EXPERIMENTAL STUDIES ON COCCIDIA (EIMERIA *TENELLA*) INFECTED BROILER TREATED WITH AQUEOUS EXTRACT OF OYSTER MUSHROOM

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ABSTRACT

The present work was undertaken to determine aqueous extract of mushroom *(Pleurotus ostreatus*) as an alternative to other coccidiosis control measures would result in improving body weight, oocyst reduction and improving pathological lesions of cecum. A total of 140 broiler chicks of 14 days age were divided into 7 groups (each contain 20 chicks). The 1st group was not infected with *Eimeria tenella* and didn't take any medicine or additives and kept as control, while the 2nd group was infected with *Eimeria tenella* at a rate of 50,000 sporulated oocyst/ml / bird at 14th day of age. Third group was infected with *Eimeria tenella* as mentioned before at 14th day of age and treated with aqueous extract of mushroom (200 mg/ml) at day 6 post infection and continued for 7 days consecutively. Fourth group was infected with *E. tenella* as mentioned before and treated with amprolium (200 mg/ml) at 6 day post infection and continued for 7 days consecutively.Fifth group was infected with *E. tenella* as mentioned before and treated with both aqueous extract of mushroom (200 mg/ml) and amprolium (200 mg/ml) at 6 day post infection, for 7 days. Sixth group was non infected with *E. tenella* but received aqueous extract of mushroom at 14th day of age (200 mg/ml) and continued till the end of experiment (at 35 days of age). Seventh group not infected with *E. tenella* but received amprolium (200 mg/ml) at 14th day of age and continued till the end of experiment (the period of experiment is three weeks). Body weight was recorded weekly, oocysts counted at 21, 28 and 35 days of age, and pathological lesions were studied at 28 and 35 days of age. The obtained data revealed that chicks of the 2nd group showed a significant decrease in body weight, while third, fifth and sixth groups showed a significant increase in body weight which appeared clearly at 35day of age. Fifth group posses the highest body weight followed by chicks of sixth group then third group. The highest oocyst count was recorded in chicks of the 2nd group while the lowest oocyst count was recorded in 5th, 4th and 3rd groups. Regarding pathological lesions, the infected and untreated 2nd group showed severe necrosis of ceca with destruction and desquamation of the lining epithelium. The necrotic mucosa was heavily infiltrated or replaced with lymphocytes, macrophages and few heterophiles. The pathological lesions were less in the 3rd group (infected and treated with mushroom) and nearly absent in both 4th group (infected and treated with amprolium) and 5th group (infected and treated with both mushroom and amprolium).

Key words: *Eimeria Tenella, Broiler, Mushroom extract.*

INTRODUCTION

Coccidiosis is an acute to chronic infectious disease caused by protozoal parasites of genus *Eimeria* which multiply in intestinal mucosa of chickens and produce severe tissue damage resulting in bloody diarrhea, reduce growth, weight and increase susceptibility to other pathogens. So, avain coccidiosis is a fatal disease which cost the industry millions of dollars annually (Champman and Shirley

2003, Lilleh *et al.,* 2004). The cost associated with prevention via drugs in the feed, adds to the economic hardship of producer. Moreover, the increased drug resistant problems occurring in poultry production has heightened public concern. In some countries the use of drug in production has been severely regulated or banned altogether there for alternative control methods are being researched with great intensity, one such alternative receiving much interest is mushroom. Mushroom a natural health promoting fungi such as (*Pleurotus ostreatus*) and (*Ganoderma lucidumare)* used as food supplemments and medicines to improve various parameters of human and animal health and immue function in certain disease conditions (Chang and Mshigeni, 2001, Anthony and Joyce, 2007 and Fasuyi, 2007).

Mushroom like probiotics are natural ingredients that contain bioactive chemical substances or polysaccharides, protein, crude fibers, unsaturated fat, minerals as (patassium, phosphorus, calcium, iron and zinc), vitamins, essential amino acids and organic acids that can be used as a good source of supplements and medicine to promote health and production (Jang and Briminghan, 1992; Chang and Mshigeni, 2001; Ogbe *et al.,* 2005; Anonymous, 2007; Ezeokeke, 2008 and Selegean *et al.,* 2009). Mushroom stablise microflora in GIT and prevents colonization of host cells by pathogens, also stimulates non specific host immune response or phagocytosis by macrophages (Sundu *et al.,* 2006). There has been a recent upsurge of interest in mushroom not only as a healthy food which is rich in protein (Baross *et al.,* 2008), but also as a source of biologically active compounds of medicinal value which include complementary medicinal dietary supplements for anticancer (Cheung *et al.,* 2003), antioxidant (Chang and Miles, 2004), antimicrobial (Lindequist *et al.,* 1990), antiviral (Brandt and Piraino, 2000 and Mothana *et al.,* 2003) and anti inflammatory (Kim *et al.,* 2003 and 2004).

