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Effect of Fungi and Fungi Secondary Metabollite (Mycotoxin) On Blood Parameters and Liver Function of an Albino Rat

[1]Odetunde S.K., [2]Saheed O.W., [3]Shofoluwe A.S. & [4]Ekum, M.I.
[1,2,4]Department of Science Laboratory Technology
(Microbiology Unit)
School of Pure & Applied Sciences
Lagos State Polytechnic
Ikorodu, Lagos State, Nigeria
odetundesimeon@yahoo.Com

[3]Department of Mathematics & Statistics
(Statistics Unit)
School of Pure & Applied Sciences
Lagos State Polytechnic
Ikorodu, Lagos State, Nigeria

ABSTRACT

This research was conducted to monitor the effect of the presence of fungi and fungi secondary metabolites on the blood parameters and liver functions, through studying the changes in the blood of experimental animals, by monitoring their blood parameters, and liver functions. The full blood count and the liver function test for each animal was carried out, before the experiment, during the experiment, and after the experiment, to observe any significant changes in their values. The randomized complete block design (two-way) analysis of variance test shows that there is a significant change in their values for both liver function and full blood count at the end of fifteenth day of feeding with contaminated feed. The weight of the experimental animals fed with contaminated feed reduced drastically while that of the control animals increased significantly. The large variance and boxplot in the exploratory data analysis (EDA) also described the variation in the values at the beginning and at the end of the experiment. Finally, the histopathology analysis of all the animals was carried out, and the result of the study-animals was compare with that of the control-animals verified that there is no damage in the liver of the control animals, but changes were observed in the liver of the studied animals. This result shows that a mold-contaminated diet results in adverse effects on blood parameters and liver morphorlogy.

Key Words: Mycotoxin, Mycotoxicosis, Blood Parameters, Liver Function and Histopathology

Aims Research Journal Reference Format:
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1. INTRODUCTION

Fungi are ubiquitous plant pathogens that are major spoilage agents of foods and feedstuffs. The infection of plants by various fungi not only results in reduction in crop yield and quality with significant economic losses but also contamination of grains with poisonous secondary metabolites called mycotoxins. Mycotoxins are produced mainly by the mycelia structure of filamentous fungi, or more specifically, the molds. These toxins are produced by saprophytic fungi during storage or by endophytic fungi during plant growth. They are found mainly in post-harvest crops such as cereal grains or forages. Mycotoxins in humans or animals are characterized as food or feed related, non-contagious, non-transferable, non-infectious, and non-traceable to microorganisms other than fungi. Since they are generally lipophilic (except for FB) they tend to accumulate in the fat fraction of plants and animals. Mycotoxins are secondary metabolites that have no biochemical significance in fungal growth and development (Moss, 1991).

Toxigenic molds are known to produce one or more of these toxic secondary metabolites. It is well established that not all molds are toxigenic and not all secondary metabolites from molds are toxic. Although there are over 300 mycotoxins that have been isolated and chemically characterized (Betina, 1984), research has focused on those forms causing significant injuries to humans and their farm or companion animals. These include AF, OT, trichothecenes, ZEN, F, and ergot alkaloids. There have also been recent concerns over other toxins such as citrinin and sterigmatocystin. Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions. Often times most factors are beyond human control (D'Mello and McDonald, 1997).

Mycotoxins are a relatively large, diverse group of naturally occurring, fungal toxins, many of which have been strongly implicated as chemical agents of toxic disease in humans and animals. They are unavoidable contaminants in foods and feeds and are a major problem all over the world (Wood, 1992). Mycotoxins are structurallydiverse compounds produced by filamentous fungi that vary in their chemistry and biological effects (Sudakin et al., 2003). Among the various mycotoxins, aflatoxins (AFs), ochratoxin A (OTA), T-2, zearalenone(ZEN) and deoxynivalenol are often encountered in foodstuffs in different parts of the world. In nature, mycotoxins rarely occur as a single contaminant since many fungal species that produce mycotoxins grow and produce their toxic metabolites under similar conditions. Furthermore, a typical animal diet is made up of several sources, each of which may be contaminated with a different mycotoxin or more than one mycotoxins.

Thus, mixed feeds, made from foodstuffs contaminated with individual mycotoxins, may have all the mycotoxins present in different individual ingredients. The consumption of multiple mycotoxin contaminated diet may induce hematological, biochemical and liver physiological changes and growth depression in animals (Awad et al., 2006, Shi et al., 2006, Razar et al., 2007, Gowda et al., 2008), and thus the presence of mycotoxins in poultry feeds causes significant economic losses to animal industries (Awad et al., 2006a).

Liver

The largest organ in the body is essential in keeping the body functioning properly. It removes or neutralizes poisons from the blood, produces immune agents to control infection and removes microbes from the blood. It makes proteins that regulate blood clotting and produces bile to help absorb fats and fat-soluble vitamins. Because of the activities it is exposed to a wide variety of insults and is therefore, one of the most frequently injured organs of the body. Yet one cannot live without a functioning liver (Kirsch *et al.*, 1995). In man, acute liver failure is observed following ingestion of mushrooms (fungi) like ***Amanita phalloides*** and ***Amanita verna***. The toxins present in these mushrooms, namely phalloidin and phalloin are extremely lethal to liver cells (Rensberg, 1977).

Blood

The blood is living tissue made up of liquid and solids. The liquid part, called plasma, is made of water, salts and protein. Over half of the blood is plasma. The solid part of the blood contains red blood cells, white blood cells, and platelets. The presence of mycotoxins in blood can result to blood disorder, which may prevents it from doing its job. Two main genera of fungi ***Aspergillus*** and***Penicillium***which may develop during storage may produce harmful mycotoxins, such as aflatoxin and ochratoxin (Akande et al., 2006). As with other major mycotoxins, ochratoxin has been detected worldwide in human blood and several food commodities (VanEgmondandSpeijers, 1994; Araguas et al., 2005; Matrella et al,. 2006; Boudra et al., 2007; Iheshiulor et al., 2011).

1.1 Scope of The Study

- The study was based on the effect of contaminated food/feed on liver and blood, of animal
- It also revealed the effect on the physiological structure of the liver.
- The significance difference in parameters was verified with statistical analysis.
- The study failed to verify the etiology of the infection in the studied animal, that is, it does not verify, if the infection is been caused by the fungi, or by the myctoxin.

2. METHODOLOGY

2.1 Preparation Of The Contaminated Food Sample

Maize was chosen as the desired food sample, because it is one of the foods that support the growth of ***molds.*** It was made to be infected by microorganism, through its exposure to an unsterile environment, and by increasing its water activity. It remained in this condition for seven days, while the growth of the microorganism increases. The organisms were ascertained to be fungi, by culturing them aseptically, on Saboroud dextrose agar. This agar only support the growth of fungi.

2.2 Acclimatization

The animals were divided into two groups. Three were housed in cage A, while the remaining two were housed in cage B. The animals in cage B, were used as the control animals. They were all acclimatized for seven days, during which they were being fed with non-contaminated feeds. Their temperature and weight was recorded throughout the acclimatization period (see Table 1a).

