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Full Length Article

Ameliorating Effects of Different Extracts of Culinary Mushroom Species on the Production Performance of Healthy and *Eimeria* Infected Commercial Broiler Birds

Muhammad Irfan Ullah[1,2*], Masood Akhtar[1,2], Muhammad Irfan Anwar[1], Mian Muhammad Awais[1], Kashfa Khaliq[2] and Nabeel Ijaz[3]

[1]*Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan, Pakistan*
[2]*Immunoparasitology Laboratory, Department of Parasitology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan*
[3]*Department of Clinical Sciences, Faculty of Veterinary Sciences Bahauddin Zakariya University Multan, Pakistan*
[*]For correspondence: irfanullah@bzu.edu.pk; irfanullahvet@gmail.com
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Abstract

In this research, three edible mushroom species including *Pleurotus (P.) ostreatus*, *P. sajor-caju* and *Lentinus (L.) edodes* were processed for hot water, methanolic and polysaccharide extracts. These extracts were administered to commercial broiler birds. Production performance was determined as weight gains and feed conversion ratios (FCR) in healthy birds on weekly basis. Further, groups were subjected to oral *Eimeria* infection and weight gains were monitored from days 4 to 12 post inoculation. Study revealed significantly higher ($P < 0.05$) weight gains in experimental groups given different mushroom extracts during 4th, 5th and 6th weeks of experiment in comparison to control. Feed conversion ratios observed in the control groups were significantly higher ($P < 0.05$) as compared to experimental groups especially methanolic extracts of all three mushroom species during 5th and 6th weeks of the experiment. After *Eimeria* inoculation, weight gains of the experimental groups given different mushroom extracts were significantly higher ($P < 0.05$) on 10th, 11th and 12th day. Results concluded that aqueous, methanolic and polysaccharide extracts of *P. ostreatus*, *P. sajor-caju* and *L. edodes* had potential to improve the production in healthy birds and may also enhance the weights in commercial broiler birds suffering from *Eimeria* infection.

Keywords: *Eimeria*; Coccidiosis; Mushroom; Production; Chicken; Broiler

Introduction

There is an increasing concern among poultry consumers regarding the irrational use of synthetic medicines in the poultry feed for prophylactic and growth promoting effects. Moreover, widespread use of antibiotics in poultry feed has resulted in rapid development of resistance against several pathogens (Cowieson and Kluenter 2019). These undesirable effects led to ban on the use of antibiotic growth promoters in the poultry feed in Europe in January 2006 (Salim *et al*. 2018). These restrictions caused the enteric pathogens to grow more to affect the health and production of the poultry birds. Therefore, it is needed to substitute substances from natural sources to maintain and increase production in the poultry industry. Intensified research proceeded in the last 2–3 decades for the development of certain substances that can be alternated to antibiotics for the improvement of health and production in poultry industry. Among these substances, probiotics, prebiotics, nutraceuticals, plant extracts and acidifiers were investigated (Dharma and Tomar 2007; Adhikari and Kim 2017; Abbas *et al.* 2020; Hazrati *et al.* 2020).

Coccidiosis is a major protozoal infection causing huge economic losses to the poultry producers in terms of compromised usage of feed and reduced growth rates of birds (Khater *et al.* 2020). Prophylactic medication to avian coccidiosis adds considerable increase in the prices of poultry feed (Lillehoj *et al.* 2004). Additionally, growth promoters are also commonly used in the poultry feed to exploit the production potential of genetically engineered birds. Different types of growth promoters including probiotics, prebiotics, enzymes, antioxidants and coccidiostats are being used in poultry industry (Angelakis *et al.* 2013). In case of withdrawal of these agents from the poultry feed, productive performance of the poultry birds severely affects the production parameters along with increasing the vulnerability of the birds towards different diseases (Yasmin *et al.* 2020).

