

HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILER FINISHER CHICKENS FED DIETS CONTAINING VARYING LEVELS OF MYCOFIX®

¹Kehinde, W. H., ²Afolayan, M. and ¹Mallam, I.

¹Department of Animal Science, Kaduna State University, Kafanchan Campus

²Samaru College of Agriculture, Ahmadu Bello University, Zaria.

Corresponding Author: yebokenny@gmail.com; +2348066245044

ABSTRACT

Three hundred (300) day old broiler birds were used for the feeding trial, while completely randomized design (CRD) was used to assign the birds to five diets, which were replicated three times each with 20 birds per replicate. The treatments were Mycofix®-containing regimens T1, T2, T3, T4, and T5 at rates of 0, 100, 200, 300, and 400g/100kg, respectively. The chickens were reared in a deep litter house. Clean water and feed were given free choice. On the 28th day of the trial, blood samples were drawn directly into vacutainer plastic bottles containing Ethylenediaminetetraacetic acid (EDTA) for haematological analysis and into bottles without EDTA for biochemical analysis. Analysis of variance (ANOVA) was used to run all collected data, and Duncan Multiple Range Test was used to distinguish between significant differences in treatment means. Packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell counts (RBC), mean corpuscular volume, and lymphocytes were not significantly different ($P>0.05$). The average levels of corpuscular haemoglobin and heterophils were significantly ($P<0.05$) influenced with higher values (MCH) 12.64 – 13.31pg and 10.30 – 13.08%. Conclusion: Mycofix® use and the inclusion levels had no negative effects on haematology and serum biochemistry, but haemoglobin and heterophils were significantly ($P<0.05$) increased.

Keywords: Broiler chickens, Mycofix®, Haematology and Mycotoxins

INTRODUCTION

Feed is an essential denominator in any farm animal's production. It is a major factor in the poultry industry, as feed value accounts for 70-80 percent of the total farm expenditure (Gunasekar, 2007; Olugbenga, et al., 2015). Rearing broiler chickens is a well-known industry with confirmed monetary advantages, they develop very rapidly and their feed conversion capacity is rapid. They can convert feed into flesh efficiently in the shortest duration, approximately 6 – 8 weeks. As an end result, their productivity demand is actualized via feed intake (Mallick, et al., 2020). Optimum benefits from feed consumption are not actualized in full capacity without some barriers, the occurrence of mycotoxins is a type of limitation.

Mycotoxins can have a critical negative impact on animal wellbeing and health, when present in animal feed. Mycotoxins are filamentous fungi that are mould derivatives, and contaminate grains effortlessly and improperly stored feeds. The increase of Moulds is normally associated with extremes in climate conditions and their spores are all around nearly everywhere, such as soil and plant debris. The field, all through harvest, storage, processing, or feeding, is the access factor of moulds in plants. The result of pollution caused in poultry feed depends on the specific mycotoxin or toxicant, the level of infection, the period of time the animal has been ingesting the feed, the animal's age, sex, and stage of stress (Jacquie, 2015).

Mycotoxin binders or adsorbents are materials utilized in feed components to bind the mycotoxins and stop them from being absorbed via the intestine and into the blood circulation (Jacela et al., 2010). The most effective dietary strategy for reducing the effects of mycotoxins has been thought to be the

inclusion of mycotoxin binders in poultry diets (Galvano et al., 2001). According to the theory, the binder decontaminates the mycotoxins within the feed through binding them firmly to prevent poisonous interactions when the animal consumes the feed and to stop mycotoxin absorption throughout the digestive tract.

The fitness and nutritional status of an animal has been assessed using haematological and serum biochemical indices, which are blood-related parameters. White blood cells (WBCs), red blood cells (RBCs), packed cell volume (PCV), haemoglobin, and lymphocytes are among the parameters that can be used to access the dietary content of a feed. As a result, it is essential for determining whether an animal's blood cell count is normal, excessive, or abnormally low, as well as whether the cells themselves are abnormal. According to Olafedehan et al. (2010), as well as Etim et al. (2014), a pathological reflection of the health of poultry exposed to other toxicants in addition to aflatoxin is provided by the haematological analysis of the birds. Birds with a healthy blood composition are more likely to perform better (Isaac et al., 2013).

