxicity of NK cells and stimulation of macrophages for increased production of nitric oxide with marked decrease in the quantity of sarcoma cells in sarcoma 180 bearing mice^[14]. Similarly, methanol, dichloromethane and n-hexane extracts of *Agaricus blazei* were investigated against Ehrlich tumor bearing mice and revealed stimulated lympho-proliferative activity of splenocytes and enhanced antibody production^[15]. Keeping in view the well documented pharmacological activities of *P. sajor-caju* in different disease models, this study was conducted to evaluate the immunotherapeutic activity of hot water and methanolic extracts of *P. sajor-caju* against *Eimeria* infection in chickens.

MATERIAL and METHODS

Procurement and Processing of Mushrooms

Fresh mushrooms (*P. sajor-caju*) were procured from the local grower at Millet Town, Faisalabad, Pakistan and its

authenticity was confirmed by the concerned Botanist from University of Agriculture, Faisalabad, Pakistan (UAF), and a specimen (Voucher No. 175) was kept in Ethnoveterinary Research Center, Department of Parasitology, UAF.

Mushroom were dried at 50°C followed by grinding and passed through sieve (2 mm) to maintain the uniformity of the dried powder. The hot water extract was prepared by the methodology described previously^[16]. In brief, the dried powder (500 g) was added in water (1500 mL) at temperature of 100°C in a water bath for 2 h. More water (500 mL) was added after each hour. The mixture obtained was centrifuged at 3000 rpm for 30 min and supernatant was collected. Supernatant thus obtained was subjected to lyophilization which yielded 85 g dried extract per 500 g of dried powder. The dried extract was stored at 4°C till further use in the experiment. The methanolic extract was prepared by the methodology described by Yang and his co-workers^[17]. Briefly, dried mushroom powder (500 g) was vortexed thrice with 2000 mL of 80% methanol at room temperature for 48 h. Pooled extract obtained was subjected to rotary evaporator. Concentrated extract was lyophilized which yielded 25 gm of methanolic extract. These extracts (hot water and methanolic) were subjected to proximate analysis to determine the concentrations of crude protein, fiber, ash, ether and nitrogen free extracts^[18].

Experimental Design

The experimental study was conducted at Experimental Station, Faculty of Veterinary Sciences, UAF. For the purpose, day old birds (n=150; Hubbard) purchased from local hatchery were reared under standard management conditions. Birds were given free access to fresh water and withdrawal feed. The study was approved for ethical consideration and humane handling of animals by Institutional Animal Care & Use Committee and Advance Studies & Research Board of UAF. After acclimatization for 5 days, the birds were divided into 3 groups i.e. 1, 2 and 3; each containing 50 randomly selected birds. On day 7th, 8th and 9th of the experiment, the groups 1 and 2 were administered with mushroom extracts as follows:

Group 1- Hot water extract of *P. sajor-caju* administered orally at the dose rate of 200 mg/kg body weight (BW)/day using phosphate buffered saline (PBS; 2 mL) as a solvent

Group 2- Methanolic extract of *P. sajor-caju* administered orally at the dose rate of 200 mg/kg BW/day using PBS (2 mL) as a solvent

Group 3- Control administered orally 2 mL of PBS.

On day 15th of the experiment, all three groups were subdivided into two equal groups (n=25 each) i.e. 1a, 1b; 2a, 2b and 3a, 3b, respectively. The subgroups 1a, 2a and 3a were designated to evaluate immunomodulatory effects through lymphoproliferative response to phytohaemagglutinin-P (PHA-P), antibody response to sheep

red blood cells (RBCs) and organ (spleen, bursa of Fabricius and cecal tonsils) to body weight ratios. The subgroups including 1b, 2b and 3b were shifted to another shed and challenged with *Eimeria* infection to assess the anti-*Eimeria* efficacy of the extracts.

