

Full Length Research Paper

Oral treatment of *Eimeria tenella*-infected broilers using aqueous extract of wild mushroom (*Ganoderma* sp): Effect on haematological parameters and histopathology lesions

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Accepted 13 April, 2010

In Nigeria, wild *Ganoderma* species of mushroom grows in abundance during the rainy season. Studies were conducted to evaluate the haematological parameters and the histopathology lesions in organs of broilers treated with aqueous extract of wild *Ganoderma* sp. Blood and organs were collected for haematology and histopathology, respectively. The haematological analysis showed a slight drop in packed cell volume (PCV) in the birds of group A ($23.5\% \pm 0.7$), B ($28.0\% \pm 2.8$) and C ($27.5\% \pm 0.7$) at 7 weeks of age, one week after infection with *Eimeria tenella*. The values of haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) were within normal range in all the groups and seemed to bear no direct relationship to the treatment using either the wild mushroom or amprolium. The values showed considerable variations characterized by a wide range of normal values (Hb = $8.5 \pm 0.0 - 14.9 \pm 1.52$ g/dl; RBC = $1.9 \pm 0.18 - 8.6 \pm 0.78 \times 10^{12}$ /L; WBC = $2.0 \pm 0.92 - 8.5 \pm 0.49 \times 10^9$ /L). Histopathology showed mild lymphocytic infiltration in the liver of the broilers. The lesions could not be linked to the use of mushroom or amprolium, as both treated and untreated birds had similar lesions in their organs. It was concluded that the mushroom has no deleterious or adverse effects on the organs of treated birds.

Key words: Medicinal mushroom, *Ganoderma lucidum*, *Eimeria tenella*, haematological values, histopathology lesions, broilers.

INTRODUCTION

In Asia and some tropical countries of Africa, edible and medicinal mushrooms such as *Pleurotus ostreatus* and *Ganoderma lucidum* are used as food supplements and medicines to improve various parameters of human health and immune functions in certain disease conditions (Chang and Buswell, 1996; Chang and Mshigeni, 2001;

Anthony and Joyce, 2007). Many literatures are available on the beneficial effects of mushrooms, particularly the *G. lucidum*, which is cultivated in China and Japan, and other western Nations. The wild species of *G. lucidum* also grows in abundance in Nigeria on wood and tree stumps during the rainy season. However, the use of wild *Ganoderma* species as feed supplement or medicine to treat chickens against some disease problems and to improve health and production has not been considered.

Haematological profile and histopathology changes in organs or tissues of animals including humans are important indices of physiological status of an individual and to make clinical predictions of health status of the

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Abbreviations: PI, Post-inoculation; EDTA, ethylene diamine tetraacetic acid; PCV, packed cell volume; Hb, haemoglobin; RBC, red blood cell; WBC, white blood cell; NK, natural killer.

individual or animal. The findings may vary with certain disease conditions, stress and toxicity (Esiebo and Sarror, 1992; Conway et al., 1993; Khan and Zafar, 2005; Fasuyi, 2007).

Mushrooms, like probiotics are natural ingredients that contain bioactive chemical substances, or polysaccharides, proteins, crude fibres, unsaturated fat, minerals, vitamins, essential amino acids and organic acids that can be used as good sources of food supplements and medicines to promote health and production (Jong and Birmingham, 1992; Chang and Mshigeni, 2001; Guo et al., 2003; Wachtel-Galor et al., 2004; Ogbe et al., 2005; Anon, 2007; Ezeokeke, 2008). They stabilise microflora in the gastrointestinal tract, and prevents colonization of host cells by pathogens and also stimulates non-specific host immune response or phagocytosis by macrophages (Spring et al., 2000; Guo et al., 2003; Sundu et al., 2006). Toxicological studies using Swiss mice, rats and guinea pigs administered with hot water extract of cultivated *G. lucidum* orally showed there were no adverse effects or toxicity in the various organs and on haematological parameters (Mamoru and Hitoshi, 1977).

The objective of the present study is to evaluate the haematological parameters and histopathology lesions in *Eimeria tenella* infected broilers treated with aqueous extract of wild *Ganoderma* sp.

MATERIALS AND METHODS

Site of study

The study was conducted at the federal college of animal health and production technology, national veterinary research institute, Vom, Nigeria.