Figure 1: Study animals , Cage A

Figure 2: control animals, Cage B

Figure 3: Weighing the rat using analytical balance

Figure 4: Observing the temperature using a thermometer

2.3 Feeding Of The Animals

The control animals were fed with non-contaminated feed, being separated from the study animals, in a separate animal cage. The other study animals were being fed with fungal contaminated feeds for fifteen (15) days. It was observed that, the study animals refused to feed on the contaminated feed. Hence, the contaminated feed was grinded into fine particles, using mortar and pestle, in a safety cabinet, to prevent the ingestion of spores. Then, 50g of the fine particle of the contaminated feed was dissolved in 500ml of water. Then, 10ml of the resulting solution was given orally, to each of the study animals, daily using a sterile syringe, while the control animals were fed with non-contaminated feeds, daily.

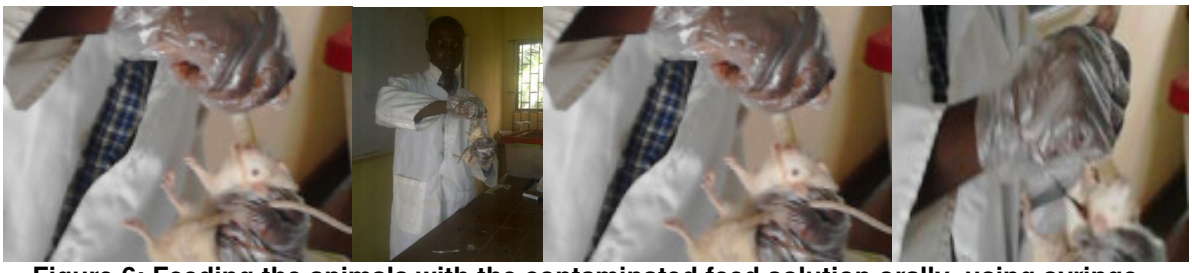


Figure 6: Feeding the animals with the contaminated feed solution orally, using syringe

The weights as well as the temperatures of the study animals and the control animals were observed at two days interval (see Table 1b).

On the seventh day, their liver function test and full blood count was carried out, to determine, if there will be a change in the previous test, carried out before being fed with the contaminated feed.
After the fifteenth days, their liver function test and the full blood count were determined for the third time. The histopathology analysis was also carried out.

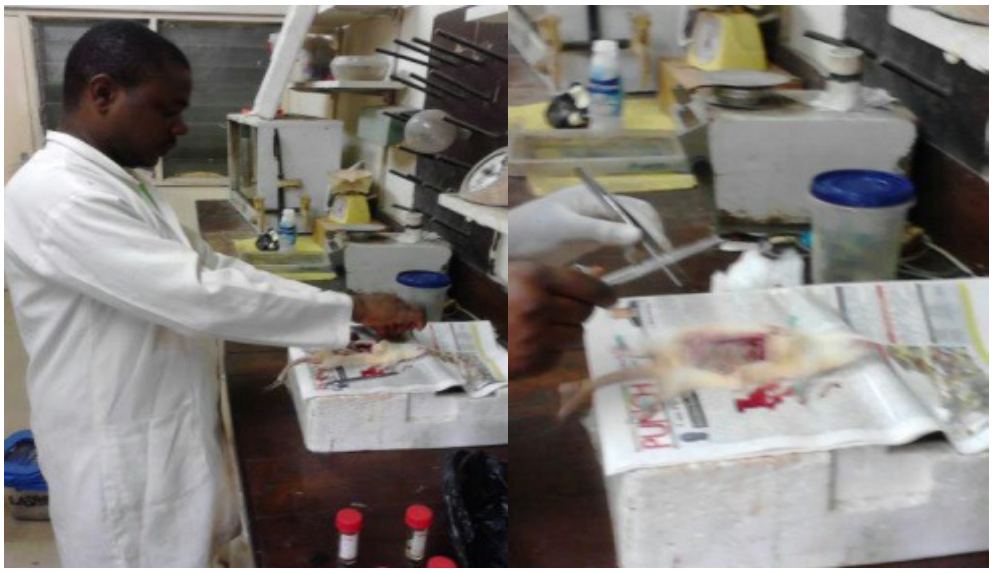


Figure 6: a technologist dissecting the experimental animal at the faculty of basic medical science, university of Lagos, idi-araba

Key For Blocking

- *R1: study rat 1*
- *R2: study rat 2*
- *R3: study rat 3*
- *CR1: control rat 1*
- *CR2; control rat*

KEY FOR PARAMETER

- *temp: temperature*
- *wt: weight*

Key For Blood and Liver Parameters

- ALT: Alanine aminotransferase
- ALT: Aspartate aminotransferase
- ALP: Alkaline phosphatase
- WBC: White blood count
- RBC: Red blood cell count
- HBg/dl: Heamoglobin
- HCT: Heamatocrite
- MCHC: Mean cell haemoglobin concentration
- MCH: Mean cell haemoglobin
- MCV fl: Mean cell volume
- PLT: Platelet

3. RESULT

The data for this study is described using exploratory data analysis (EDA), which includes using line graphs, mean plot, boxplot, measures of central tendency, measure of partition and variation, skewness and kurtosis to present and explained the nature and shape of the data. The analysis was carried out using completely randomized design (CRD) and randomized complete block design (RCBD) and duncan comparison test for the post hoc to see whether there are differences in the parameters measured for the rats (R1, R2, R3, CR1 and CR2). The various readings are tabulated in Tables 1a to 3c.

Table 1a: Table of constant temperature and increasing body weight of experimental animals, during acclimatization

	R1		R2		R3		CR1		CR2	
Day	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)
1	37	240.7	37	170.5	37	181.4	37	175.3	37	170.4
2	37	248	37	170.5	37	184.3	37	179.8	37	172.8
3	37	250	37	173.3	37	185.6	37	195.3	37	172.9
4	37	253	37	175.5	37	192.9	37	198.9	37	183.4
5	37	253	37	175.9	37	195.3	37	201.5	37	187.3
6	37	258	37	178.9	37	197.1	37	210.5	37	201.8
7	37	260.7	37	180.4	37	198.3	37	215	37	205.9

Table 1b: Table of constant temperature and increasing body weight of experimental animals observed at two days interval

	R1		R2		R3		CR1		CR2	
Day	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)	Temp (^{0}C)	Wt (g)
1	37	260.6	37	180.4	37	198.3	37	215	37	205.9
3	37	240.3	37	178.4	37	195.2	37	219.5	37	210.3
5	37	233.5	37	173.2	37	190.4	37	224.3	37	217.3
7	37	228.8	37	170.1	37	185.3	37	230.6	37	227.6
8	37	208.6	37	168.4	37	180.4	37	234.7	37	233.4
10	37	198.5	37	162.4	37	177.5	37	240.6	37	240.4
12	37	195.3	37	158.4	37	169.4	37	243.9	37	243.2
13	37	192.5	37	155.3	37	163.8	37	251.6	37	246.3
15	37	189.4	37	153.3	37	160.6	37	253.3	37	248.5