The study focus was to assess the effect on varying levels of Mycofix®, a toxin binder, in the diets on the haematological and serum biochemical parameters of broiler chickens.

MATERIALS AND METHODS

Location and Area of the Experiments

The Poultry Unit of the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture, Ahmadu Bello University, Samaru, Zaria, was the site for the experiment. The site is situated on Latitude 11° 9' 46"N, Longitude 7°37'45"E, and altitude of 610m above sea level in

Nigeria's Guinea Savannah Zone. Depending on the season, the temperature ranges from 26 to 40°C, and the relative humidity varies between 21 and 72 percent during the dry and wet seasons. According to the Institute for Agricultural Research Meteorological Unit (2016), Zaria experiences its wettest months between May and October, when the city receives an average of 1500mm of rain.

Experimental Design and Management

Three hundred (300) broiler chickens were used for the feeding trial. The birds were weighed on replicate basis, having 20 birds per replicate and 60 per treatment. The birds were assigned to the various treatments in a completely

randomized design arrangement. The experiment, which lasted 28 days, involved managing chickens in a deep litter house with unlimited access to feed and water.

Experimental Diets

Five isonitrogenous and isocaloric diets were formulated to meet the nutrient needs as endorsed by NRC (1994) for broiler finisher diets. The experimental finisher diets (Table 1) had 20.54% crude protein and 2929kcal/kg ME. There were 5 diets, T1 (control) had no Mycofix®, T2, T3, T4 and T5 had Mycofix® included at 100, 200, 300, and 400g respectively in a 100kg feed.

Table 1: Ingredient composition of experimental broiler finisher diets supplemented with varying Inclusion levels of Mycofix® (5 – 8 weeks)

Ingredients	Levels of Mycofix® g/100kg				
	0	100	200	300	400
Maize	58.00	58.00	58.00	58.00	58.00
Soya bean cake	20.00	20.00	20.00	20.00	20.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00
Maize offal	4.70	4.70	4.70	4.70	4.70
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Vitamin premix ^A	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME Kcal/kg	2929	2929	2929	2929	2929
Crude protein (%)	20.54	20.54	20.54	20.54	20.54
Crude fibre (%)	4.11	4.11	4.11	4.11	4.11
Ether extract (%)	3.35	3.35	3.35	3.35	3.35
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.27	1.27	1.27	1.27	1.27
Methionine (%)	0.50	0.50	0.50	0.50	0.50
AfB1(ppb)	99.03	99.03	99.03	99.03	99.03
Cost/ kg diet(₦)	79.44	79.50	79.56	79.62	79.68

ABiomix Broiler Finisher premix provides per kg diet Vit. A, 10,000 I.U; Vit D₃, 2000 I.U; Vit E 23mg; Vit. K, 2mg; =Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B₁ 1.8mg, Vit. B₂, 5mg; Vit B₆, 3mg; Manganese, 40mg Iron, 20mg; Copper, 3mg; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 50mg. AfB1 = Aflatoxin B1, ppb = parts per billion

Blood Collection Procedure

On the 28th day of the feeding trial, blood samples were taken from 3 birds per replicate for haematological and serum biochemistry analysis. Two (2) milliliters of blood was drawn from the wing vein and placed into heparinized tubes with EDTA. Using the Cell-Dyn 3500 blood evaluation machine (Abbott Diagnostics, Abbott Park, IL), which was calibrated for chicken blood (Balog *et al.*, 2003). The procedure of Davies *et al.* (1984) was used to evaluate packed cell volume (PCV), total white blood cell (WBC), haemoglobin (Hb), percentage of heterophils (HET), and lymphocytes (LYM). Mean corpuscular haemoglobin (MCH), mean cell volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were calculated as derivatives.

Another set of 2ml blood sample was collected into a second set of tubes without EDTA for blood serum analysis. For the purpose of determining parameters relating to liver characteristics, the samples were taken to the clinical pathology laboratory of the Veterinary Teaching Hospital, Ahmadu Bello University Zaria. Centrifuging the plasma at 3500 rpm for 15 minutes and freezing it at -200° C allowed for its separation. Using Reinhold's (1995) guidelines, the biuret method of total serum protein determination was used for this assay. With the help of the Randox Kit, glucose was measured calorimetrically. As described by Bush (1991), liver enzymes including serum transaminases covering aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase were measured. Following the method developed by Bush (1991), the kidney function parameters were measured along with urea, creatinine, sodium ion, chloride ion, and hydrogen carbonate..