Different mushrooms have been reported to possess growth promoting and therapeutic activities against several ailments (Willis *et al.* 2012, 2013; Gargano *et al.* 2017). Mushrooms have dietary priority over other sources from plant origin because of higher contents of good quality protein, dietary fibres and vitamin B with less fat and no cholesterol (Ghorai *et al.* 2009). Exudate secreted from the cell surface of mycelia of mushrooms contains natural antibiotics and some of these antibiotics target specific pathogens (Willis *et al.* 2009).

Keeping in view the beneficial effects of mushrooms, present study was designed to investigate the effects of extracts obtained from different culinary mushroom species on the production performance of healthy as well as commercially grown broiler birds infected experimentally with *Eimeria*.

Materials and Methods

Preparation of methanolic and polysaccharide extracts

Mushroom species including *Pleurotus ostreatus* (PO), *P. sajor-caju* (PSC) and *Lentinus edodes* (LE) procured from the local grower in Millat Town Faisalabad were identified (Voucher no. 173 (LE), 174 (PO) & 175 (PSC), dried, pulverized and sieved. All three mushroom species were processed for hot water extract (HWE), methanolic extract (ME) and polysaccharide extract (PSE). For HWE, mushroom powder (500 g) was added in 1500 mL of distilled water and stirred followed by boiling (4 h), centrifugation (3000 rpm for 30 min) and lyophilization. For ME, mushroom powder (500 g) was added in 1000 mL of 80% methanol and vortexed for 24 h followed by rotary evaporation and lyophilization. For PSE, mushroom powder (200 g) added in 95% ethanol for 24 h followed by ultrasonication, centrifugation (4000 rpm/30 min) and washing with ethanol, acetone and ether. Crude extract thus obtained was fractionated through diethylaminoethyl (DEAE–52) cellulose column followed by Sephadex G-100 columns to obtain polysaccharide extracts (Ullah *et al.* 2014).

Preparation of mixed species *Eimeria* infection

Intestines of the broiler birds were collected from the butcher shops and poultry farms around Faisalabad. After direct microscopic examination of the intestinal contents, positive samples were separated and contents were squeezed to collect the infected material. Equal quantity of 2.5 per cent $K_2Cr_2O_7$ added to the contents having mixed species of *Eimeria* (for 48–72 h). The mixture was centrifuged at 1500 rpm for 10 min. Sediment thus obtained was processed for $ZnSO_4$ floatation technique. Supernatant thus collected was washed with PBS three times and challenged dose was adjusted at 6.5–7.0 × 10^4 sporulated oocyst (Mumtaz *et al.* 2021).

Experimental design

Present study was divided into two experiments. In the experiment 1, HWE and ME, of LE, PO and PSC mushrooms were investigated. Briefly, a total of 350 birds were kept at experiment station of Parasitology Department, University of Agriculture-Faisalabad Pakistan. After 5 days, acclimatized birds were divided randomly into 7 (n=50) groups and given mushroom extracts on day 7th, 8th and 9th of the experiment. In the experiment 2, PSE of LE, PO and PSC were investigated for production performance of broiler birds. A total of 200-day old birds were kept in the experiment station of Microbiology Department Veterinary Sciences Faculty University of Agriculture Faisalabad. On day 5th, birds were divided into 4 (n=50) groups and given PSE on day 7th, 8th 9th (Table 1) Experimental groups assigned were given different mushroom extracts dissolved in PBS (Phosphate Buffer Saline) as solvent. However, control groups of both experiments were given 2 mL of PBS.

In both experiments, following routine vaccination schedule for Newcastle Disease, Infectious Bursal Disease and Hydropericardium Syndrome was done and birds were offered withdrawal feed and water *ad-libitum*. All the experimental groups including controls were given mixed specie *Eimeria* infection orally, on day 21st of the experiment at the dose rate of 6.5–7.0 × 10^4 oocysts/bird.