Immunological Evaluation

Classical toe web assay was performed to quantify cell mediated immunity. In this assay, PHA-P was injected intra-dermally in the right toe web of experimental birds. Meanwhile, PBS was also injected in the left toe web of same birds. Lymphoproliferative response was observed in terms of increase in the thickness of toe web after 24, 48 and 72 h by using the following formula:

Lymphoproliferative response = (Thickness of PHA-P injected toe web - Thickness of PBS injected toe web).

Microplate haemagglutination test was used to demonstrate humoral immune response. Sheep RBCs (5%) were injected twice as T-cell mitogen in the breast muscles of experimental birds at an interval of 14 days. Immunoglobulin (total Ig, IgG and IgM) titers were determined at day 7th and 14th after primary and secondary injections of sheep RBCs [19].

Lymphoid organs including spleen, thymus, bursa of Fabricius and cecal tonsils were collected from chickens of the experimental groups after humane slaughtering on day 42 of the experiment. All the organs were weighed to determine the per cent organ to live body weight ratios [20].

Evaluation of Anti-*Eimeria* Effects of Mushroom Extracts

To assess the anti-*Eimeria* effect, subgroups (1b, 2b and 3b) were challenged with infective dose (6.5×10^4 - 7.0×10^4) of sporulated oocysts of mixed *Eimeria* (*E.*) species viz. *E. tenella*, *E. maxima*, *E. acervulina* and *E. necatrix* (local isolates; maintained at Immunoparasitology Laboratory, UAF) on day 15th of experiment. These groups were monitored for oocyst per gram of droppings, lesion scoring and per cent protection from day 4th to 12th post challenge with *Eimeria* species [20-22].

Statistical Analysis

Data obtained was analyzed using completely randomized design and one-way analysis of variance (ANOVA) using SPSS ver. 16. The differences among mean values were determined using Tukey's range test and differences were considered significant at $P < 0.05$. For immunoglobulin titers, geometric mean titers (GMT) were calculated [23].

RESULTS

Proximate Analysis

Proximate analysis revealed 29.22% and 17.59% crude protein, 11.93% and 6.50% ash contents, 1.52% and 2.39% ether extract, 57.33% and 73.52% nitrogen free

extract from hot water and methanol extracts of *P. sajor-caju*, respectively. However, no fibers were found in both extracts (Table 1).

Immunological Evaluation

Lymphoproliferative Response to PHA-P: Cell mediated immune response was evaluated through Toe web assay. Thickness observed in the toe web of birds belonging to experimental groups treated with hot water and methanol extracts showed significantly higher values ($P < 0.05$) at 24, 48 and 72 h post PHA-P injection as compared to control. The results revealed that both extracts showed excellent T-cell mitogen activity (Fig. 1).

Humoral Immune Response Against Sheep RBCs: Total immunoglobulins, IgG and IgM titers observed in the hot water and methanol extracts at days 7th and 14th post-primary and post-secondary injections of sheep RBCs were significantly higher as compared to control. However, the titers (total Ig, IgG and IgM) observed in the methanolic extract treated groups were highest followed by those of hot water extract (Table 2).

Lymphoid Organs to Body Weight Ratios: Birds belonging to experimental and control groups were weighed and

Table 1. Results of proximate analysis of hot water and methanolic extract

Extract Type	Hot Water Extract (%)	Methanol Extract (%)
Crude protein	29.22	17.59
Crude fiber	-	-
Ash	11.93	6.50
Ether extract	1.52	2.39
Nitrogen free extract	57.33	73.52