Table 2a: Full Blood Count of the Experimental Animals Before Feeding

S/N	WBC (× $10^{9/1}$)	HB g/dl	RBC ×$10^{12/1}$	HC T%	MCV fl	MCHC g/dl	PLT ×$10^{9/1}$	MCH Pg
CR1	5.7	12.2	6.36	36.20	51.85	33.7	542	9.63
CR2	8.2	12.5	6.13	37.43	46.98	32.3	671	19.21
R1	4.2	11.4	8.43	32.87	51.87	33.7	830	22.62
R2	6.4	10.4	6.43	35.67	48.67	32.9	925	21.98
R3	5.8	11.6	7.42	39.30	55.23	32.5	815	23.67

Table 2b: Full Blood Count of the Experimental Animals During Feeding

S/N	WBC (× $10^{9/1}$)	HB g/dl	RBC ×$10^{12/1}$	HC T%	MCV fl	MCHC g/dl	PLT ×$10^{9/1}$	MCH Pg
CR1	5.75	12.2	6.42	36.29	51.85	33.2	540	19.48
CR2	8.00	12.9	6.50	37.98	47.87	31.8	669	19.92
R1	6.70	12.7	10.42	40.89	61.76	37.5	851	30.52
R2	7.90	12.9	9.91	43.67	55.87	36.9	952	29.3
R3	6.80	13.4	10.41	47.56	62.65	36.5	839	31.95

Table 2c: Full Blood Count of the Experimental Animals After Feeding

S/N	WBC (× $10^{9/1}$)	HB g/dl	RBC ×$10^{12/1}$	HC T%	MCV fl	MCHC g/dl	PLT ×$10^{9/1}$	MCH Pg
CR1	5.7	12.2	6.36	36.2	51.8	33.7	542	19.63
CR2	8.2	12.5	6.13	37.43	46.98	32.3	671	19.21
R1	8.2	16.5	13.07	51.5	72.3	41.8	871	39.1
R2	8.6	16.3	12.29	50.2	65.3	39.8	987	37.3
R3	9.2	17.5	13.05	53.6	73.92	40	953	38.3

Table 3a: Liver Function Test Of The Experimental Animals Before feeding

	AST	ALT	ALP
CR1	24.9	16.8	62.5
CR2	42.4	11.7	63
R1	23.6	11.5	37.6
R2	26.8	10.5	12
R3	26.6	10	54.7

Table 3b: Liver Function Test Of The Experimental Animals During Feeding (seventh day)

	AST	ALT	ALP
CR1	24.4	16.3	61.4
CR2	42	11.8	63.5
R1	26.6	15.6	42.6
R2	30.8	14.7	12
R3	31.6	13.8	60.7

Table 3c: Liver Function Test of the Experimental Animals After Feeding (fifteenth day)

	AST	ALT	ALP
CR1	23.5	14.2	63.4
CR2	42.2	12.9	64.8
R1	31.4	21.7	49.9
R2	37.9	20.7	18.5
R3	38.8	22.6	67.4

3.1 Exploratory Data Analysis (EDA)

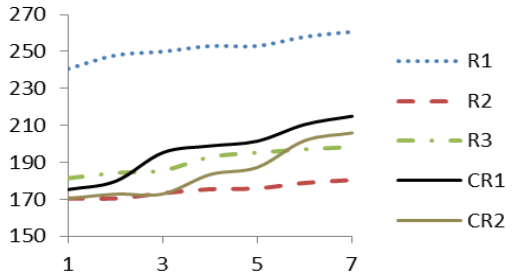


Figure 7a: Line graph showing body weight of experimental animals during acclimatization

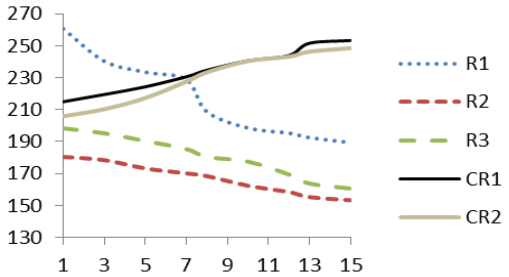


Figure 7b: Line graph showing body weight of experimental animals observed at two days interval

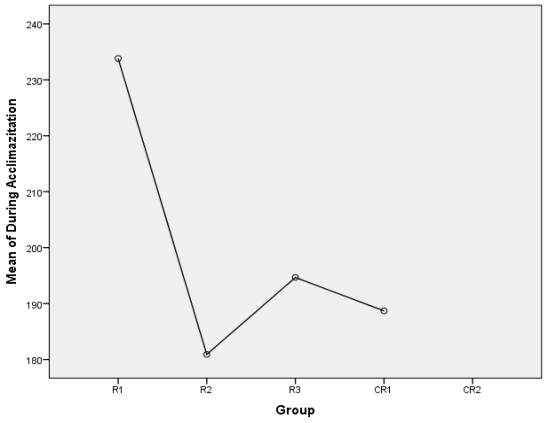


Figure 8a: Mean plot showing increasing body weight of experimental animals during acclimatization

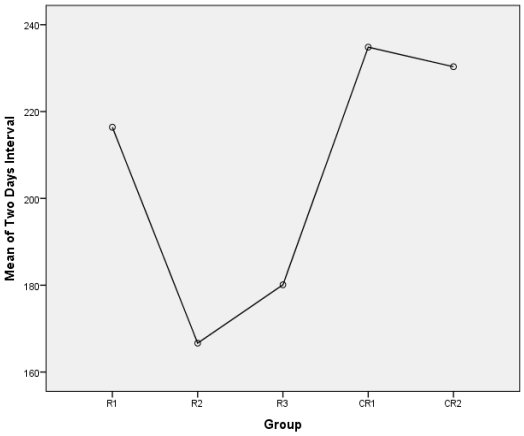


Figure 8b: Mean Plot showing body weight of experimental animals observed at two days interval

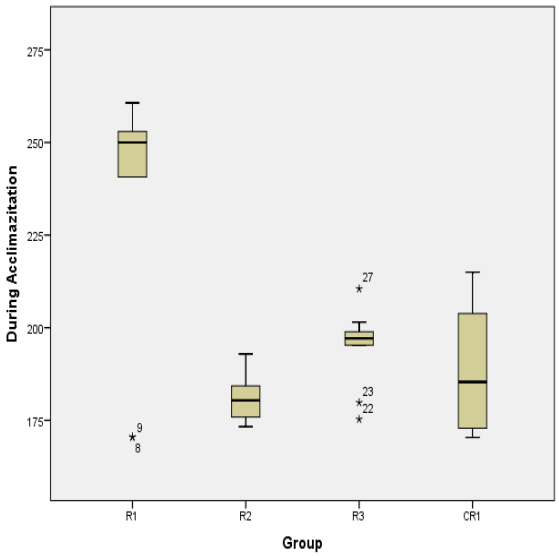


Figure 9a: Boxplot showing increasing body weight of experimental animals during acclimatization