Weight gains and feed conversion ratios (FCR) of healthy birds

Birds belonging to all groups from 1st and 2nd experiments were kept in separate cages and monitored for feed consumption and weight gains weekly for up to 6 weeks of age and similarly, feed conversion ratios (FCR) were also calculated by using the following formula:

$$FCR = \frac{\text{Total feed consumed (gms)}}{\text{Total weight gains (gms)}}$$

Weight gains post experimentally induced *Eimeria* infected birds

Half of birds from all experimental and control groups were separated in both 1st & 2nd experiments and were given mixed specie infection of *Eimeria* at the dose rate of (6.5–7.0 × 10^4) sporulated oocysts on day 21st of the experiment and weights were recorded from day 4th to day 12th post *Eimeria* challenge. Oocysts per gram of feces, lesion scoring, and mortality percentages were also calculated (Ullah *et al.* 2014. 2015, 2018a, b).

Statistical analysis

Data obtained in the experiments was subjected to S.P.S.S. v. 21 and Tuckey's range test was used for one-way ANOVA to determine the differences among various groups at $P < 0.05$.

Results

Experiment 1

Weekly weight gains: In the experiment 1, birds from all groups were weighed and feed consumed by each group was noted during 1st to 6th weeks of the experiment. Groups given Hot Water Extracts (HWE) and Methanolic Extracts (ME) of *P. ostreatus* (PO), *L. edodes* (LE) and *P. sajor-caju* (PSC), respectively showed significantly higher ($P < 0.05$) body weights as compared to control during the last three weeks (4th, 5th and 6th) of the experiment. In the 4th week of the experiment, among different groups highest weights (g) were observed in group given ME of LE (1016) followed by HWE of LE (967), ME of PSC (950), ME of PO (910), HWE of PSC (892) and HWE of PO (858). In the 5th week, highest weights were observed in the ME of LE (1396) followed by ME of PSC (1295), HWE of LO (1253), ME of PO (1238), HWE of PSC (1218) and HWE of PO (1140). In the last week of the experiment, Maximum weights were observed in HWE and ME of LE (1722, 1669) followed by PSC (1631, 1608) and PO (1610, 1555), respectively (Table 2).

Feed conversion ratios (FCR): In control group, FCR observed during 6th weeks of the experiment was significantly higher ($P < 0.05$) as compared to groups treated with HWE of LE, PSC and ME of PO, LE and PSC. In 5th week of the experiment, groups given ME of LE and PSC showed significantly higher ($P < 0.05$) performance in terms of FCR as compared to control. In 4th and 2nd weeks of experiment, groups given ME of all three mushroom species showed significantly better performance in FCR. Among different experimental groups, groups administered with ME of LE showed better performance followed by ME of PSC and PO (Fig. 1).

Weight gains post *Eimeria* infection: The experimental and control groups were subjected to infective dose of *Eimeria* and weight gains were monitored from day 4th to day 12th post *Eimeria* infection. In the experiment 1, on day 6th, 10th and 11th weight gains observed in all experiment groups were statistically higher ($P < 0.05$) as compared to control. Among different groups, in most of the days highest weights post infection were observed in ME and HWE of LE followed by ME and HWE of PSC and PO, respectively (Fig. 2).

Experiment 2

Weekly weight gains: Polysaccharide extracts (PSE) of PO, LE and PSC were evaluated in term of weekly weight gains. Weights (g) observed during 5th and 6th weeks of the experiment were significantly higher ($P < 0.05$) in PSE of PO, LE and PSC. Among the mushroom treated groups in the 5th week of the experiment, highest weights (g) were observed in PSE of LE (1352) followed by PO (1335) and PSC (1272). During 6th week of the experiment, highest weights were observed in PSE of LE (1851) followed by PO (1820) and PSC (1580), respectively (Table 3).