Analytical Statistics

Using the general linear model of the Statistical Analysis System (SAS, 2003) Software package, all the experiment-related data were subjected to an analysis of variance (ANOVA). Using Dunnett's Test, significant differences between means were distinguished.

Findings and Discussion

Table 2 shows the haematological parameters of broiler finisher chickens fed diets with various concentrations of Mycofix®. The outcomes confirmed no significant (P>0.05) variations in packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell count (RBC), mean corpuscular volume and lymphocytes. Significant (P<0.05) differences which were recorded for the values of mean corpuscular haemoglobin (MCH) and

heterophils, with the treatment groups having an appreciably (P<0.05) higher values than the control.

Therefore, using blood parameters is recommended for medical and nutritional evaluation. The various metabolites that make up blood offer useful information on nutritional status and may be used for scientific research and diagnosis of an individual organism (Egbunike *et al.*, 2009). According to Mitruka and Rawnsley (1977) the normal range for healthy chickens is 24 to 44 percent for PCV. In the present study observed packed cell volume of 24 to 27 percent fell within this range. This outcome demonstrated that the high Mycofix® inclusion in broiler diets had no negative effects on the proportion of PCV, RBC, and Hb. The observed PCV is close to the report of Adebiyi *et al.* (2010) when different concentrations of Diatomaceous Earth (DE) were used as binder in a broiler feed. The researchers found that PCV ranged from 27.88 to 28.88 percent. The haemoglobin concentration of 7.97- 8.92mg/dl, also fell within the reference values of 9.10 - 13.9mg/dl as recorded by Mitruka and Rawnsley (1977) for healthy chickens. The outcome indicates that even with Mycofix® toxin binder, the birds' health was stable.

Sufficient haemoglobin has been used as an index of available oxygen needed for the transportation of digested foods. The haemoglobin is responsible for oxidation of digested foods to release energy for different functions in addition to the transportation of carbon dioxide out of the body of animals as supported by means of its physiological function because it delivers oxygen to tissues of the animal (Soetan *et al.*, 2013).

The total white blood cell count of broiler chickens in the treatment groups and the control all fell within the reference values of 9.20 – 31.00 x 10¹²/l reported by Swenson (1970). It was found that 400g Mycofix® inclusion had the least white blood cells numerically compared to the other treatments. With the overall performance recorded in this group, it may suggest that Mycofix® might boost immunity regardless of a challenge in the health of birds. The red blood cells (3.92- 4.55 x 10⁹/l) were not within the reference value of 1.58-4.10 x 110⁹/L for healthy chickens as reported by Mitruka and Rawnsley (1977).

The heterophils values significantly increased as the levels of Mycofix® was increased from 100 to 200g (10.30 and 12.00%) and then declined. This observed increase in the treatment groups may be as a result of the impact of the toxin binder's protection against possible

toxins within the feed ingredients thereby improving the immunity of the birds. Heterophils are a common type of white blood cell which reacts to infections and attack bacteria and different foreign invaders directly. They are

phagocytes that engulf the invading organisms and kill them. The values of the lymphocytes indicate that the birds did not succumb to pathogenic fungi, viruses, bacteria, and protista.

Table 2: Haematological parameters of broiler finisher chickens fed diets containing varying levels of Mycofix®

Parameters	0	100	200	300	400	SEM
Packed cell volume (%)	25.83	24.00	25.50	27.00	26.83	1.32
Haemoglobin (g /dl)	8.58	7.97	8.47	8.90	8.92	0.45
White blood cell (x 10 ¹² /L)	11.23	7.17	11.63	8.10	5.00	2.53
Heterophils (%)	2.60 ^a	10.30 ^b	12.00 ^b	4.08 ^b	5.72 ^b	3.94
Lymphocytes (%)	92.00	86.17	85.17	89.80	89.50	2.98
Red blood cell (x 10 ⁹ /L)	4.42	3.92	4.20	4.50	4.55	0.24
MCV (fl)	58.62	60.99	60.99	60.68	59.19	1.08
MCH (pg)	9.36 ^a	12.64 ^b	9.84 ^b	12.61 ^b	21.31 ^b	3.33

ab Means with different superscripts along the same rows show significant differences (P < 0.05) SEM = Standard Error of Means, MCHC = Mean corpuscular haemoglobin concentration, MCV =Mean corpuscular volume, MCH = Mean corpuscular haemoglobin *Mitruka and Rawnsley (1977),**