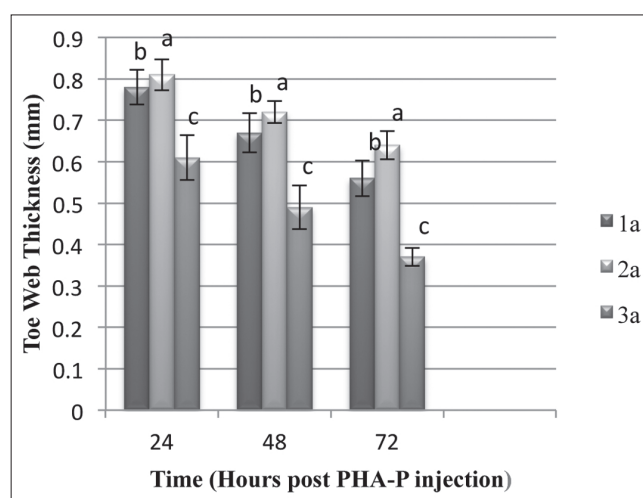


Fig 1. Lymphoproliferative response to Phytohaemagglutinin-P in mushroom extracts administered and control groups. Bars sharing different letters present a significant difference ($P < 0.05$). Group 1a: Hot water extract of *Pleurotus sajor-caju*, Group 2a: Methanol extract of *Pleurotus sajor-caju*, Group 3a: Control

Table 2. Anti sheep-RBCs antibody titers (Total Ig, IgG, IgM) after day 7 and day 14 post-primary and post-secondary injection of Sheep RBCs

Total Immunoglobulin	7ppi	14ppi	7psi	14psi
Group 1a PSC/HWE	48.53	42.27	55.72	36.73
Group 2a PSC/ME	63.97	48.53	73.45	55.72
Group 3a Control	31.99	27.86	36.73	27.86
IgM				
Group 1a PSC/HWE	32.53	18.00	34.58	8.87
Group 2a PSC/ME	42.84	27.39	45.59	13.45
Group 3a Control	22.18	11.16	20.73	9.49
IgG				
Group 1a PSC/HWE	16.00	24.27	21.13	27.86
Group 2a PSC/ME	21.13	21.13	27.86	42.27
Group 3a Control	9.18	16.00	16.00	18.37

PSC/HWE - *Pleurotus sajor-caju*/Hot Water extract, PSC/ME = *Pleurotus sajor-caju*/Methanolic extract, 7ppi = day 7 post-primary injection of sheep RBCs, 14ppi = day 14 post-primary injection of sheep RBCs, 7psi = day 7 post-secondary injection of sheep RBCs, 14psi = day 14 post-secondary injection of sheep RBCs

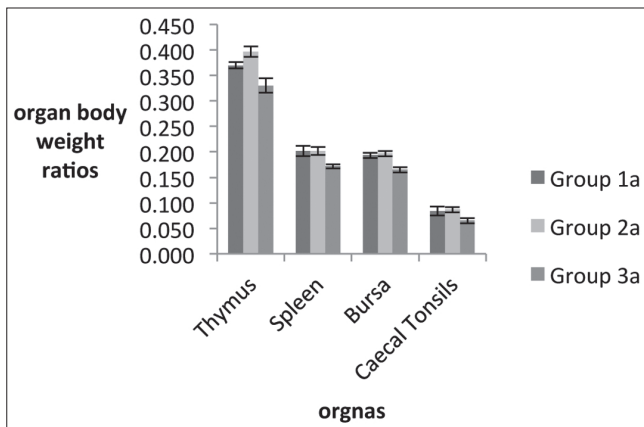


Fig 2. Organ to body weight ratios of experimental and control groups. Group 1a: Hot water extract of *Pleurotus sajor-caju*, Group 2a: Methanol extract of *Pleurotus sajor-caju*, Group 3a: Control

sacrificed to obtain lymphoid organs including thymus, spleen, bursa of Fabricius and cecal tonsils. Organ to body weight ratios of these organs were statistically non-significant ($P > 0.05$) (Fig. 2).