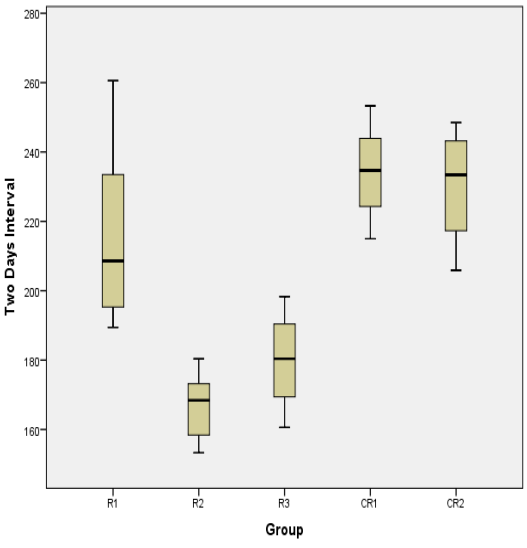
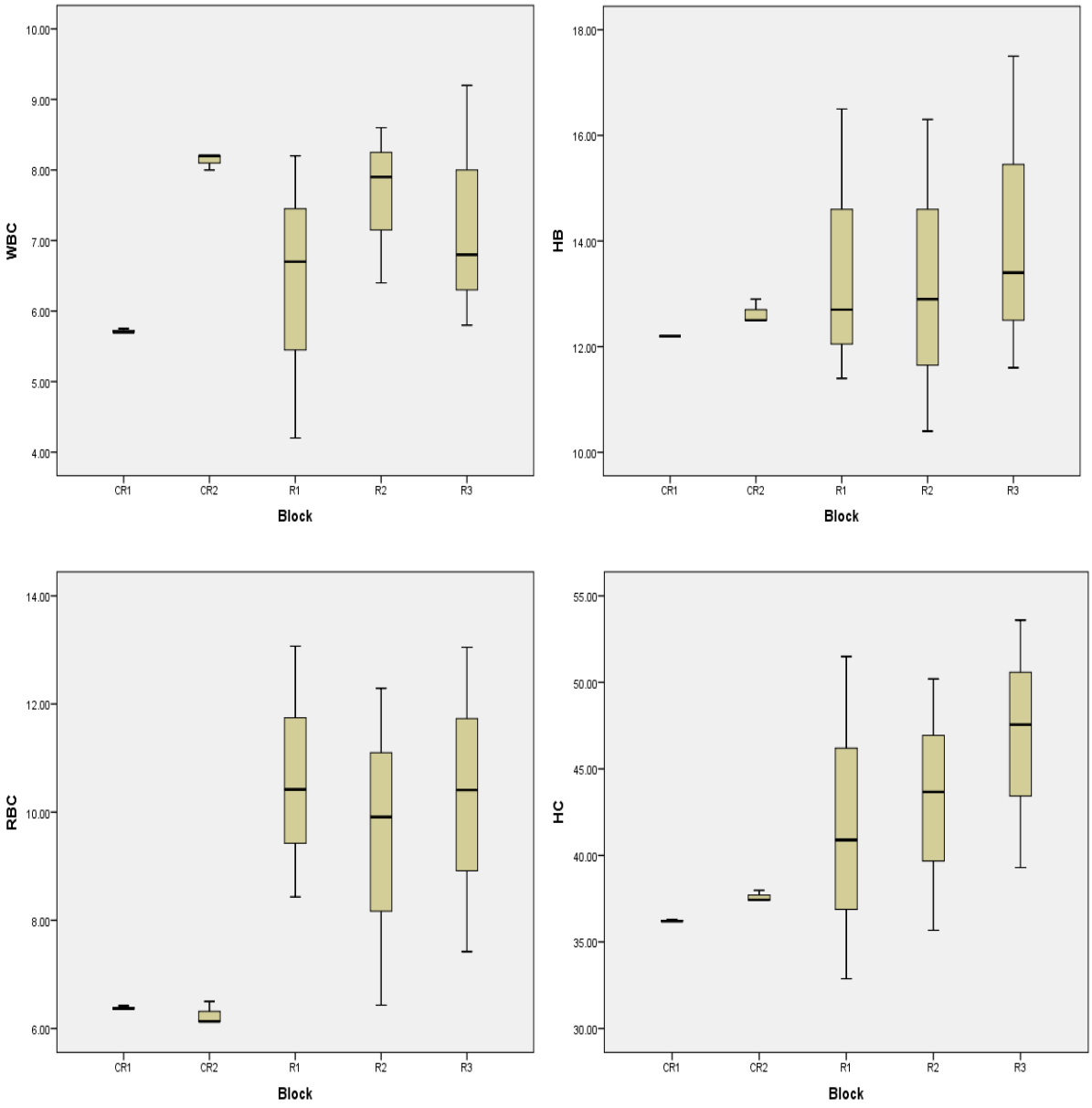


Figure 9b: Boxplot showing body weight of experimental animals observed at two days

interval


WBC
10.00
9.00
8.00
7.00
6.00
5.00
4.00
CR1
CR2
R1
R2
R3
Block
HB
18.00
16.00
14.00
12.00
10.00
CR1
CR2
R1
R2
R3
Block
RBC
14.00
12.00
10.00
8.00
6.00
CR1
CR2
R1
R2
R3
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HC
55.00
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CR1
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R1
R2
R3
Block