In addition, it has immunopotentiating (Reshetinkov *et al.,* 2001) hypocholestroemic, (Ishikawa *et al.,* 1984) and anticoocidial effect against *Eimeria tenella* (Ogbe *et al.,* 2009; Naphade *et al.,* 2010; Willis *et al.,* 2012 and Hossain *et al.,* 2013).

Infection with *Eimeria* is known to stimulate a protective immune response in chicken (Yun *et al.,* 2000). The polysaccharide extract from mushroom *(Pleurotus ostreatus)* was shown to have immunomodulating effects in chicken (Selegean *et al.,* 2009). Mushroom can reduce *Eimeria tenella* oocycts output in infected broilers, improve body weight gain and hematological changes that may occur (Ogbe *et al.,* 2009).

This study was carried out to investigate the performance and health promoting effects of aqueous extracts of mushroom (*Pleurotus ostreatus)* on broiler chicks infected with *Eimeria tenella.*

MATERIALS and METHODS

1- Experimental birds: One hundred and forty chicks of 14 day age were obtained from a commercial hatchery. The chicks were weighed, divided into 7 groups (20 chicks each) and housed on wire cages labeled 1 to 7. All groups of birds were fed with standard ration.

2- Preparatio n of aqueous extract of *Pleurotus ostreatus* (according to Ogbe *et al.,* 2009): Mushroom was washed in distilled water, sun dried then ground to powder using a mortar pestle and then blended. The mushroom powder was again sun dried for about 3hours and then stored in plastic polythene bags and kept at room temperature until required. A 20% w/v solution of aqueous extract of mushroom was prepared by soaking in hot water boiled to 100°C for 3 hours brining the concentration to 200mg/ml. The solution was sieved, solid matter discarded and the filtrate allowed to cool to room temperature befor use.

3- Experimental infection and treatment:

1st group: Control group, non infected and non treated.

2nd group: Infected with *Eimeria tenella* at a rate of 50.000 sporulated oocyst/ml per bird using an insulin syring introduced directly into crop of each bird at 14th day of age. (Oocyst per gram (OPG) of feces was counted following McMaster technique.

***3rd group*:** Infected with *Eimeria tenella* as mentioned before at 14th day of age and treated with aqueous extract of mushroom (200mg/ml) by day 6 post infection and continued for 7 days consecutively.

4th group: Infected with *Eimeria tenella* at 14th day of age as mentioned before and treated with amprolium (200mg/ ml drinking water) by 6 day post infection and continued for 7 days consecutively.

5th group: Infected with *Eimeria tenella* as mentioned befor at 14th day of age , by 6 day post infection and treated with both aqueous extract of mushroom (200mg/ml) and amprolium (200mg/ml) given for 7 days consecutively.

6th group: Non infected with *Eimeria tenella* but received aqueous extract of mushroom (200mg/ml) at 14th day of age and continued till the end of experiment.

7th group: Non` infected with *Eimeria tenella* but received amprolium (200mg/ml) at14th day of age and continued till the end of experiment.

1 - Determination of body weight: Body weight of broilers in all groups was monitored weekly using a weighting balance every morning prior to feeding.

2 - Collection of fecal sample and laboratory examination: The faeces of broiler chicks were

collected at 21,28,35 day of age in plastic bags for parasitological examination. Oocysts per gram (OPG) were counted by McMaster's slide (Hodgson, 1970).

3 - The lesion score of *E.tenella* infestation: was carried out according to (Johonson and Reid 1970).

4 - Pathological examination: Clinical signs and post mortem findings were recorded on the experimental birds before and after the day of sacrifice (1 and 2 weeks post infection or post treatment). Specimens from the ceci were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin, five micron sections were prepared and then routinely stained with hematoxylin and eosin (H&E) (Bancroft and Gambl 2008) and then examined microscopically.

5 - Statistical analysis: The obtained data were analyzed using the liner model programs of SAS (1990). The difference among means were tested using Duncan Multiple range test (Duncan, 1955).

RESULT

1. Body weight record: The recurrent results in table (1) showed that at 21 day of age (before treatment), second group (infected, untreated chicks) showed a significant decrease in body weight (465.50±1.5) compared to control 1st group (uninfected, untreated) (540.50±4.5) and compared to all another groups (3rd,4th,5th,6th,7th) which showed non significant difference between each other and between control 1st group. At 28 days of age (one week PT.), 2nd group (infected, untreated) showed a significant decrease in body weight (590.60±9.6) in comparison with control 1stgroup (879.90±13.6) and in comparison with all another groups. Both 5th group (infectd, treated with mushroom and amprolium) and 6th group (uninfected, treated with mushroom) recorded the highest body weight (899.60±16.9,899.60±8.5), they showed non significant difference between each other and between control 1stgroup (879.90±13.6), followed by 3rd group (infectd, treated with mushroom) (886.20±3.9) then 7thgroup (uninfected, treated with amprolium (870.40±3.9) which showed non significant difference between each other and between control 1st group. At 35days of age (2week PT.), 2nd group showed significant decrease in body weight (800.70±8.5) as compared to control 1stgroup (1189.5±14.4) and in comparison with all another groups. Fifth Group recorded the highest body weight (1378.5±18.5) which showed significant increase as compared to control 1stgroup and all another groups, followed by 6th group (1303.4±8.1) then 3rd group (1299.7±2.4) which showed significant increase in body weight in comparison with control 1stgroup. Wherease7th and4th groups (1220.4±5.2, 1208.8±6.9) showed non significant difference in body weight between each other and between contol 1st group.