Serum biochemical indices of broiler finisher chickens fed diets containing varying levels of Mycofix®

Table 3 displays the results of the serum biochemical parameters of broiler finishers given varying doses of Mycofix®. Differences in any of the measured parameters were not statistically significant (P>0.05). Serum biochemical indices can be used to measure a dependable biochemical system that can be used as a good indicator to tell the condition of the organism with regard to the changes occurring to it under the influence of internal and external factors (Toghyani *et al.*, 2010). Liver function is evaluated using serum enzyme analyses for alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) (Kwari *et al.*, 2011).

In this study, there was no discernible difference between the binder fed group and the control group for the serum

indices ALP, AST, and ALT, though there was a slight increase in these values. T. Abdelaziz *et al.* (2015) had reported that there were no significant differences in the values of AST, ALT, and ALP when compared with the control in their study with antimycotoxins. The workers reported that the use of a natural anti-mycotoxin (toxin binders) improved liver activities without any negative effects. Regardless of the treatment, the diet's energy content was sufficient as evidenced by non-significant blood glucose levels. The 400g Mycofix® diet had the highest glucose value, indicating that birds fed that diet likely had more readily available metabolizable glucose as a source of energy. The inclusion of Mycofix® was therefore implied to have improved the feed's energy source as there was no difference between the treatment diets and the control The addition of Mycofix® had no impact on total serum protein.

Table 3: Biochemical indices of broiler finisher chickens fed diets containing levels of Mycofix®

Parameters	Levels of Mycofix inclusion (g/100kg diet)					SEM
	0	100	200	300	400	
Glucose (mg/dl)	149.50	128.00	153.00	143.00	159.83	16.06
Total Protein (g/dl)	2.03	2.80	2.17	2.70	2.13	0.46
ALT (µ/l)	19.33	24.83	28.00	25.33	33.83	7.24
ALP (µ/l)	138.50	150.17	132.17	167.83	173.67	31.20
AST (µ/l)	29.00	33.67	35.17	36.33	40.55	6.42

SEM = Standard error means, AST= aspartate aminotransferase, ALT= alanine aminotransferase. ALP = alkaline phosphatase

CONCLUSION AND RECOMMENDATION

This study showed no adverse effect on the haematological and biochemical profiles of those birds placed on Mycofix®. Haematological and serum indices all fell within the normal range suggesting that the health status of the birds was not compromised even with increasing levels of Mycofix®.

It is advised that poultry farmers use of Mycofix® up to 400 g/100kg diet in broiler finisher feed because it is safe.