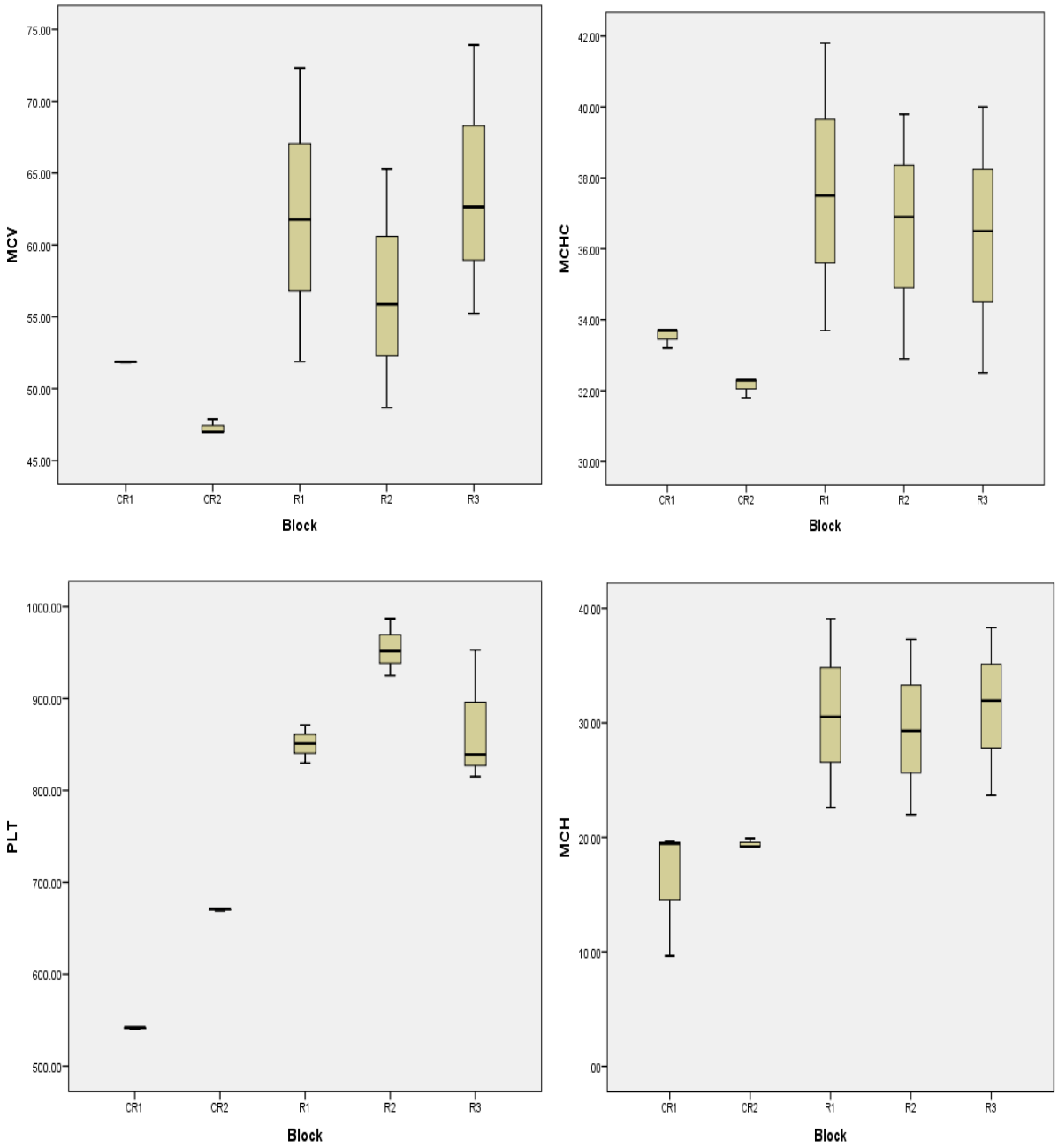

Figure 10: Box Plot Showing Full Blood Count of the Experimental Animals for 8 Parameters

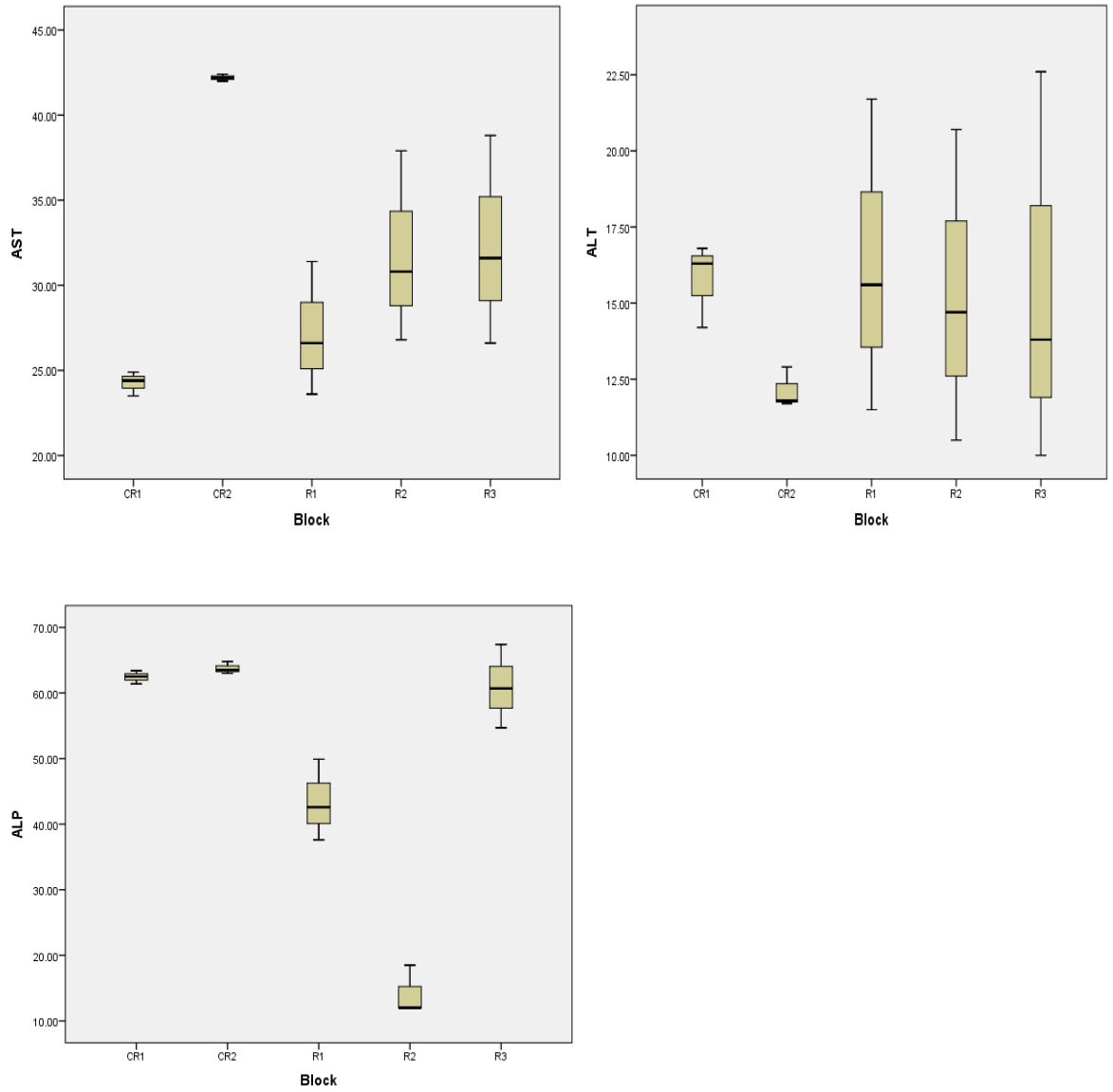

Figure 11: Box Plot Showing Liver Function Test of the Experimental Animals for 8 Parameters

3.2 Randomize Complete Block Design (RCBD) – Two Way Anova – F-Test

3.2.1 Full Blood Count of The Experimental Animals

Table 4a: ANOVA SUMMARY TABLE FOR WBC × $10^{9/1}$

Source	Sum of Squares	df	Mean Square	F	P-value
Group	11.406	4	2.851	2.997	0.087
Period	9.216	2	4.608	4.843	0.042
Error	7.612	8	.951		
Total	768.143	15			
R Squared = 0.990 (Adjusted R Squared = 0.981)					