Table 1: Mean values of body weight of the 7 groups of broilers under experiment.

Parameters	1st group (unchallenged ,untreated)	2ndgroup (challenged ,usntreated)	3rdgroup (challenged, treated with mushroom)	4thgroup (challenged, treated with amprolium)	5thgroup (challenged, treated with mushroom and amprolium)	6th group (unchallenged , treated with mushroom)	7th group (unchallenged ,treated with amprolium)
14 days of age	321.80 ± 14.6 ^{d}	360.20± 2.9^{b}	363.70± 2.1^{ab}	370.70± 3.8^{a}	333.80± 3.81^{c}	363.60± 3.02^{ab}	360.30± 3.8^{b}
21days of age	540.50 ± 4.5^{b}	465.50± 1.5^{c}	554.70± 2.7^{ab}	558.50± 3.5^{ab}	568.20± 13.9^{ab}	560.10± 7.6^{ab}	548.20± 4.1^{ab}
28days of age	879.90 ± 13.6 ^{abc}	590.60± 9.6^{d}	886.20± 3.9^{ab}	864.20± 5.9^{bc}	899.60± 16.9^{a}	899.60± 8.5^{a}	870.40± 3.9^{ab}
35days of age)	1189.5 ± 14.4 ^{C}	800.70± 8.5^{d}	1299.7± 2.4^{b}	1208.8± 6.9^{c}	1378.5± 18.5^{a}	1303.4± 8.1^{b}	1220.4± 5.2^{c}

Value are means ± standard error, means with different letters at the raw differ significantly at (P <0.05).

1 - Ooycsts per gram count: The analysis of results in table (2**)** showed that, at 21 day of age (befor treatment): The oocysts output was (47100±458.25) 2nd group, (45000±1414.21) 3rd group, (45700±422.95) 4th group and

2 - (44200±840.64) 5th group. At 28 day of age (one week post treatment), 2nd group showed high significant increase in OPG counts (70000±1626.17) while 3rd, 4th, 5th groups showed significant decrease in oocysts count (7553±2.33, 2567±1.86, 2067±1.7) respectively as compared to control untreated 2nd group, at the same time 3rd, 4th and 5th groups showed non significant difference in oocyst count between each other. At 35 days of age (2week post treatment), 2nd group showed significant increase in oocysts count (60000±4216.37) while all treated groups (3th, 4th, 5th) showed significant decrease (50±2.58, 2±0.36,1±0.2) respectively as compared to control 2nd group, as well as, these groups showed non significant difference in oocyst count between each other.

Table 2: Mean values of OPG count of broilers

Parameters	1st group (unchallenged, untreated)	2ndgroup (challenged ,usntreated)	3rdgroup (challenged , treated with mushroom)	4thgroup (challenged , treated with amprolium)	5thgroup (challenged , treated with mushroom and amprolium)	6th group (unchallenged, treated with mushroom)	7th group (unchallenged ,treated with amprolium)
21 days of age	0	47100± 458.25^{c}	45000± 1414.21^{a}	45700± 422.95^{a}	44200± 840.64^{a}	0	0
28 days of age	0	70000± 1626.17^{a}	7553± 2.33^{b}	2567± 1.86^{b}	2067± 1.7^{b}	0	0
35 days of age	0	60000± 4216.37^{b}	50± 2.58^{c}	2± 0.36^{c}	1± 0.2^{c}	0	0