Experimental birds

One hundred and twenty Ross broilers were obtained at day-old from a hatchery in Jos, Plateau State, Nigeria. The birds were selected for uniformity and fitness and randomly distributed into treatment groups of 20 chicks each and housed on wire cages labeled A to F. Each group of birds was provided with broiler feed containing 22% crude protein *ad libitum*.

Preparation of aqueous extract of *Ganoderma* sp.

Wild *Ganoderma* species of mushrooms with red open caps were harvested during the rainy season (June-September) from wooden logs and tree stumps on some farm land in Vom, Plateau State, Nigeria. They were washed in sterile distilled water, sun dried and milled into powder using a mortar and pestle and then blended with a Corona grinder (Landers and CIA, S.A). The mushroom powder was again sun dried for about 3 h and then stored in plastic polythene bags and kept at room temperature until required for treatment. A 20% w/v solution of aqueous extract of *Ganoderma* was prepared by soaking in hot water boiled to 100°C for 3 h. The solution was sieved, solid matter discarded and the filtrate allowed to cool to room temperature before use. Amprolium was used at concentration of 200 mg/ml to compare its efficacy with that of the aqueous extract of *Ganoderma* sp.

Inoculation and treatments

The birds in groups A, B and C were inoculated with *E. tenella* (Houghton strain) at the rate of 36,250 sporulated oocysts per ml per bird using insulin syringe introduced directly into the crop of each bird at 6 weeks of age. By day 6 post-inoculation (PI), they were treated with aqueous extract of *Ganoderma* (group A) and amprolium (group B) in drinking water each for 7 days consecutively with only the medicated water given *ad libitum*. Broilers in group C and D were not treated, while those in group E and F were treated with *Ganoderma* and amprolium, respectively.

Haematological analysis

Blood was collected from five birds per group. Bleeding was done via the jugular veni-puncture using sterile syringes and needles. The blood was transferred immediately into a set of sterile tubes containing anticoagulant, disodium-salt of ethylene diamine tetraacetic acid (EDTA). The microhaematocrit method and cyanmethaemoglobin method were used to determine packed cell volume (PCV) and haemoglobin (Hb), while red blood cell (RBC) and white blood cell (WBC) counts were determined using the Neubauer haemocytometer method (Esiebo and Saror, 1992). The tests were conducted within 2 h of collecting the blood.

Histopathology

Histopathological examination of organs in each group was carried out (Durry and Wallington, 1976). This was done by first harvesting the liver, kidneys, heart, spleen, brain, caeca and bursa and fixing them in 10% neutral buffered formalin in plastic containers for 24 h. The tissues were then trimmed to 3 - 5 mm thickness. They were processed in an automatic tissue processor in different chambers containing different grades of alcohol (70, 80, 95 and 100%) for a period of 24 h, as tissue stay for 2 h per chamber. The processed tissues were embedded in paraffin wax; block sectioned with microtome to a size of about 5 micrometer, floated in a water bath, dewaxed and mounted on a slide, allowed to dry, then stained using haematoxylin and eosin (H and E) stain. The slides were allowed to dry before examination under the light microscope for any microscopic lesions at different (x10, x20 and x40) magnifications.

Statistical analysis

Mean \pm SD of haematological values and lesions in percentages were subjected to statistical analysis of variance, ANOVA (Olawuyi, 1996; Ronald, 1972).

RESULTS

Clinical observations and performance

Clinical observations, feed intake, weight gain and faecal oocysts output of birds were reported else-where by Ogbe (2008) and Ogbe et al. (2009 a,b).

Haematological parameters

Results showed that the haematological values of broilers were inconsistent and seemed to bear no direct relationship

Table 1. Haematological values (mean \pm SD) in *Eimeria tenella*-infected broilers treated with wild *Ganoderma* sp.