REFERENCES

- Abdelaziz, M.A.M., El-Faham, A.I. and Nematallah, G.M. (2015). Using Natural Feed Additives as Alternative to Anti-Mycotoxins in Broiler diets. *Egyptian Poultry Science*, 35:291-310.
- Adebiyi, O.A., Ologhobo, A.D and Ogunwole, O. (2010). Growth and haematological variables of broiler chicken feed. *International Journal of Current Research*, 2:62-66.
- Balog, J.M., Kidd, B.D., Huff, W.E., Huff, G.R., Rath, N.C. and Anthony, N.B. (2003). Effect of cold stress on broilers selected for resistance or susceptibility ascites syndrome. *Poultry Science*, 82:1383-1387.
- Bush, B.M. (1999). Interpretation of Laboratory Results for Small Animal Clinician. Black Well scientific publication U.K.32-67.
- Davies, E. T., Benson, J. A., Bicknell, S. R. and Gray, D. E. (1984). Ministry of Agriculture and Food Manual of Veterinary Investigation (MAFI) Laboratory Techniques, 2:35.
- Egbunike, G.N. Agiang., E. A., Owoyibo, A.O and Fafute, A.A. (2009). Effects of protein on performance and haematology of broilers fed cassava peel-based diets. *Archivos de zootecnia*, 58: 224 :656 – 670.
- Etim, N.N. and Oguike, M.A. (2010). Egg production of the domestic fowls (*Gallus gallus*). Factors affecting it and implications for food security. *Proceedings of the 35th Annual Conference of the Nigerian Society for Animal Production (NSAP)*, held at the University of Ibadan, Ibadan Oyo State, Nigeria. March 21st- 24th Pg. 660 – 663.
- Galvano, F.A., Piva, A. and Galvano, G. (2001). Dietary strategies to counteract the effects of mycotoxins: A review. *Journal Food Production*, 64:120-131.
- Gunasekar, K. R. (2007). Formulating feed for performance. Gurgaon, India: Courtesy of Avitech Animal Health Pvt. Limited.
- IAR, (2016). Annual Weather Report. Institute of Agricultural Research Meteorological Unit, Ahmadu Bello University, Zaria.
- Isaac, L.J., Abah, G., Akpan, B. and Ekaette, I.U. (2013). Haematological properties of different breeds and sexes of rabbits. *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria*, held at Abuja September 8th – 12th Pp.24-27.
- Jacela, J.Y., DeRouchey, J.M. and Tokach, M.D. (2010). Feed additives for swine: Fact sheets – flavours and mould inhibitors, mycotoxin binders, and antioxidants. *Journal of Swine Health Production*, 18(1):27-32.

- Jacque, J. (2015). Mycotoxins in Poultry Feed – [articles.extension.org /pages /65716/mycotoxins-in-poultry-feed](https://articles.extension.org/pages/65716/mycotoxins-in-poultry-feed).
- Kwari, I.D., Diarra, S.S., Saleh, B., Bovo, P.R., Ramat, O.A. and Tochukwu, D. (2011) Growth, Haematology and Serology of Broiler chickens fed different cultivars of sorghum as a replacement for maize in the semi-arid zone of Nigeria. *International Journal of Poultry Science*, 10(8): 608-612.
- Mallick, P., Muduli, K., Biswai, J. N. and Pumwa, J. (2020). Broiler poultry feed cost optimization using linear programming technique. *Journal of Operations and strategic planning*, 3(1):31-57.
- Mitruka, B.M. and Rawnsley, H.M. (1977). Clinical biochemical and haematological reference values in normal experimental animal, Mason publishing, USA Inc. N.V. Pp. 88:142.
- NRC., (1994) National Research Council. Nutrient Requirement for Poultry, 9th Revised Edition National Academy Press, Washington, DC.
- Olafedehan, C.O., Obun, A.M., Yusuf, M.K., Adewumi, O.O., Oladefedehan, A.O., Awofolaji, A. O. and Adeniji, A.A. (2010). Effects of residual cyanide in processed cassava peel meals on haematological and biochemical indices of growing rabbits. *Proceedings of 35th Annual Conference of Nigerian Society for Animal Production, held at the University of Ibadan, Ibadan Oyo State, Nigeria. March 21st- 24th P Pg. 212-*
- Olugbenga, S. O., Abayomi, O. O., Oluseye, A. A. and Taiwo, A. T. (2015). Optimized nutrients diet formulation of broiler poultry rations in Nigeria using linear programming. *Journal of Nutrition and Food Sciences*, 14:2.
- Reinhold, J. G. (1953). Manual determination of total serum proteins, albumin and globulin fractions by Biuret method In Standard Methods in Clinical Chemistry (Reiner M.Ed.) Academic Press, New York, 1: 88.
- SAS, (2003). Statistical analysis Systems users guide, version 9.0 SAS Institute Inc., Cary, NC. The USA.
- Soetan, K.O., Akinrinde, A.S. and Ajibade, T.O. (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production, held at River State University of Science and Technology, PortHarcourt, Nigeria. Pg. 49-52.*
- Toghyani, M., Toghyani, M.A., Gheisari, G. and Mohammadrezaei, M. (2010). Growth performance, serum biochemistry and blood haematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). *Livestock Science*, 129:173-178.