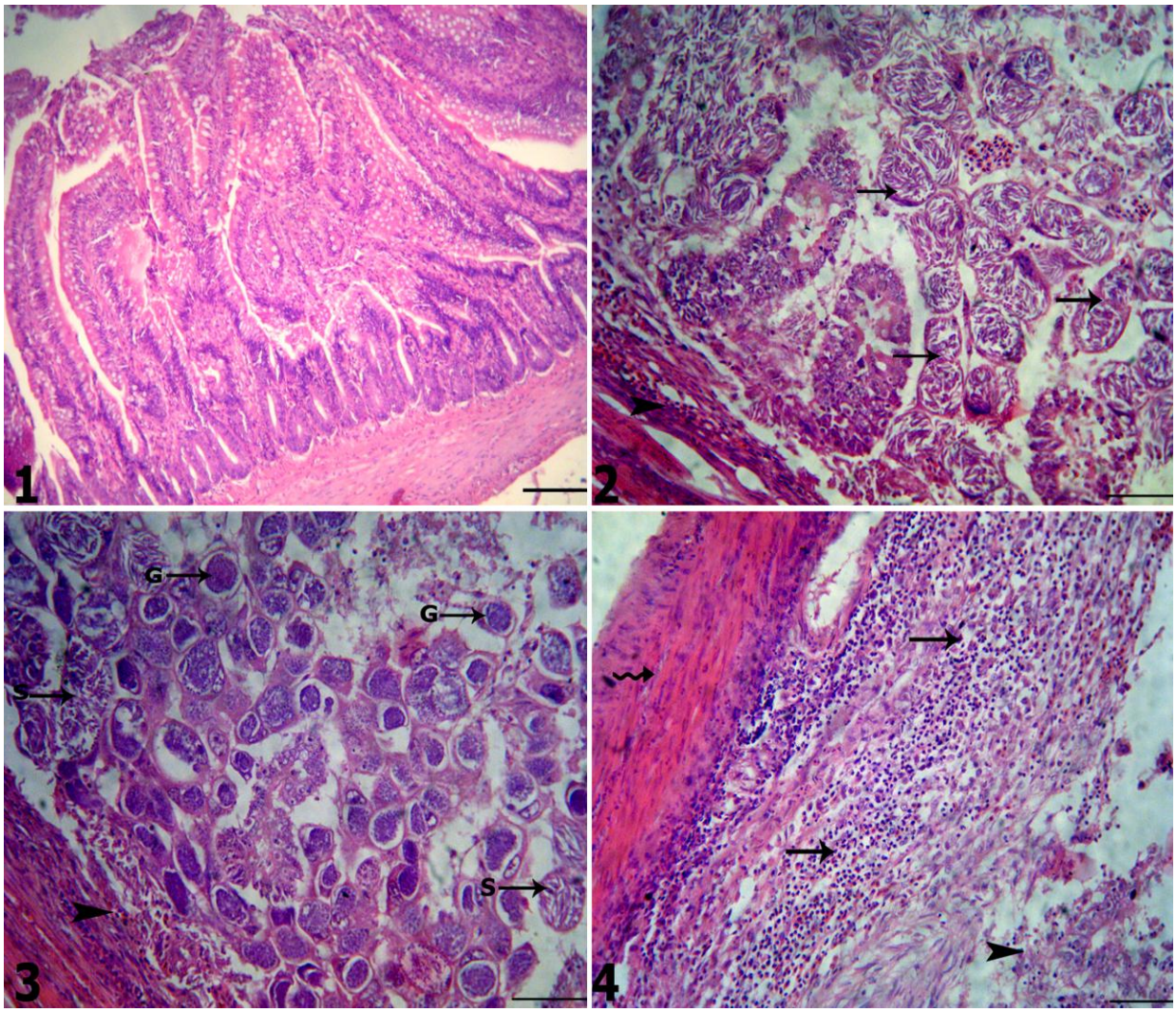
Value are means ± standard error, means with different letters at the raw differ significantly at (P <0.05).

3. Mortality rate and morbidity:
No mortality occurred in any of the experimental groups till the end of the experiment while on day 5 PI. all the birds in the infected groups (2, 3, 4 and 5) appeared dull, weak, loss of appetite, their feces became bloody and watery and *Eimeria tenella* oocysts were detected in their feces. These symptoms nearly disappeared in chicks of third group at (one week post treatment) and disappeared completely at (two week post treatment.). While in chicks of fourth and fifth groups the symptoms disappeared completely at (one week post treatment). On the other hand, in chicks of second group the symptoms continued till (two week PI.) then decreased gradually.

4. Pathological findings
The ceci of control (**group 1**): "Non-infected, non-treated" showed neither gross nor microscopic abnormalities along the experimental periods (Fig 1). However, the ceci of (group 2): "Infected,non-treated"macroscopically showed extensive hemorrhages in the cecal lumen and petechiation on the mucosa and serosa, particularly at 1 week PI and hemorrhagic cecal core on 2 weeks PI. Microscopically, the cecum revealed severe necrosis, destruction and desquamation of the lining epithelium with the presence of developmental stages of Eimeria including mature schizonts (Fig 2) and gamonts (Fig 3) in the enterocytes and lumens with extensive extravasated erythrocytes, 1 and 2 weeks PI. Sometimes, the necrotic mucosa was heavily infiltrated or replaced with lymphocytes and few heterophils besides hyalinization of the muscular coat (Fig 4). Hemorrhages, edema and necrosis were seen in the submucosa particularly near the muscular layer. Almost all the epithelial lining of the cecal mucosa were invaded with different developmental stages of coccidia (schizonts and gamonts), 2 weeks PI (Fig 5). The submucosa was edematous and severely infiltrated with lymphocytes, macrophages and few heterophils.