Feed conversion ratios: FCR observed in control groups

Table 1: Different treatment groups assigned in Experiment 1 and 2

Experiment 1:	Experiment 2:
G1: HWE of PO @ 200 mg/kg B.Wt.	G1a: PSE of PO @ 25 mg/kg B. Wt.
G2: HWE of LE @ 200 mg/kg B.Wt.	G2b: PSE of LE @ 25 mg/kg B. Wt.
G3: HWE of PSC @ 200mg/kg B.wt.	G3c: PSE of PSC @ 25 mg/kg B. Wt.
G4: ME of PO @ @ 200mg/kg B.wt.	G4d: Control: 2 mL PBS saline
G5: ME of LE @ 200 mg/kg B. wt.	
G6: ME of PSC@ 200 mg/kg B. wt.	
G7: Control: 2 mL PBS saline	

HWE=Hot water Extract, ME=Methanol extract, PSE= Polysaccharide extract, PO= *Pleurotus ostreatus*, LE=*Lentinus edodes*, PSC=*Pleurotus sajor-caju*, B. Wt.=Body Weight

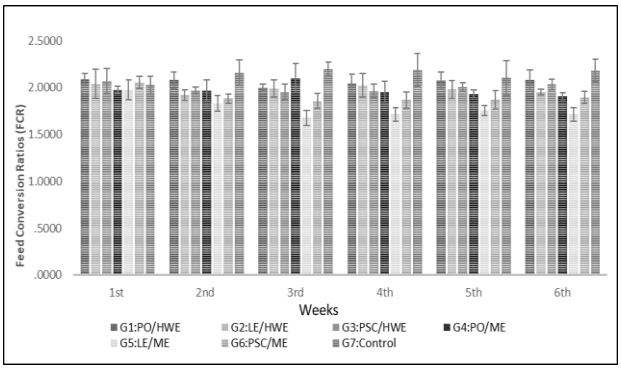

Fig. 1: Weekly feed conversion ratios (FCR) of experimental and control groups in experiment 1.

PO=*Pleurotus ostreatus*, LE= *Lentinus edodes*, PSC=*Pleurotus sajor-caju*, HWE= Hot Water Extract, ME= Methanol Extract. G1: Hot water extract of *Pleurotus ostreatus* administered @ 200 mg /kg body weight. G2: Hot water extract of *Lentinus edodes* administered @ 200 mg /kg body weight. G3: Hot water extract of *Pleurotus sajor-caju* administered @ 200 mg /kg body weight. G4: Methanolic extract of *Pleurotus ostreatus* administered @ 200 mg /kg body weight. G5: Methanolic extract of *Lentinus edodes* administered @ 200 mg /kg body weight. G6: Methanolic extract of *Pleurotus sajor-caju* administered @ 200 mg /kg body weight. G7: Control

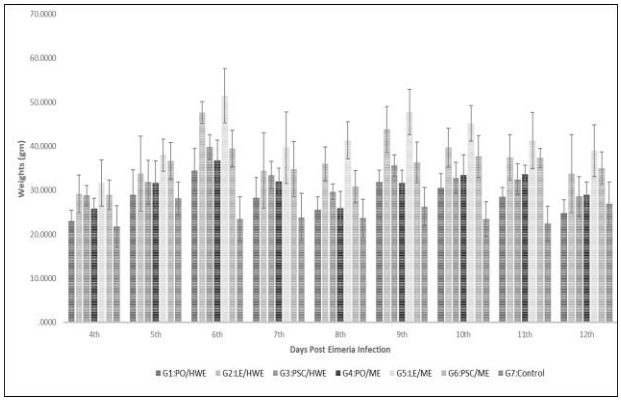

Fig. 2: Weight gains of experimental and control groups post *Eimeria* infection in experiment 1.