Evaluation of Anti-Eimeria Effects of P. sajor-caju Extracts

Post-challenge with *Eimeria* species, the birds in experimental groups administered with mushroom extracts and control group were monitored for OPG, percent protection and lesion scoring. In control group, significantly higher ($P < 0.05$) OPG of droppings were observed as compared to *P. sajor-caju* extracts treated experimental groups. Among the mushroom administered groups, minimum OPG was observed in the methanolic extract followed by hot water extract (Fig. 3).

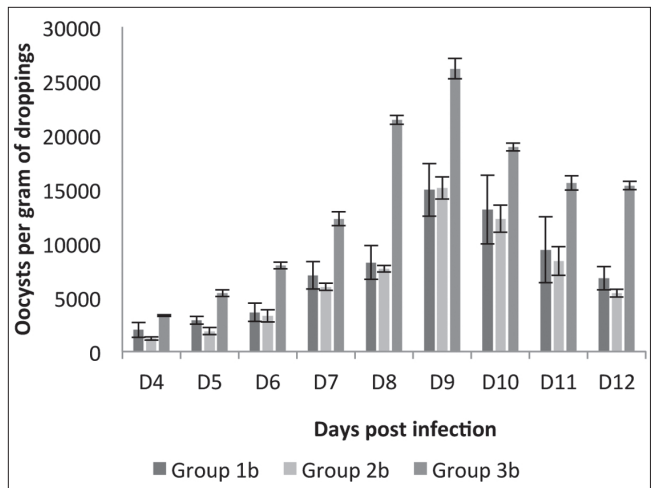


Fig 3. Oocysts per gram of droppings from day 4 to day 12 post challenge with *Eimeria* species. Group 1b: Hot water extract of *Pleurotus sajor-caju*, Group 2b: Methanolic extract of *Pleurotus sajor-caju*, Group 3b: Control

Table 3. Lesion Scoring observed in intestines of experimental and control birds

Groups	Lesion Scoring of the Birds				
	0	1	2	3	4
Group 1b	4	7	5	4	5
Group 2b	3	6	5	4	7
Group 3b	1	2	4	6	12

Group 1b: Birds given Hot water extract of *Pleurotus sajor-caju*, Group 2b: Birds given Methanol extract of *Pleurotus sajor-caju*, Group 3b: Control

Highest protection (45%) was observed in group treated with methanolic extract followed by hot water extract treated group (31%) and control group (24%). Birds of all the experimental groups designated for anti-*Eimeria* activities were evaluated for intestinal lesion scorings on a scale of 0 to 4. Results showed that severe lesion scores (3-4) were lesser in birds of groups administered with hot water (09) and methanolic extracts (11) as compared to those of control group (18) (Table 3). It indicated the protective efficacy of *Pleurotus* extracts against adverse effects of *Eimeria* infection on the intestine of the affected birds.

DISCUSSION

Oysters (*Pleurotus*) are among the popular edible mushrooms consumed by human being worldwide. This group of mushroom is cosmopolitan for their nutritional and therapeutic properties. Several studies in the past revealed medicinal potentialities of different species of oyster mushrooms and classified them as "mushroom nutraceuticals" [14,24]. Keeping in view the medicinal properties of *Pleurotus* species, current study was designed to evaluate immunotherapeutic activities of hot water and methanolic extracts of *P. sajor-caju* against coccidiosis in broiler.

In a recent study, proximate analysis of 10 mushrooms (*Cantharellus cibarius*, *Rusula delica* var *chloroides*, *Ramaria largentii*, *Hygrophorus russula*, *Amanita caesarea*, *Fistulina hepatica*, *Boletus aureus*, *Armillaria tabesceus*, *Armillaria mellea* and *Lepista nuda*) were evaluated to determine the concentration of carbohydrates (55.33-66.87), fats (2.10-6.00), proteins (21.57-34.77), and ash (5.61-9.44) [25]. In another study, nutritional composition of different *Pleurotus* species including *P. pulmonaris*, *P. floridanus*, *P. cystidiosus* and *P. sajor-caju* was demonstrated on dry weight basis. Carbohydrates, proteins, crude fat, crude fibers and ash were found in the range of 85.86-88.38%, 0.98-2.17%, 0.62-0.84%, 2.76-3.12% and 1.03-2.20%, respectively [12]. Results found in the current study have slight variations with the above-mentioned studies. It may be due to different ways and times of harvesting, different storage conditions, species differences, difference of compost used during mushroom growth, different conditions of management and other parameters including temperature, relative humidity, water availability and geographical distribution [26-28].