The ceci of group (**3**): "Infected and treated with Mushroom" showed slight lowering in the lesions of group (2). Bloody contents and petechial hemorrhages on the mucosa were also visualized. Microscopically, the developmental stages of *Eimeria* were evident in the lining epithelium of the cecum similar to those described in the infected chickens besides necrotic mucosa and lymphocytes infiltrations, 1 and 2 weeks PT (Fig 6). The mucosa of the cecum was slightly intact and the submucosa was infiltrated with lymphocytes and few macrophages, 2 weeks PT. Some hyperplastic changes were seen in the cecal epithelium and crypts. These findings in this group were presented as regenerative attempts. The lamina propria and

submucosa showed edema and focal fibrosis. Mild hyperplasia of the lymphoid follicles of cecal tonsils was also seen. Meanwhile **the ceci of group (4)**: "Infected and treated with amprolium" showed nonspecific changes, particularly at 2 weeks PT. Microscopically, the ceci showed intact mucous membrane with few developmental stages of Eimeria with numerous lymphocytes and macrophages infiltrations, particularly in the crypts, 1 week PT (Figs 7 and 8). Partial desquamation of the lining epithelium was also detected. However, the ceci at 2 weeks PT showed intact intestine with mild mucinous degeneration and few round cells infiltrations. Severe hyperplasia in the lining epithelium was noticed with no evidence of coccidial stages (Fig 9) besides congested blood vessels and thick eosinophilic membrane coated the luminal surface of the lining epithelium. **The ceci of group** (**5**): "Infected and treated with both amprolium and Mushroom" were apparently normal particularly at 2 weeks PT. Microscopically, the lesions of such group were completely ameliorated with intact mucosa, complete absence of the developmental stages of Eimeria and few lymphocytes infiltrations in the submucosa (Fig 10). Few birds, sacrificed 1 week PT, showed few) degenerated or necrotic gamonts in the lining epithelium of crypts (Fig 11). The cecal mucosa was focally regenerated with hyperplastic lining epithelium in both sacrificed periods. The submucosa and lamina propria were infiltrated with round cells of mostly lymphocytes besides hyperplasia in the lymphocytes of the cecal lymphoid follicles and tonsils (Fig 12). The ceci of groups (6,7): Showed neither gross nor microscopic abnormalities along the experimental periods.



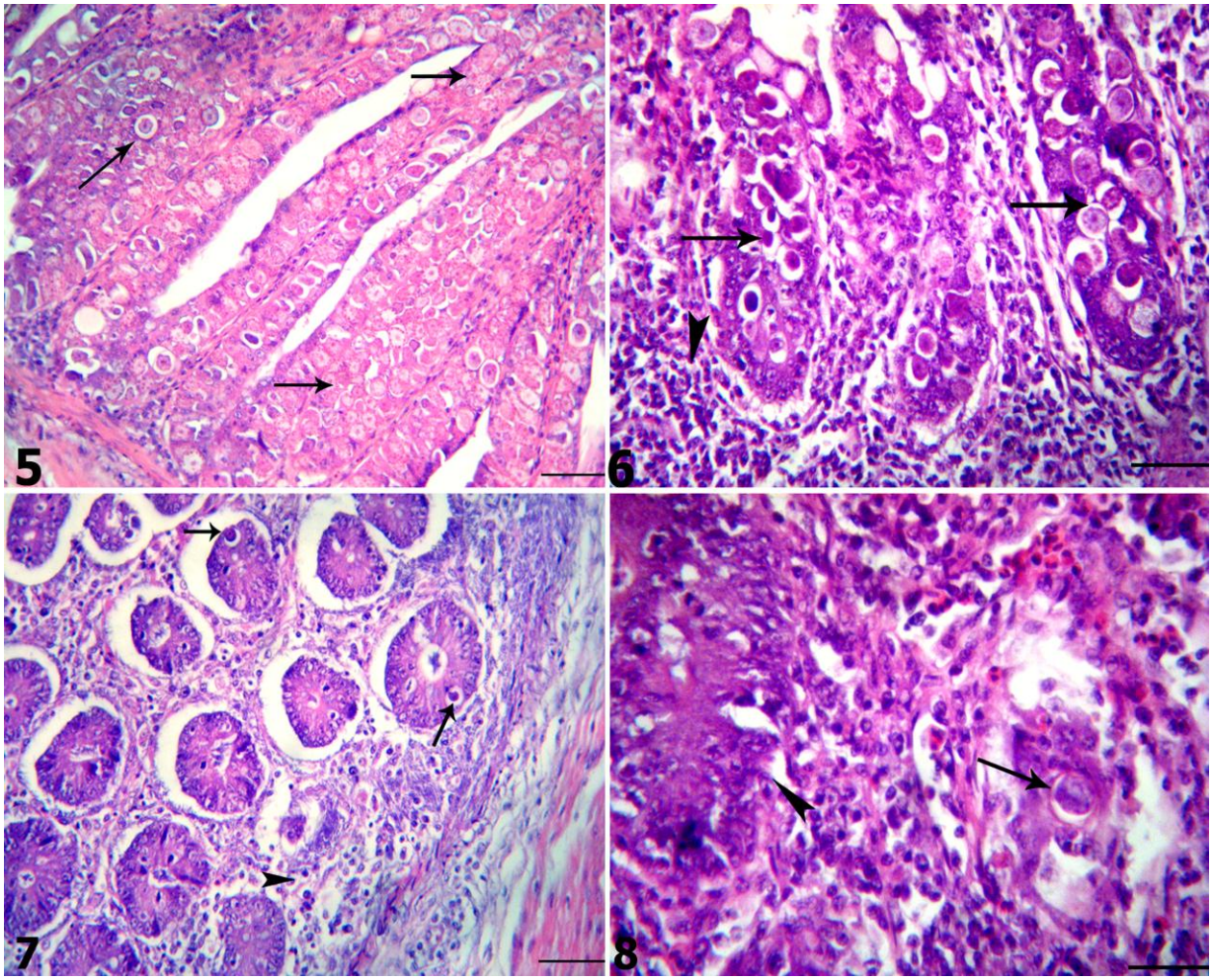
Figs. (1-4): Cecum from groups:

Fig.(1), (group1): shows normal intact mucosa and lymphoid follicles. H&E x 100.

Fig.(2), (group 2): shows several schizonts (arrows) in the enterocytes. H&E x 400.

Fig.(3),(group 2): shows numerous gamonts (arrows) in the enterocytes. H&E x 400.

Fig.(4), (group2): shows severe necrosis and lymphocytic infiltrations (arrow) with hyalinization of muscular coat (irregular arrow). H&E x 200.



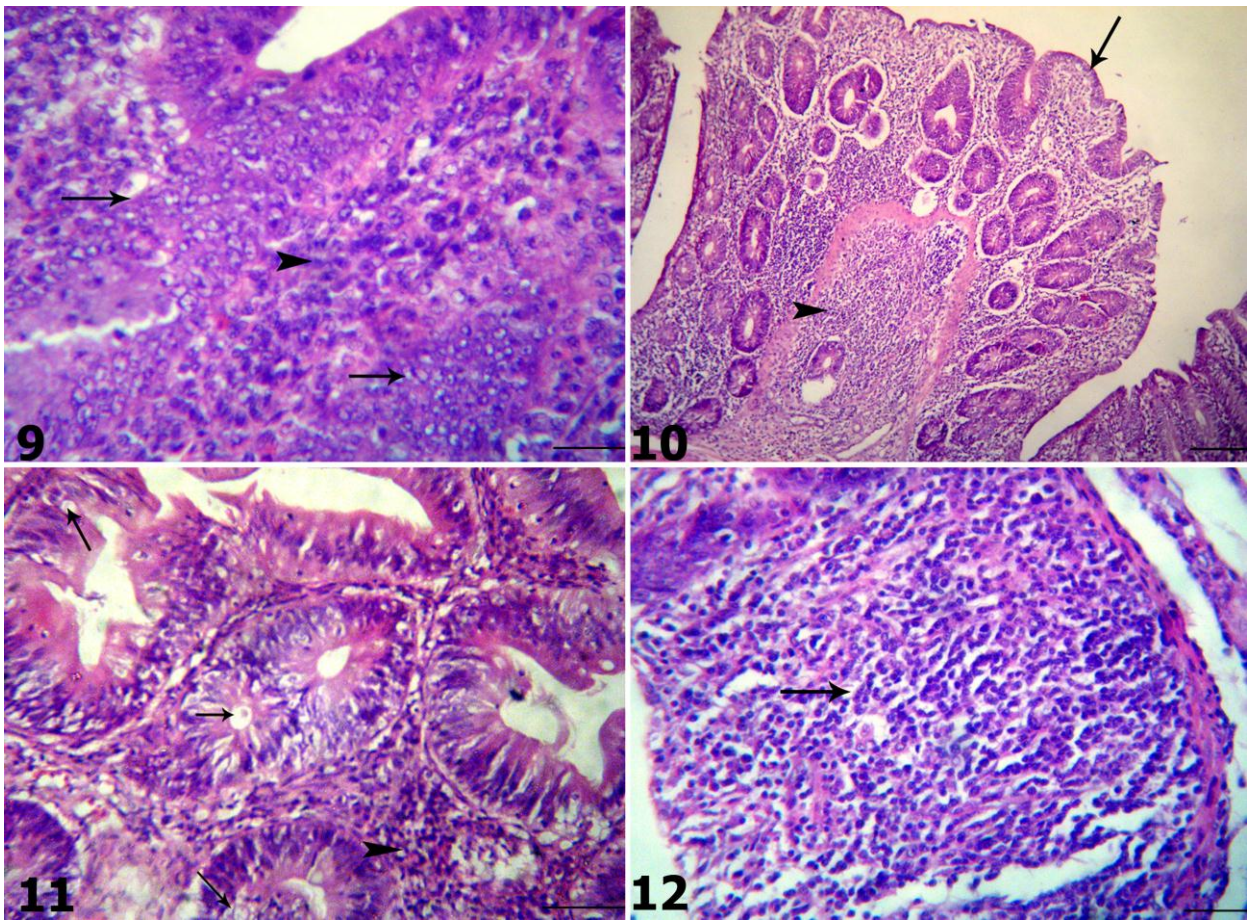
Figs. (5-8): Cecum from groups:

Fig.(5), (group 2): shows developmental stages in almost all the intestinal epithelium (arrows). H&E x 200.