PO=*Pleurotus ostreatus*, LE= *Lentinus edodes*, PSC=*Pleurotus sajor-caju*, HWE= Hot Water Extract, ME= Methanol Extract. G1: Hot water extract of *Pleurotus ostreatus* administered @ 200 mg /kg body weight. G2: Hot water extract of *Lentinus edodes* administered @ 200 mg /kg body weight. G3: Hot water extract of *Pleurotus sajor-caju* administered @ 200 mg /kg body weight. G4: Methanolic extract of *Pleurotus ostreatus* administered @ 200 mg /kg body weight. G5: Methanolic extract of *Lentinus edodes* administered @ 200 mg /kg body weight. G6: Methanolic extract of *Pleurotus sajor-caju* administered @ 200 mg /kg body weight G7: Control

Table 2: Weekly weight gains (g) in experimental and control groups (Experiment 1)

Weeks	Groups						
	G1 (mean ± SE)	G 2 (mean ± SE)	G 3 (mean ± SE)	G 4 (mean ± SE)	G 5 (mean ± SE)	G 6 (mean ± SE)	G 7 (mean ± SE)
1st	103 ± 5.41^{b}	120 ± 8.37^{ab}	108 ± 4.32^{b}	110 ± 4.42^{b}	134 ± 2.99^{a}	124 ± 3.85^{ab}	110 ± 4.76^{b}
2nd	241 ± 3.82^{cd}	276 ± 6.51^{ab}	251 ± 3.91^{bcd}	265 ± 6.24^{abc}	293 ± 6.73^{a}	241 ± 3.82^{cd}	232 ± 11.62^{d}
3rd	367 ± 10.86^{abc}	408 ± 6.10^{ab}	397 ± 8.15^{abc}	394 ± 5.29^{abc}	435 ± 5.63^{a}	403 ± 7.82^{abc}	345 ± 31.58^{c}
4th	858 ± 8.14^{d}	967 ± 7.93^{b}	892 ± 12.0^{cd}	910 ± 4.70^{c}	1016 ± 8.15^{a}	950 ± 8.4^{b}	802 ± 8.03^{e}
5th	1140 ± 9.39^{d}	1253 ± 14.39^{bc}	1218 ± 7.75^{c}	1238 ± 11.39^{c}	1396 ± 10.62^{a}	1295 ± 7.88^{b}	1023 ± 17.97^{e}
6th	1555 ± 9.09^{d}	1669 ± 12.93^{ab}	1608 ± 14.27^{cd}	1610 ± 13.09^{cd}	1722 ± 9.00^{a}	1631 ± 10.31^{bc}	1363 ± 18.71^{e}

Means sharing similar letters in a row are statistically non-significant ($P > 0.05$).
G1: Hot water extract of *Pleurotus ostreatus* administered @ 200 mg /kg body weight. G2: Hot water extract of *Lentinus edodes* administered @ 200 mg /kg body weight. G3: Hot water extract of *Pleurotus sajor-caju* administered @ 200 mg/kg body weight. G4: Methanolic extract of *Pleurotus ostreatus* administered @ 200 mg/kg body weight. G5: Methanolic extract of *Lentinus edodes* administered @ 200 mg /kg body weight. G6: Methanolic extract of *Pleurotus sajor-caju* administered @ 200 mg /kg body weight. G7: Control

Table 3: Weekly weight gains (g) in experimental and control groups (Experiment 2)

Weeks	Groups			
	G1a (mean ± SE)	G1b (mean ± SE)	G1c (mean ± SE)	G1d (mean ± SE)
1st	104 ± 3.15^{b}	124 ± 2.95^{a}	97 ± 2.197^{b}	85 ± 2.07^{c}
2nd	212 ± 5.72^{b}	253 ± 3.45^{a}	209 ± 7.03^{bc}	187 ± 5.71^{c}
3rd	320 ± 4.04^{bc}	359 ± 11.62^{a}	339 ± 3.94^{ab}	298 ± 5.26^{c}
4th	920 ± 6.85^{bc}	1001 ± 7.07^{a}	943 ± 16.76^{b}	884 ± 18.87^{c}
5th	1335 ± 12.35^{ab}	1352 ± 10.38^{a}	1272 ± 16.10^{b}	1059 ± 24.09^{c}
6th	1820 ± 16.15^{a}	1851 ± 10.41^{a}	1580 ± 11.40^{b}	1411 ± 13.04^{c}