Table 4b:POST-HOC TEST: DUNCAN COMPARISON TEST FOR WBC × $10^{9/1}$

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	5.70	5.75	5.70	5.7167±0.02887[b]
	CR2	8.20	8.00	8.20	8.1333±0.11547[ab]
	R1	4.20	6.70	8.20	6.3667±2.02073[ab]
	R2	6.40	7.90	8.60	7.6333±1.12398[ab]
	R3	5.80	6.80	9.20	7.2667±1.74738[a]
	Mean	6.06±1.44499[b]	7.03±0.93515[ab]	7.98±1.33866[a]	7.0233±1.42012

Table 5a: ANOVA SUMMARY TABLE FOR HBg/dl

Source	Sum of Squares	df	Mean Square	F	P-value
Group	7.057	4	1.764	0.685	0.622
Period	29.361	2	14.681	5.696	0.029
Error	20.619	8	2.577		
Total	2649.560	15			
R Squared = 0.992 (Adjusted R Squared = 0.985)					

Table 5b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR HBg/dl

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	12.2000	12.2000	12.2000	12.2000±0.00000[a]
	CR2	12.5000	12.9000	12.5000	12.6333±0.23094[a]
	R1	11.4000	12.7000	16.5000	13.5333±2.02073[a]
	R2	10.4000	12.9000	16.3000	13.2000±2.96142[a]
	R3	11.6000	13.4000	17.5000	14.1667±3.02379[a]
	Mean	11.62±0.81363[b]	12.82±0.43243[ab]	15.00±2.46374[a]	13.1467±2.01844

Table 6a: ANOVA SUMMARY TABLE FOR RBC × $10^{12/1}$

Source	Sum of Squares	Df	Mean Square	F	P-value
Group	55.055	4	13.764	6.096	.015
Period	26.108	2	13.054	5.782	.028
Error	18.063	8	2.258		
Total	1214.310	15			
R Squared = 0.985 (Adjusted R Squared = 0.972)					

Table 6b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR RBC × $10^{12/1}$

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	6.3600	6.4200	6.3600	6.3800±0.03464[b]
	CR2	6.1300	6.5000	6.1300	6.2533±0.21362[b]
	R1	8.4300	10.4200	13.0700	10.6400±2.32781[a]
	R2	6.4300	9.9100	12.2900	9.5433±2.94716[a]
	R3	7.4200	10.4100	13.0500	10.2933±2.81681[a]
	Mean	6.95±0.96204[b]	8.73±2.08446[ab]	10.18±3.60680[a]	8.6220±2.66226

Table 7a: ANOVA SUMMARY TABLE FOR HC T%

Source	Sum of Squares	Df	Mean Square	F	P-value
Group	220.031	4	55.008	2.778	0.102
Period	225.434	2	112.717	5.692	0.029
Error	158.417	8	19.802		
Total	25965.875	15			
R Squared = 0.994 (Adjusted R Squared = 0.989)					

Table 7b; POST-HOC TEST: DUNCAN COMPARISON TEST FOR HC T%

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	36.2000	36.2900	36.2000	36.2300 ± 0.05196^{b}
	CR2	37.4300	37.9800	37.4300	37.6133 ± 0.31754^{b}
	R1	32.8700	40.8900	51.5000	41.7533 ± 9.34496^{ab}
	R2	35.6700	43.6700	50.2000	43.1800 ± 7.27738^{ab}
	R3	39.3000	47.5600	53.6000	46.8200 ± 7.17866^{a}
	Mean	36.29 ± 2.36899^{b}	41.28 ± 4.50257^{ab}	45.79 ± 8.29014^{a}	41.1193 ± 6.56768

Table 8a: ANOVA SUMMARY TABLE FOR MCV fl

Source	Sum of Squares	df	Mean Square	F	P-value
Group	575.876	4	143.969	5.370	0.021
Period	311.049	2	155.525	5.801	0.028
Error	214.479	8	26.810		
Total	48691.805	15			
R Squared = 0.996 (Adjusted R Squared = 0.992)					

Table 8b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR MCV fl

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	51.8500	51.8500	51.8000	51.8333 ± 0.2887^{bc}
	CR2	46.9800	47.8700	46.9800	47.2767 ± 0.51384^{bc}
	R1	51.8700	61.7600	72.3000	61.9767 ± 10.21672^{ab}
	R2	48.6700	55.8700	65.3000	56.6133 ± 8.33988^{abc}
	R3	55.2300	62.6500	73.9200	63.9333 ± 9.41086^{a}
	Mean	50.92 ± 3.19881^{b}	56.00 ± 6.33909^{ab}	62.06 ± 12.13145^{a}	56.3267 ± 8.86971

Table 9a: ANOVA SUMMARY TABLE FOR MCHC g/dl

Source	Sum of Squares	df	Mean Square	F	P-value
Group	63.963	4	15.991	3.686	0.055
Period	50.652	2	25.326	5.839	0.027
Error	34.701	8	4.338		
Total	18777.180	15			
Squared = 0.998 (Adjusted R Squared = 0.997)					

Table 9b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR MCHC g/dl

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	33.7000	33.2000	33.7000	33.5333±0.28868^{ab}
	CR2	32.3000	31.8000	32.3000	32.1333±0.28868^{b}
	R1	33.7000	37.5000	41.8000	37.6667±4.05257^{a}
	R2	32.9000	36.9000	39.8000	36.5333±3.46458^{a}
	R3	32.5000	36.5000	40.0000	36.3333±3.75278^{a}
	Mean	33.02±0.65727^{b}	35.18±2.52131^{ab}	37.52±4.22812^{a}	35.2400±3.26580

Table 10a: ANOVA SUMMARY TABLE FOR PLT x10$^{9/1}$

Source	Sum of Squares	df	Mean Square	F	P-value
Group	337117.733	4	84279.433	90.198	0.000
Period	6175.600	2	3087.800	3.305	0.090
Error	7475.067	8	934.383		
Total	9411366.000	15			
R Squared = 0.999 (Adjusted R Squared = 0.999)					

Table 10b; POST-HOC TEST: DUNCAN COMPARISON TEST FOR PLT x10$^{9/1}$

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	542.0000	540.0000	542.0000	541.3333±1.15470^{d}
	CR2	671.0000	669.0000	671.0000	670.3333±1.15470^{c}
	R1	830.0000	851.0000	871.0000	850.6667±20.50203^{b}
	R2	925.0000	952.0000	987.0000	954.6667±31.08590^{a}
	R3	815.0000	839.0000	953.0000	869.0000±73.72923^{b}
	Mean	756.60±150.45^{b}	770.20±163.97^{ab}	804.80±191.38^{a}	777.2000±158.28735

Table 11a: ANOVA SUMMARY TABLE FOR MCH Pg

Source	Sum of Squares	df	Mean Square	F	P-value
Group	599.147	4	149.787	11.526	0.002
Period	322.990	2	161.495	12.427	0.004
Error	103.968	8	12.996		
Total	10745.205	15			
R Squared = 0.990 (Adjusted R Squared = 0.982)					