Fig.(6), (group 3): shows developmental stages of *Eimeria* in the lining epithelium and numerous lymphocytes infiltrations. H&E x 200.

Fig.(7), (group 4): shows few developmental stages of Eimeria in the lining epithelium of crypts (arrows) with numerous lymphocytes and macrophages infiltrations (arrowhead). H&E x 200.

Fig.(8): A higher magnification of fig (7). H&E x 400.



Figs. (9-12): Cecum from groups:
Fig.(9), (group 4): shows severe hyperplasia in the lining epithelium (arrows) with no evidence of Eimeria. H&E x 400.
Fig.(10), (group 5): shows intact mucosa with no evidence of developmental stages (arrow) and lymphocytes infiltrations (arrowhead). H&E x 100.
Fig.(11), (group 5): shows few degenerated gamonts in the lining epithelium of crypts (arrows). H&E x 400.
Fig.(12), (group 5): shows hyperplasia in lymphocytes of the lymphoid follicles (arrow). H&E x 200.

Scoring of histopathological findings in the ceca.

Groups	Necrosis	Viable stages	Degenerated stages	Cecal tonsils and lymphoid follicles	Hemorrhages and congestion	Leukocytes infiltrates
1	-	-	-	**N**	-	-
2	++++	++++	+	**Slightly edematous**	++++	+++
3	+++	+++	++	**Mild hyperplasia**	++	++++
4	+	+	+++	**N**	+	+++
5	±	±	++++	**Hyperplasia**	±	++++
6	-	-	-	**N**	-	-
7	-	-	-	**N**	-	-

++++ Serious +++ Severe ++ Moderate + Mild ± rare - Nil

DISCUSSION

The present study investigated the anticoccidial efficacy of Oyster mushroom in relation to body weight, OPG count of faeces, morbidity, mortality, score lesion and pathological lesions. All broilers were infected (100%) by 7days PI., showed clinical signs of weakness, reduced appetite and bloody diarrhea. Regarding to the body weight, there was a significant decrease in the body weight of infected, untreated birds (2nd group); while the infected chickens which were treated with mushroom (3rd group) or with both mushroom and amprolium (5th group) showed a marked increase in body weight. Those results agreed with that obtained by (Guo *et al.,* 2003; Ogbe *et al.,* 2009 and willis *et al.,* 2012) who found that mushroom have polysaccharides that stimulating the activities of T and B lymphocytes, macrophage and natural killer cells (NK) inducing production and secretion of cytokines and complement, so it controls certain parasitic diseases. Among the reason why the treated broilers gained weight more than the non treated birds could be due to that aqueous extract of mushroom like amprolium may affect or prevent development of *E.tenella* stages. It appeared that this wild mushroom may contain compounds that are active against *E.tenella.* It was found to be non toxic in animal toxicity studies even when used at high therapeutic dose (Harkonen, 1998 and James, 2002). As well as the mushroom extract and amprolium stimulate appetite so the uninfected broilers which was treated with mushroom (6th group) or treated with amprolium (7th group) performed even high body weight.

Large numbers of oocysts were detected in the faeces of challenged birds that had received the primary infection challenge but not treated (2nd group). That data clearly showed that birds were adversely affected by this protozoan while broilers in treated groups (3, 4 and 5) showed the lowest oocyst count. Those results demonstrate the effectiveness of the mushroom in reducing the shedding of oocysts and agreed with those obtained by (Ogbe *et al.,* 2009; Willis *et al.,* 2010 and willis *et al.,* 2012) who showed that diet supplementation with aqueous extrat of mushroom (FMG) exhibited a reduction in oocyst excretion and mortality in *Eimeria* challenged broiler chicks. Mushroom have bioactive compounds or polysaccharides are known to play vital roles in enhancing health. They block colonization of the intestine by pathogens, thereby improving their elimination from the body (Guo *et al.,* 2003 and Elmusharafa *et al.,* 2006) and also it contains organic acids, resin and glycosides which include steroid are known to have therapeutic use against microbes and parasites (Anon 2007; Deihk *et al.,* 2007).

Regarding to histopathological findings the cecum of infected, untreated chicks (2nd group) showed severe necrosis, destruction and desquamation of the lining epithelium with presence development stages of *Eimeria* sometimes the necrotic mucosa was heavily infilterated with lymphocytes, haemorrhages, oedema and necrosis were seen near the muscular layer. Those result agreed with that obtained by Soomro *et al.* (2001); Ogbe *et al.* (2005) and Enas (2011). There were reduction in those lesions in broilers infected and treated with mushroom (3rd group) and the lesions were ameliorated in broilers of (4th group) (treated with amprolium) and (5th group) (treated with both amprolium and mushroom). The regeneration of cecal mucosa was observed and there were decrease in the number of oocysts of *E. tenella* in epithelial at 2 week PT. From those results, it could be demonstrated that both amprolium and mushroom lead to improve the pathological finding induced by *E. tenella* as mushroom lead to improve innate immune responses against coccidiosis. Those finding, however, correspond to those recorded by (Naphade *et al.,* 2010 and Hossain *et al.,* 2013).