Group/type of bird	Haematological values/week	Initial sample	Before Infection	Time of Infection	During Infection	After Infection
A	PCV	31.50 \pm 0.71	30.00 \pm 2.83	29.50 \pm 0.71	23.50 \pm 0.71	31.50 \pm 2.48
	Hb	12.00 \pm 4.38	10.10 \pm 0.57	9.50 \pm 0.85	8.50 \pm 0.00	14.83 \pm 0.95
	RBC	2.12 \pm 0.69	2.61 \pm 0.13	6.60 \pm 1.41	8.55 \pm 0.78	3.30 \pm 0.26
	WBC	2.55 \pm 0.35	2.65 \pm 0.71	3.70 \pm 0.99	4.80 \pm 0.57	2.80 \pm 0.64
B	PCV	29.50 \pm 0.71	32.50 \pm 2.12	29.50 \pm 3.54	28.00 \pm 2.83	30.75 \pm 2.47
	Hb	9.50 \pm 0.28	11.10 \pm 0.28	10.45 \pm 2.19	11.45 \pm 2.47	13.45 \pm 0.11
	RBC	1.85 \pm 0.18	2.58 \pm 0.21	7.31 \pm 0.70	6.69 \pm 1.76	3.14 \pm 0.80
	WBC	2.20 \pm 0.28	1.95 \pm 0.92	4.30 \pm 0.28	8.30 \pm 0.14	2.78 \pm 0.25
C	PCV	31.50 \pm 2.12	28.50 \pm 0.71	30.00 \pm 1.97	27.50 \pm 0.71	29.00 \pm 0.35
	Hb	9.70 \pm 0.57	11.10 \pm 0.28	9.50 \pm 0.28	9.50 \pm 0.28	13.52 \pm 1.10
	RBC	1.89 \pm 0.17	2.59 \pm 0.42	3.46 \pm 0.64	3.32 \pm 0.17	2.94 \pm 0.41
	WBC	2.80 \pm 0.14	2.90 \pm 0.14	8.45 \pm 0.49	5.65 \pm 0.72	3.48 \pm 1.24
D	PCV	31.50 \pm 0.17	31.00 \pm 1.41	30.00 \pm 0.00	31.00 \pm 1.41	29.50 \pm 0.71
	Hb	11.05 \pm 2.47	11.05 \pm 1.34	11.00 \pm 1.41	11.00 \pm 1.41	12.73 \pm 1.24
	RBC	2.04 \pm 0.11	2.89 \pm 0.08	3.74 \pm 0.95	6.65 \pm 1.48	3.50 \pm 1.04
	WBC	2.15 \pm 0.07	3.35 \pm 0.35	6.36 \pm 1.77	4.35 \pm 0.07	3.08 \pm 0.81
E	PCV	32.50 \pm 3.54	29.50 \pm 0.71	30.00 \pm 1.14	29.50 \pm 0.71	29.50 \pm 0.71
	Hb	8.90 \pm 1.13	10.30 \pm 0.28	10.10 \pm 0.57	9.70 \pm 1.13	12.63 \pm 1.52
	RBC	2.40 \pm 0.33	2.26 \pm 0.04	6.10 \pm 1.00	5.61 \pm 0.20	3.70 \pm 0.81
	WBC	2.65 \pm 0.21	3.20 \pm 0.57	4.70 \pm 0.78	5.80 \pm 0.14	3.08 \pm 0.81
F	PCV	32.50 \pm 3.54	31.50 \pm 3.54	30.50 \pm 0.71	31.50 \pm 2.12	29.50 \pm 0.71
	Hb	10.70 \pm 0.28	10.50 \pm 0.57	11.90 \pm 1.27	11.90 \pm 1.27	14.85 \pm 1.52
	RBC	2.37 \pm 0.37	2.40 \pm 0.56	4.78 \pm 0.76	5.85 \pm 0.64	3.82 \pm 0.76
	WBC	2.50 \pm 0.57	2.20 \pm 0.28	7.60 \pm 0.85	4.75 \pm 0.49	3.75 \pm 1.13

*Mean values are all within normal range (PCV = 23.5 - 32.5%; Hb = 8.5 - 14.9 g/dl; RBC = 1.9 - 8.6 $\times 10^{12}$ /L; WBC = 2.0 - 8.5 $\times 10^9$ /L). A = Infected with *E. tenella* and treated with *Ganoderma*; B = infected with *E. tenella* and treated with amprolium; C = infected with *E. tenella* but not treated; D = not infected and not treated; E = not infected but treated with *Ganoderma*; F = not infected but treated with amprolium..

with the treatments as the values showed considerable variations characterized by a wide range of normal values. The values gotten for the PCV was 23.5 - 32.5%; Hb, 8.5 - 14.9 g/dl; RBC, 1.9 - 8.6 $\times 10^{12}$ /L and WBC, 2.0-8.5 $\times 10^9$ /L (Table 1). Although the PCV in all the groups were within normal values, there was slight drop in PCV observed in the broilers in group A (23.5% \pm 0.7), B (28.0% \pm 2.8) and C (27.5 % \pm 0.7) at 7 weeks of age (Table 1).