Mushroom extracts in the current study revealed remarkable cellular immune response in terms of increased thickness of toe web in birds treated with hot water and methanolic extracts of *P. sajor-caju*. These immunomodulatory activities may be due to activation of NK cells, higher production of interferon, enhanced complement activation, potentiation of phagocytic activities and prevention of leukocyte reduction [29,30]. In another study, after administration of *Lentinus edodes*' polysaccharides, chickens challenged and infected with Marek's disease showed significant increase in T-cell proliferation and interleukin production [31,32]. Studies in other animals including rats showed almost similar results as after administration of different mushrooms resulted in the enhancement of T-cell mediated immune responses including lymphocyte proliferation, increasing spleen thymus indexes and corrections of immunosuppression after administration of immune inhibitors such as cyclophosphamide and dexamethasone [33,34]. Moreover, mushroom extracts obtained from different mushrooms also enhanced the production of interleukin 1 and 8 [35]. Likewise, polysaccharide isolated from *Lentinus edodes* enhanced cell-mediated immune response through delayed type of hypersensitivity reaction shown through enhancement in proliferation of splenocytes and increased production of TNF- α and IFN- γ [36]. However, the actual mechanism involved in the enhancement of cellular immune response is still obscure.

Enhanced immunoglobulin production in chicken mucosa had been reported in the past after administration of different mushrooms including *Lentinus edodes*, *Tremella fuciformis* and *Fomitella fuciformis* [33,37]. This enhanced production may be due to proliferation and activation of immune effector cells including macrophages, T-helper, natural killer cells and dendritic cells. Enhanced activation and proliferation of these cells increase the production of

certain cytokines such as IL-12 and IFN- γ after administration of different extracts of *Agaricus brazelliance* and *Antrodia camphorate*. These cytokines may be responsible for activation of effector phase of innate and adaptive immunity [38,39].

After challenge infection of sporulated oocysts of *Eimeria*, oocysts per gram of droppings, mortality percentage and lesion scores in experimental groups were observed. In this regard, limited research has been conducted on the effects of mushrooms particularly against *Eimeria* infection in chicken [40-42]. Birds administered with hot water and methanolic extracts of *P. sajor-caju*, showed excellent protection against coccidiosis in chicken. This might be due to polysaccharides present in the crude extracts [33-35]. Similar findings had also been reported in some previous studies [43,44]. In conclusion, results of this study revealed the immunopotentiating and anti-*Eimeria* efficacy of hot water and methanolic extracts of *Pleurotus sajor-caju*. Further studies to rule out the specific mechanism of action of these extracts for such activities and their commercial feasibility are underway in our lab.