CONCLUSION

Treatment with aqueous extract of oyster mushroom lead to improve body weight more than amporilum, while treatment with amprolium lead to the reduction of faecal oocysts and improve pathological lesions more than oyster mushroom. On the other hand best results were obtained when we used oyster mushroom mixed with amporilum.

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دراسات تجريبية على عدوي الكوكسيديا فى دجاج التسمين والمعالج بالمستخلص المائي للمشروم

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أجريت هذه الدراسة للتعرف على تأثير المستخلص المائي للمشروم على الدجاج المصاب بالإيميريا تينيلا ومقارنته بتأثير الامبرول علي معدل الزيادة فى الوزن وفي تقليل عدد البويضات التي تخرج من الدجاج وكذلك تحسين التغيرات الباثولوجية فى الأعورين. أجريت هذة الدراسة على ١٤٠ من دجاج التسمين عمر ١٤ يوم حيث تم تقسيمها إلى ٧ مجموعات ، يحتوى كل منها علي ٢٠ كتكوت. المجموعة الأولى لم يتم عدواها بطفيل الايميريا ولم يتم استخدام أي علاج أو إضافات لها (المجموعة الضابطة) ، المجموعة الثانية تم عدواها عند عمر ١٤ يوم بعدد ٥٠٠٠٠ حويصلة متجرثمة من الايميريا لكل طائر والمجموعة الثالثة تم عدواها كما سبق بنفس الجرعة وفي نفس العمر ثم تم علاجها بإضافة المستخلص المائي للمشروم بجرعة (٢٠٠مجم/مللي) وذلك فى اليوم السادس من العدوى ويستمر ذلك لمدة اسبوع متواصل. المجموعة الرابعة يتم عدواها كما سبق وفي نفس العمر تم علاجها بالامبرول (٢٠٠ مجم/مللي ماء شرب) وذلك فى اليوم السادس من العدوي ولمدة أسبوع ، المجموعة السادسة لم يتم عدواها بالايميريا ولكن يتم اعطائها المستخلص المائي للمشروم (٢٠٠مجم/مللي) عند عمر ١٤ يوم ويستمر ذلك يوميا حتى نهاية التجربة. المجموعة السابعة لم يتم عدواها بالايميريا ولكن يتم إضافة الامبرول بجرعة (٢٠٠مجم/مللى ماء الشرب) عند عمر ١٤ يوم ويستمر ذلك حتى نهاية التجربة وقد تم أخذ وزن الطيور أسبوعيا حتي نهاية التجربة. واجراء عدد بويضات الكوكسيديا التي تخرج من البراز فى اليوم السابع من العدوي (عند عمر ٢١ يوم) وكذلك بعد أسبوع واسبوعين من العلاج (عند عمر ٢٨ يوم ، ٣٥ يوم) وتم الفحص الباثولوجي فى اليوم ٢٨ ، ٣٥ من العمر. وقد أسفرت النتائج عن وجود نقص ي فى وزن الطيور فى (المجموعة الثانية) التي تم اصابتها ولم يتم علاجها فى كل الأسابيع وكان هناك زيادة معنوية فى وزن الطيور التي تم علاجها بالمشروم والامبرول معا (المجموعة الخامسة) وظهر ذلك بوضوح بعد اسبوعين من العلاج (عند عمر ٣٥ يوم) وكان هناك زيادة معنوية فى وزن الطيور التي تم علاجها بالمشروم فقط (المجموعة الثالثة) وكذلك هناك زيادة معنوية فى وزن الطيور فى المجموعة السادسة (التي لم يتم عدواها وتم علاجها بالمشروم) وبالتنبيه لعدد الحويصلات فكان أعلى ما يمكن فى المجموعة الثانية (التي تم عدوتها ولم يتم علاجها) اما المجموعات التي تم علاجها بالمشروم أوبالامبرول أو بالاثنين (المجموعة الثالثة والربعة والخامسة) فكان هناك نقص ملحوظ فى عدد البويضات مقارناً بالمجموعة الضابطة. واسفر الفحص الظاهري للاعورين (فى المجموعات المصابة) عن وجود انزفة على الجدار الخارجى ووجود زيادة فى سمك الجدار ومحتويات الاعورين كانت ما بين اللون البني الى الدم المتجلط، وبالفحص الباثولوجي للمجموعة الثانية فقد وجد نخر وتكسير فى الخلايا المحيطة بجدار الاعورين مع وجود مراحل الايميريا بداخلها وتجمع خلايا الليمفوسيت ووجود نزيف وتورم ونخر بالقرب من طبقة العضلات ولكن فى المجموعات ٣، ٤ ، ٥ والتي تم علاجها بالمشروم أو بالامبرول او بالمشروم والامبرول معاً فقد وجد تحسن ملحوظ فى التغيرات الهستوباثولوجية.