Table 11b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR MCH Pg

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	9.6300	19.4800	19.6300	16.2467±5.73069^{bc}
	CR2	19.2100	19.9200	19.2100	19.4467±0.40992^{bc}
	R1	22.6200	30.5200	39.1000	30.7467±8.24234^{ab}
	R2	21.9800	29.3000	37.3000	29.5267±7.66251^{abc}
	R3	23.6700	31.9500	38.3000	31.3067±7.33619^{a}
	Mean	19.42±5.71717^{b}	26.23±6.03999^{ab}	30.71±10.32527^{a}	25.4547±8.56114

3.2.2 Liver Function Test Of The Experimental Animals

Table 12a: ANOVA SUMMARY TABLE FOR AST

Source	Sum of Squares	df	Mean Square	F	P-value
Block	558.253	4	139.563	13.670	0.001
Period	88.801	2	44.401	4.349	0.053
Error	81.679	8	10.210		
Total	15675.550	15			
R Squared = 0.995 (Adjusted R Squared = 0.990)					

Table12b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR AST

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	24.9000	24.4000	23.5000	24.2667±0.70946[c]
	CR2	42.4000	42.0000	42.2000	42.2000±0.2000[a]
	R1	23.6000	26.6000	31.4000	27.2000±3.93446[b]
	R2	26.8000	30.8000	37.9000	31.8333±5.62168[b]
	R3	26.6000	31.6000	38.8000	32.3333±6.13297[b]
	Mean	28.86±7.68167[b]	31.08±6.78764[ab]	34.76±7.40966[a]	31.5667±7.21473

Table 12a: ANOVA SUMMARY TABLE FOR ALT

Source	Sum of Squares	df	Mean Square	F	P-value
Group	32.151	4	8.038	.704	0.611
Period	102.097	2	51.049	4.469	0.050
Error	91.389	8	11.424		
Total	3594.640	15			
R Squared = 0.975 (Adjusted R Squared = 0.952)					

POST-HOC TEST: DUNCAN COMPARISON TEST FOR ALT

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	16.8000	16.3000	14.2000	15.7667±1.37961[a]
	CR2	11.7000	11.8000	12.9000	12.1333±0.66583[a]
	R1	11.5000	15.6000	21.7000	16.2667±5.13258[a]
	R2	10.5000	14.7000	20.7000	15.3000±5.12640[a]
	R3	10.0000	13.8000	22.6000	15.4667±6.46323[a]
	Mean	12.10±2.71937[b]	14.44±1.75014[ab]	18.42±4.51962[a]	14.9867±4.01459

Table 13a: ANOVA SUMMARY TABLE FOR ALP

Source	Sum of Squares	df	Mean Square	F	P-value
Group	5357.960	4	1339.490	161.862	0.000
Period	122.949	2	61.475	7.429	0.015
Error	66.204	8	8.276		
Total	41464.180	15			
R Squared = 0.998 (Adjusted R Squared = 0.997)					

Table 13b: POST-HOC TEST: DUNCAN COMPARISON TEST FOR ALP

		Period			
		Before Feeding	During Feeding	After Feeding	Mean
Group	CR1	62.5000	61.4000	63.4000	62.4333±1.00167[a]
	CR2	63.0000	63.5000	64.8000	63.7667±0.92916[a]
	R1	37.6000	42.6000	49.9000	43.3667±6.18574[b]
	R2	12.0000	12.0000	18.5000	14.1667±3.75278[c]
	R3	54.7000	60.7000	67.4000	60.9333±6.35321[a]
	Mean	45.96±21.58409[b]	48.04±21.83032[b]	52.80±20.33728[a]	48.9333±19.90534

Table 14: Histopathology Analysis of the Experimental Animals

SLIDES	HISTOLOGIC FINDINGS
CONTROL LIVER 1	Normal
CONTROL LIVER 2	Normal
STUDY R1	No abnormality seen
STUDY R2	ld Focal Microvesicular Steatosis (fatty changes)
STUDY R3	Moderate to severe portal inflammation

Observations

1. It was observed that, the study-animals, were losing appetite, which led to their loss of weight gain.
2. The temperature of both the study animals and the control animals remains constant, indicating the absence of fever.
3. The rate of weight loss of the study animals is not uniform.
4. The rate of weight gain of the control animals is not uniform.
5. The control animals were active throughout the research work.
6. The study animals become dull as they were being fed with the contaminated feed.
7. There is absence of diarrhoea in both the study and control animals.

4. DISCUSSION OF FINDINGS

Table 1a shows that the animals have constant temperature and a daily increasing body mass as depicted by figure 7a. After two days of the experiment, the body mass of rats R1, R2 and R3 decreased while that of CR1 and CR2 increased as shown in Table 1b and figure 7b. The mean plots and the box plots summarized the mean, measure of partition and variations. Figure 10 shows the box plots of the full blood count of the experimental animals displaying for 8 parameters. The box plot clearly depicts that there is a large variation in the values of these parameters for rats R1, R2 and R3 while that of CR1 and CR2 are almost the same. In addition, figure 11 shows the box plots of the liver function test of the experimental animals displaying for 3 parameters. The box plot clearly depicts that there is a large variation in the values of these parameters for rats R1, R2 and R3 while that of CR1 and CR2 are almost the same. The whiskers of the box plots depict the extreme values.

The full blood count and the liver function test were performed to test whether there is a significant difference among the body mass of the rats in the different groups before, during and after feeding with the contaminated feed. The randomized complete block design, two-way Analysis of variance (ANOVA) test is used for this test and a P-value less than 0.05 shows a significant difference, while the Duncan Comparison Test was used to classify the animals into different homogeneous subsets if the ANOVA test is significant. The same super script implies homogeneity of weight. The result of the analysis at 5% level of significance for the blood count parameters shows that RBC, MCV, PLT and MCH are significant while WBC, HB, HC and MCHC are not significant. The RBC of animals R1, R3 and R2 are significantly greater than those of CR1 and CR2 and it is also shown in their large standard deviation. Superscript “a” shows the highest mean value, followed by superscript “b”, then “c” in that order.

The HC of R3, R2, R1 are significantly greater than those of CR2 and CR1. The MCV of R3, R1 and R2 are significantly greater than those of CR1 and CR2. The PLT of $R2^{a}$ is significantly greater than those of $R1^{b}$ and $R3^{b}$ and those of R1 and R3 are significantly greater than that of $CR2^{c}$ and CR2 is significantly greater than $CR1^{d}$. More so, the result of the analysis at 5% level of significance for the liver function test shows that AST and ALP are significant while ALT is not significant. The AST of animals $CR2^{a}$ is significantly greater than $R3^{b}$, $R2^{b}$ and $R1^{bc}$ and R3 and R2 are greater than $CR1^{c}$. The ALP of $CR2^{a}$, $CR1^{a}$ and $R3^{a}$ are significantly greater than that of $R1^{b}$ and that of R1 is significantly greater than that of $R2^{c}$.