Means sharing similar letters in a row are statistically non-significant ($P > 0.05$)
G1a: Polysaccharide Extract of *Pleurotus ostreatus* administered @ 25 mg /kg body weight. G1b: Polysaccharide Extract of *Lentinus edodes* administered @ 25 mg /kg body weight. G1c: Polysaccharide Extract of *Pleurotus sajor-caju* administered @ 25 mg /kg body weight. G1d: Control

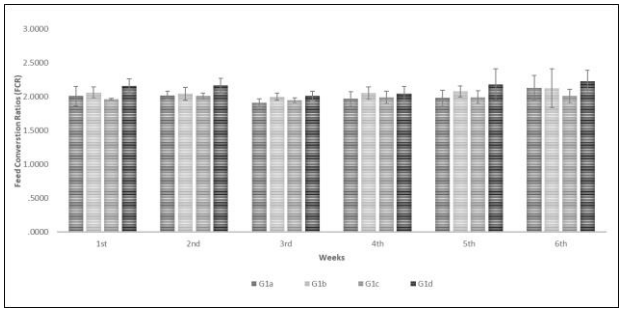

Fig. 3: Weekly Feed Conversion Ratios (FCR) of experimental and control groups in experiment 2
PO=*Pleurotus ostreatus*, LE= *Lentinus edodes*, PSC=*Pleurotus sajor-caju*, PSE= Polysaccharide Extract. G1a: Polysaccharide Extract of *Pleurotus ostreatus* administered @ 25 mg /kg body weight. G1b: Polysaccharide Extract of *Lentinus edodes* administered @ 25 mg /kg body weight. G1c: Polysaccharide Extract of *Pleurotus sajor-caju* administered @ 25 mg /kg body weight. G1d: Control

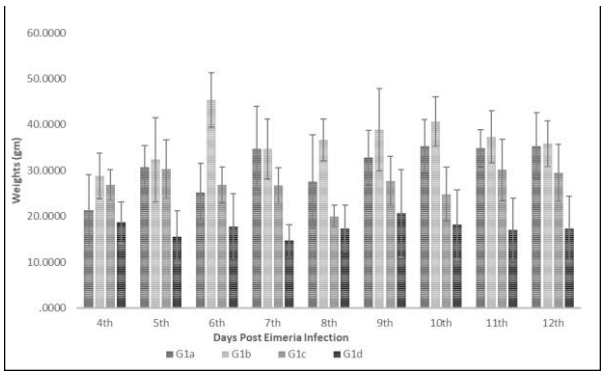

Fig. 4: Weight gains of experimental and control groups post *Eimeria* infection in experiment 2.
PO=*Pleurotus ostreatus*, LE= *Lentinus edodes*, PSC=*Pleurotus sajor-caju*, PSE= Polysaccharide Extract. G1a: Polysaccharide Extract of *Pleurotus ostreatus* administered @ 25 mg /kg body weight. G1b: Polysaccharide Extract of *Lentinus edodes* administered @ 25 mg /kg body weight. G1c: Polysaccharide Extract of *Pleurotus sajor-caju* administered @ 25 mg /kg body weight. G1d: Control

were statistically non-significant ($P > 0.05$) as compared to all experimental groups during 6th, 5th and 4th week of the experiment. However, during 3rd week, FCR observed in group given PSE of PO and in the 2nd week, groups given PSE of PO and PSC showed significant improvement in FCR as compared to control (Fig. 3).

Weight gains post *Eimeria* infection: On day 7th, 11th and 12th weight gains observed in the experimental groups were statistically higher ($P < 0.05$) as compared to control. Among different experimental groups, in most of the days highest weight gains were observed in PSE of LE followed by PO and PSC (Fig. 4).