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REFERENCES

1. Valverde ME, Hernández-Pérez T, Paredes-López O: Edible mushrooms: Improving human health and promoting quality life. *Int J Microbiol*, 2015:376387, 2015. DOI: 10.1155/2015/376387
2. Correa RCG, Brugnari T, Bracht A, Peralta RM, Ferreira ICFC: Biotechnological, nutritional and therapeutic uses of *Pleurotus* spp. (Oyster mushroom) related with its chemical composition: A review on the past decade findings. *Trends Food Sci Technol*, 50, 103-117, 2016. DOI: 10.1016/j.tifs.2016.01.012
3. Grujic M, Dojnov B, Potocnik I, Duduk B, Vujcic Z: Spent mushroom compost as substrate for the production of industrially important hydrolytic enzymes by fungi *Trichoderma* spp. and *Aspergillus niger* in solid state fermentation. *Int Biodeterior Biodegradation*, 104, 290-298, 2015. DOI: 10.1016/j.ibiod.2015.04.029
4. Gonzaga MLC, Ricardo NMPS, Heatley F, Soares SA: Isolation and characterization of polysaccharides from *Agaricus blazei* Murill. *Carbohydr Polym*, 60, 43-49, 2005. DOI: 10.1016/J.CARBPOL.2004.11.022
5. Wasser SP: Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Appl Microbiol Biotechnol*, 89, 1323-1332, 2011. DOI: 10.1007/s00253-010-3067-4
6. Carbonero ER, Ruthes AC, Freitas CS, Utrilla P, Galvez J, da Silva EV, Sasaki GL, Gorin PAJ, Iacomini M: Chemical and biological properties of a highly branched β -glucan from edible mushroom *Pleurotus sajor-caju*. *Carbohydr Polym*, 90, 814-819, 2012. DOI: 10.1016/j.carbpol.2012.06.005
7. Heleno SA, Barros L, Sousa MJ, Martin A, Ferreira ICFR: Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchem J*, 93, 195-199, 2009. DOI: 10.1016/j.microc.2009.07.002
8. Shao BM, Dai H, Xu W, Lin ZB, Gao XM: Immune receptors for polysaccharides from *Ganoderma lucidum*. *Biochem Biophys Res Commun*, 323, 133-141, 2004. DOI: 10.1016/j.bbrc.2004.08.069
9. Lin KI, Kao YY, Kuo HK, Yang WB, Chou A, Lin HH, Alice LY, Wong