Generally, the full blood count parameters values for animals R1, R2 and R3 are significantly greater than those of the control animals CR1 and CR2 while on the contrary, the liver function test parameters of control animals CR1 and CR2 are greater than animals R1, R2 and R3. The histopathology analysis verified that, there is no damage in the hepatic tissue of photomicrograph 1 and 2, i.e the control animals, as well as the study rat 1. Photomicrograph 4, showed a mild microvessiclessteatoesis or mild fatty changes, while photomicrograph 5, showed moderate to severe portal inflammation. Fatty acid changes seen, is indicative of mycotoxins induced fatty hepatitis. This conforms with the report given by Gelderblom and Snyman, 1991, who reported that mycotoxins such as AFB1 also have been implicated in hepatic tumor formation in rats.

Full blood count (FBC), sometimes referred to, as full blood examination, or complete blood count, is one of the most commonly performed blood test, as it can tell us more about the health status. According to the result, there is a significant increase in the level of all the blood parameters of the experimental animals, compared to the values of the control animals. This measurement detects an abnormal high concentration of heamoglobin, which may occur due to chronic lung disease. This verified that, the ingestion of fungal-contaminated feeds by the experimental animals, had led to damages in their lungs. This might have occurred due to an abnormal increase in their red cell production by the bone marrow. Abnormally high number of red blood cells may indicate congenital heart disease, some lung disease, dehydration, kidney disease. There is an increase in the MCV, which indicated nutritional deficiencies, bone marrow abnormalities, liver disease, chronic lung disease. The result showed an increase in the MCH concentration in the study animal, indicating the presence of anaemia.There is an abnormal increase in the levels of the white blood cell of the study animals, indicating infection, damages to the tissue, leukaemia, or inflammatory diseases. This is in agreement with Schwarzer, 2009, who verifies that the toxic action of trichothecene results in an acute effect on the immune function. It also conformed with the report of Wannemacher et al., 1991, who reported that symptoms of acute T-2 toxicity in rats include reduced feed intake and increase in white blood cells and lymphocytes.

According to the liver functions, alanine aminotransferase (ALT), aspartate aminotransferase (ALT), and alkaline phosphatase (ALP), are important enzymes located in the hepatocytes, i.e. the liver cell. The significance increase in the level of these enzymes indicated that, they have been present in the serum of the animals, as a result of damages caused to the liver cell. The liver cell had been inflamed, and the enzymes are being leaked to the cell environment, i.e. the serum. It may also indicate muscle damage elsewhere in the body. This result is also in agreement with the report of Beri et al., 1991, who stated that the activities of enzymes, such as the alkaline phosphate in liver of animals also, were altered by ingesting feed contaminated with ***Fusariumroseum***. Such change in enzyme activity resulted in metabolic and cellular respiratory disorders, reduced body weight gain, and tissue necrosis.Table 1b and figure 7b, also indicated that there is a reduction in the body weight of the animals being fed with contaminated feed, which is in agreement with the report of Smith et al.,1992, who reported that feeding animals with mycotoxins, reduces body weight.

5. CONCLUSION

Fungi cause human illness in different ways. Mycoses are the best-known diseases of fungal etiology, but toxic secondary metabolites produced by saprophytic species are also an important health hazard. Conclusion can be drawn from this research that mycotoxins usually enter the body through ingestion of contaminated foods, but inhalation of toxigenic spores and direct dermal contact are also important routes. It is difficult to prove that a disease is a mycotoxicosis. Molds may be present without producing any toxin. Thus, the demonstration of mold contamination is not the same thing as the demonstration of mycotoxin contamination. Moreover, even when mycotoxins are detected, it is not easy to show that they are the etiological agents in a given veterinary or human health problem. Nevertheless, there is sufficient evidence from animal models and human epidemiological data to conclude that mycotoxins pose an important danger to human and animal health. It is clear that mycotoxins will be of increasing importance for all those involved in feed manufacturing, farming and food production. Mycotoxins are harmful to animals and can greatly affect their performances and productivity. Because there is a wide range of different mycotoxins, with different chemical structures, a simple approach cannot efficiently solve the problem. Quality of raw materials, prevention of the occurrence of mycotoxins, control and testing systems are all essential to reducing the exposure of humans and animals to mycotoxin.

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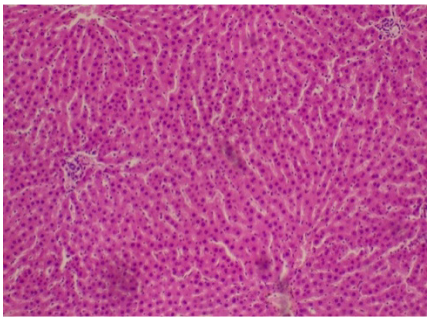
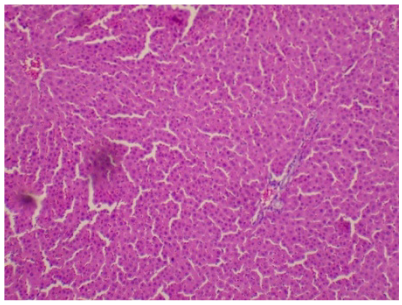
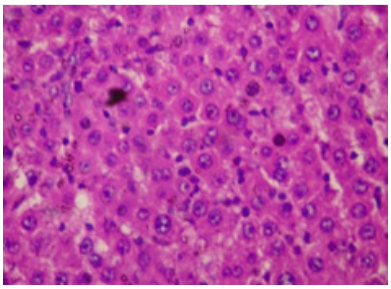
APPENDIX



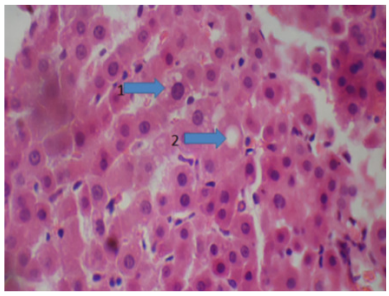
CONTROL LIVER 1: (Photomicrograph 1: Normal liver H&E ×100)



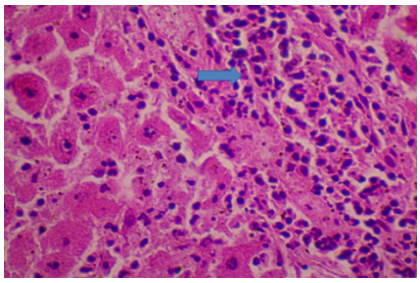
CONTROL LIVER 2: (Photomicrograph 2: Normal liver, no abnormality seen H&E × 100)



STUDY R1 LIVER: (Photomicrograph 3: No abnormality seen)



STUDY R2 LIVER (Photomicrograph 4: LIVER showing mild microvesiclessteatosis or Mild fatty changes. H&E × 400)



STUDY R3 (Photomicrograph 5: Liver – Showing moderate to severe portal inflammation {see arrow} H&E × 400)