Histopathology

Histopathology showed varying degree of lesions in the liver, bursa and caeca (Table 2). There was mild lymphocytic infiltration in the liver of the birds in all the groups. In group A, there was marked lymphoid depletion of the bursa in two birds (28.6%). In group C, the bursal follicles were fibrotic in two birds (28.6%) with diffuse lymphocytic infiltration in two other birds (28.6%) and marked congestion of lamina propria of the caeca in three birds (42.9%).

DISCUSSION

Although wide ranges of normal haematological values were reported in the broilers, the decline of PCV values observed in the broilers in group A, B and C may be attributed to *E. tenella* infection. According to some workers, PCV may be sensitive to, or affected in coccidiosis (Natt and Herrick, 1955; Conway et al., 1993). A mean PCV of 19.0% at 6 days PI was observed to be the minimum for survival in birds (Conway et al., 1993). In this experiment, there was no mortality and the minimum PCV recorded was 23.5% PI with *E. tenella*.

Wide ranges of normal haematological values were also reported by other authors (Bell and Freeman, 1971; Mitruka and Rawnsley, 1977; Oyewale, 1985; Mhatre and Joshi, 1993; Ogbe et al., 2003; Iheukwumere et al., 2006). Fluctuations in haematological values of avian blood are normal phenomenon and in most instances the variations in haematological values may depend on the physiological state of birds (Islam et al., 2004).

The histopathology lesions and gross lesions observed in birds that were infected but not treated were more

Table 2. Histopathology lesions in *Eimeria tenella*-infected broilers treated with wild *Ganoderma* sp.

Broilers	Histopathology examination	Number (%) with histopathological lesions				
		A	B	C	D	E - F
A	Focal periportal lymphocytic infiltration	1 (14.28)	1 (14.3)	2 (28.57)	2 (28.57)	2 (28.57)
B	Bursal follicles depleted of lymphoid cells	2 (28.57)	0 (0)	0 (0)	0 (0)	0 (0)
C	Marked bursal follicular fibrosis	0 (0)	0 (0)	2 (28.57)	0 (0)	0 (0)
D	Marked conestion of laminar propria of caeca/tonsils	0 (0)	0 (0)	3 (42.85)	0 (0)	0 (0)
E	Lymphocytic infiltration of caeca (diffuse)	0 (0)	0 (0)	2 (28.57)	0 (0)	0 (0)

A = infected with *E. tenella* and treated with *Ganoderma*; B = infected with *E. tenella* and treated with amprolium; C = infected/not treated; D = not infected/not treated; E = not infected but treated with *Ganoderma*; F = not infected but treated with amprolium.

severe because of the injuries caused by the coccidial parasite, *E. tenella*. The parasite is pathogenic and affects the caeca of young chickens due to invasion of the mucosal lining of the caeca by second generation merozoites. The associated microscopic changes include congestion of the intestinal mucosa, diffuse or patchy haemorrhagic enteritis, lymphocytic and mononuclear cells infiltration of intestinal mucosa and sloughing of epithelial lining (McDougald and Reid, 1997). The damage that occurs with the changes in the functional integrity of the intestine may lead to secondary bacterial infection and cellular infiltration (Rose et al., 1979; Lillehoj et al., 1989). Some mushrooms have polysaccharides which play a role in stimulating the activities of many interdependent cell types such as T and B-lymphocytes, macrophages, natural killer (NK) cells, inducing secretion and production of cytokines and complements of T-cells and NK cells (Wang et al., 1997; Guo et al., 2003), which can be exploited for their use as medicines to control coccidiosis.

Conclusions

The study showed that treatment with wild *Ganoderma* did not adversely affect haematological parameters and organs of broilers. It was therefore concluded that wild *Ganoderma* mushroom harvested from Vom, Nigeria can be recommended in broilers as health supplement.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the following for their assistance; Dr. M. S. Ahmed, Dr. Tai Cole, Dr. A. O. Olabode and Professor S. A. Akpavie.

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