- CH:** Reishi polysaccharides induce immunoglobulin production through the TLR4/TLR2-mediated induction of transcription factor Blimp-1. *J Biol Chem*, 281, 24111-24123, 2006. DOI: 10.1074/jbc.M601106200
- 10. Ullah MI, Akhtar M, Awais MM, Anwar MI, Khaliq K:** Evaluation of immunostimulatory and immunotherapeutic effects of tropical mushroom (*Lentinus edodes*) against eimeriasis in chicken. *Trop Anim Health Prod*, 50, 97-104, 2018. DOI: 10.1007/s11250-017-1407-4
- 11. Zhang M, Cui SW, Cheung PCK, Wang Q:** Antitumor polysaccharides from mushroom: a review on their isolation, process, structural characteristics and antitumor activity. *Trends Food Sci Technol*, 18, 4-19, 2007. DOI: 10.1016/j.tifs.2006.07.013
- 12. Inacio FD, Ferreira RO, de Araujo CAV, Brugnari T, Castoldi R, Peralta RM, de Souza CGM:** Proteases of wood rot fungi with emphasis on the genus *Pleurotus*. *BioMed Res Int*, 2015:290161, 2015. DOI: 10.1155/2015/290161
- 13. Schepetkin, IA, Quinn MT:** Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *Int Immunopharmacol*, 6, 317-333, 2006. DOI: 10.1016/j.intimp.2005.10.005
- 14. Sarangi I, Ghosh D, Bhutia SK, Mallick SK, Maiti TK:** Anti-tumor and immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. *Int Immunopharmacol*, 6, 1287-1297, 2006. DOI: 10.1016/j.intimp.2006.04.002
- 15. Kaneno R, Fontanari LM, Santos SA, Di Stasi LC, Rodrigues FE, Eira AF:** Effects of extracts from Brazilian sun- mushroom (*Agaricus blazei*) on the NK activity and lymphoproliferative responsiveness of Ehrlich tumor-bearing mice. *Food Chem Toxicol*, 42, 909-916, 2004. DOI: 10.1016/j.fct.2004.01.014
- 16. Han SSR, Cho CK, Lee YW, Yoo HS:** Antimetastatic and immunomodulating effect of water extracts from various mushrooms. *J Acupunct Meridian Stud*, 2, 218-227, 2009. DOI: 10.1016/S2005-2901(09)60058-3
- 17. Yang JH, Lin HC, Mau JL:** Antioxidant properties of several commercial mushrooms. *Food Chem*, 77, 229-235, 2002. DOI: 10.1016/S0308-8146(01)00342-9
- 18. Ahmad I, Hussain M, Ahmad MSA, Hameed M, Ashraf MY, Shinwari ZK, Nawaz T, Batool R, Fatima S:** Spatio-temporal variations in some medicinally important biochemical constituents of *Peganum harmala* (hermal). *Pak J Bot*, 45, 601-607, 2013.
- 19. Awais MM, Akhtar M, Anwar MI, Khaliq K:** Evaluation of *Saccharum officinarum* L. bagasse-derived polysaccharides as native immunomodulatory and anticoccidial agents in broilers. *Vet Parasitol*, 249, 74-81, 2018. DOI: 10.1016/j.vetpar.2017.11.012
- 20. Khaliq K, Akhtar M, Awais MM, Anwar MI:** Evaluation of immunotherapeutic effects of *Aloe vera* polysaccharides against coccidiosis in chicken. *Kafkas Univ Vet Fak Derg*, 23 (6): 895-901, 2017. DOI: 10.9775/kvfd.2017.17957
- 21. Ryley JF, Meade R, Hazelhurst J, Robinson TE:** Methods in coccidiosis research: Separation of oocysts from faeces. *Parasitol*, 73, 311-326, 1976. DOI: 10.1017/S0031182000046990
- 22. Guven E, Avcioglu H, Aslan A, Hayirli A:** Anticoccidial efficacy of usnic acid in broilers. *Kafkas Univ Vet Fak Derg*, 22 (4): 551-556, 2016. DOI: 10.9775/kvfd.2016.15008
- 23. Burgh M:** A Simple method for recording and analyzing serological data. *Avian Dis*, 22, 362-365, 1978. DOI: 10.2307/1589552
- 24. Patel Y, Naraian R, Singh VK:** Medicinal properties of *Pleurotus* species (oyster mushroom): A review. *World J Fungal Plant Biol*, 3, 1-12, 2012.
- 25. Ouzouni PK, Veltsistas PG, Paleologos EK, Riganakos KA:** Determination of meat content in wild edible mushroom species from regions of Greece. *J Food Compos Anal*, 20, 480-486, 2007. DOI: 10.1016/j.jfca.2007.02.008
- 26. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L:** Nutrients in edible mushrooms: An inter-species comparative study. *Food Chem*, 65, 477-482, 1999. DOI: 10.1016/S0308-8146(98)00212-X