Discussion

In production animals, growth augmentation due to the feeding of nondigestible carbohydrates, terpenes, tannins, flavonoids, saponins, alkaloids, phenolics, resins and minerals from plant source is well reported (Stanley *et al.* 1997; Grizard and Barthomeuf 1999; Toghyani *et al.* 2012; Ashraf *et al.* 2019; Elghobashy *et al.* 2020). In the present study, growth performance of commercial broiler birds was evaluated by monitoring the weekly weight gains and feed consumption ratios. In comparison to control, all experimental groups which were given mushroom extracts revealed significantly higher ($P < 0.05$) weight gains. However, in birds of groups given polysaccharide extracts (PSE), highest gains in the weight and FCR were observed in comparison to hot water extracts (HWE) and methanolic extracts (ME). Additionally, during comparison of different species of mushrooms, *L. edodes* (LO) showed highest values of

gains in the weights and FCR followed by *P. sajor-caju* (PSC) and *P. ostreatus* (PO), respectively. These encouraging results of weight gains and FCR in mushrooms extracts administered chickens are pinpointing that feed utilization in these groups was relatively better as compared to control group. These results agree with the findings of another study in which broilers when supplemented with *L. edodes* and *Tremella fuciformis* revealed statistically significant values of weight gains and lesser conversion ratios of feed as compared to those experimental groups which were not supplemented with mushroom extracts. Moreover, values of feed conversions were lower in groups given *L. edodes* supplementation showed lower FCR than *Tremella fuciformis* administered birds (Guo *et al.* 2004). Similar findings were reported in other studies in which *Agaricus bisporous*, *L. edodes* and *Fomitella fraxinea* were supplemented (Guo *et al.* 2003; Dalloul *et al.* 2006). However, Daneshmand and his co workers in 2011 reported that inclusions of mushrooms alone in the feed had no promotional effects on the production performance, nevertheless if given with probiotics better growth performance can be achieved. Further, it was suggested that the differences observed may be due to the differences of structure, physical characteristics, sugar composition, molar ratios and geographical distribution of mushrooms belonging to different parts of the world. Nevertheless, current study results may suggest that HWE, ME and PSE of PO, PSC and LE can be used as production enhancer in commercial poultry farming.

In this study, post experimental *Eimeria* infection of all the groups, gains in the weight of birds from day 4^{th} to 12^{th} was recorded and found significantly higher ($P < 0.05$) in groups given different mushroom extracts than the birds of control groups. Among the different extracts, ME revealed highest gains in weights followed by HWE and PSE. Among the different mushrooms (LE) showed highest gains in weights after *Eimeria* infection followed by PSC and PO. During the experiment, all groups of chickens were also observed for general behaviour, attitude and health status. Birds belonging from control groups were emaciated, weak and lethargic with ruffled feathers and were slow to choose and pick feed particles and water. However, birds which were given different extracts of mushroom species were active and approach the feed anxiously and similar trend was also observed in the consumption of drinking water among different experimental groups. These changes in the behaviour of different groups may be due to variation in the intestinal homeostasis and ultimately causing variations in bird metabolism followed by lowered weight gains and reduced feed intake (Fernando and McCraw 1973; Adams *et al.* 1996; Pan and Yu 2014).

Conclusion

The study concludes that mushroom species including *P. ostreatus*, *L. edod*es and *P. sajor-caju* have potential to improve the production parameters of broiler birds in healthy animals. Moreover, above mentioned mushrooms can also be used with excellent production results in birds suffering from *Eimeria* infection.

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Author Contributions

MIU, MA and MMA planned the experiment, MIU & MIA contributed equally in experimentation and interpreted the results, KK and NI finalized the write up.

Conflict of Interest

The authors declares no conflict of interest

Data Availability

Data is available

Ethical Approval

All procedures performed in studies were in accordance with the ethical committee of University of Agriculture.

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