- 27. Shamtsyan M, Konusova V, Maksimova Y, Goloshchev A, Panchenko A, Simbirtsev A, Petrishchev N, Denisova N:** Immunomodulating and anti-tumor action of extracts of several mushrooms. *J Biotechnol*, 113, 77-83, 2004. DOI: 10.1016/j.jbiotec.2004.04.034
- 28. Reis FS, Barros L, Martins A, Ferreira ICFR:** Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food Chem Toxicol*, 50, 191-197, 2012. DOI: 10.1016/j.fct.2011.10.056
- 29. She QB, Ng TB, Liu WK:** A novel lectin potent immunomodulatory activity isolated from both fruiting bodies and cultured mycelia of the edible mushroom *Volvariella volvacea*. *Biochem Biophys Res Commun*, 247, 106-111, 1998. DOI: 10.1006/bbrc.1998.8744
- 30. Awais MM, Akhtar M, Iqbal Z, Muhammad F:** *Saccharum officinarum* derived high molecular mass glycoproteins as native biological response modifiers in chickens. *Pak Vet J*, 34, 464-468, 2014.
- 31. Wei P, Li QZ, Hao YH:** Effect of *Astragalus mongholicus* Bga and *Lentinus edodes* polysaccharide on Lipid peroxide (LPO) content in VMDV infected chicks. *J Trad Chin Vet Med*, 4, 3-4, 1997.
- 32. Liu JJ, Huang TS, Hsu ML, Chen CC, Lin WS, Lu FJ, Chang WH:** Antitumor effects of the partially purified polysaccharides from *Antrodia camphorate* and the mechanism of its action. *Toxicol Appl Pharmacol*, 201, 186-193, 2004. DOI: 10.1016/j.taap.2004.05.016
- 33. Liang JL, Hu TJ, Fan BT:** Experiment of 8301 polysaccharide including IFN production in rats. *J Trad Chin Vet Med*, 4, 12-13, 1995.
- 34. Guo FC, Kwakkel RP, Williams BA, Parmentier HK, Li WK, Yang ZQ, Verstegen MW:** Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of *Eimeria tenella*-infected chickens. *Poult Sci*, 83, 1124-1132, 2004. DOI: 10.1093/ps/83.7.1124
- 35. Guo FC, Kwakkel RP, Williams BA, Suo X, Li WK, Verstegen MWA:** Coccidiosis immunization: effects of mushroom and herb polysaccharides on immune responses of chickens infected with *Eimeria tenella*. *Avian Dis*, 49, 70-73, 2005. DOI: 10.1637/7227-062504R1
- 36. Chen HL, Li DF, Chang BY, Gong LM, Piao XS, Yi GF, Zhang JX:** Effects of lentinan on broiler splenocytes proliferation, Interleukin-2 production, and signal transduction. *Poult Sci*, 5, 760-766, 2003. DOI: 10.1093/ps/82.5.760
- 37. Girard F, Fort G, Yvore P, Quere P:** Kinetics of specific immunoglobulin A, M and G production in the duodenal and caecal mucosa of chicken infected with *Eimeria acervulina* or *Eimeria tenella*. *Int J Parasitol*, 27, 803-809, 1997. DOI: 10.1016/S0020-7519(97)00044-1
- 38. Zhang Q, Lin Z:** Study on antitumor activity and mechanism of *Ganoderma* polysaccharides B. *Chin J Integ Trad West Med*, 19, 544-547, 1999.
- 39. Borchers AT, Keen CL, Gershwin ME:** Mushrooms, tumors and immunity: An update. *Exp Biol Med*, 5, 393-406, 2004. DOI: 10.1177/153537020422900507
- 40. Moradali MF, Mostafavi H, Ghods S, Hedjaroude GA:** Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). *Int Immunopharmacol*, 7, 701-724, 2007. DOI: 10.1016/j.intimp.2007.01.008
- 41. Dalloul RA, Lillehoj HS, Lee JS, Lee SH, Chung KS:** Immunopotentiating effect of a *Fomitella fraxinea* - derived lectin on chicken immunity and resistance to coccidiosis. *Poult Sci*, 85, 446-451, 2006. DOI: 10.1093/ps/85.3.446
- 42. Willis WL, Wall DC, Isikhuemhen OS, Ibrahim S, Minor RC, Jackson J, Hurley SL, Anike F:** Effect of different mushrooms fed to *Eimeria*-challenged broilers on rearing performance. *Int J Poult Sci*, 11, 433-437, 2012.
- 43. Sarı B, Çakmak A:** Preventive effects of anticoccidial drug supplementation in chicken feed against coccidiosis in broiler farming. *Kocatepe Vet J*, 1, 1-10, 2008.
- 44. Ozkan O, Sarı B, Bayazit M, Dogan A, Akpulat HA, Erdag D:** Anticoccidiosis effect of *Thymus serpyllum* in rabbits: Oocyst shedding and body weight changes. *Kafkas Univ Vet Fak Derg*, 16, 323-327, 2010. DOI: 10.9775/kvfd.2009.790