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Oxidative Enzyme Status of Brain and Meat of Two Breeds of Broiler Chicken Fed *Ficusthonningii* and Vitamin C Supplemented Diets

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A B S T R A C T

The study was carried out to examine the effect of dietary supplementation of *Ficusthonningii* leaf powder and vitamin C on the antioxidative status of the meat and brain of two breeds of broiler chicken. Two hundred and forty day-old chicks, each of Arbor Acre (AA) and Cobb 500 (CO) breeds of broiler chicken with an average weight of 37.50 ±0.47 were randomly assigned to diets 1, 2, 3, 4, 5, 6, 7 and 8. Diet 1 and 2 had no supplement (negative control) and were fed to AA and CO respectively; diets 3 and 4 with supplement of vitamin C at the rate of 200mg/kg of basal diet were fed to AA and CO respectively; diets 5 and 6, supplemented with 1g FTLP/Kg of basal diet were fed to AA and CO respectively while diets 7 and 8 supplemented with 1g FTLP/kg of basal diet + 200mg of vitamin C and were fed to AA and CO respectively. Each diet had six replicates with ten birds per replicate. It was observed that the interactive effect of breed, vitamin C and FTLP had no significant effect on the dressing percentage while gizzard, spleen, heart and proventriculus were significantly affected. Brain catalase and glutathione were significantly influenced by the breed, vitamin C and FTLP inclusion while there was no interactive effect between the factors. Dietary supplementation of FTLP and vitamin C significantly lower the lipid peroxidation of the meat. There was significant interactive effect of all the factors on reduced lipid peroxidation while the meat catalase and glutathione were significantly increased by the inclusion of FTLP. This study revealed that the supplementation of broiler diets with FTLP and vitamin C significantly influenced carcass and organs weight, meat and brain antioxidant status of broiler chicken.

Keywords: Antioxidative, interactive, supplementation, catalase, glutathione, peroxidation.

1. Introduction

Research on the utilization of phytochemicals and other phytogens of plant source as natural feed additives in livestock feed production have gained prominence in recent years due to their potentials for reduction in antibiotic residues in products, reduction of pathogenic loads and enhanced performance in livestock as well as lowering the cost of production (Dhama*et al*., 2015). In comparison to inorganic growth promoters, plant based phytogens are economical and readily available, edible and low in toxicity, rich in nutrients, and useful in improving the health of animals and consumers (Dhama*et al*., 2015).

1.1 Utilization of Natural Antioxidants in Enhancing Meat Quality

Utilization of organic substances in poultry diets have been reported to improve nutrient metabolism, catalyses endogenous antioxidants, and enhance the quality of meat by lowering cholesterol content and disrupts lipid peroxidation (Oloruntola*et al*., 2018; Oloruntola*et al*., 2019). In a study carried out by Osowe*et al.* (2021), *Ficusthonningii*was reported to contain significant number of minerals and phytochemicals with high antioxidant activity andcould be employed as a natural feed supplement in animal nutrition.

1.2Ficusthonningii as a source of natura natural antioxidant

The common wild fig, *Ficusthonningii,* is one of the many fruit-bearing trees that have been traditionally used for treating diseases in Africa and beyond (Dangarembizi*et al*., 2013). The tree is mainly distributed in the upland forests of tropical and sub-tropical Africa, at altitudes of between 1,000-2,500m and grows best in light, deep and well drained soils (Agroforestry tree Database, 2011). The protein content of the leaves was reported to be 7.63g/100g dry matter (Osowe *et al*., 2021) and therefore have potential to mitigate against protein deficiency diseases especially in drought prone area with threatening food security challenges (Dangarembizi*et al*., 2013). *F. thonningii* contains various biologically active compounds that exhibit physiological effects which could be responsible for its curative potentials for a wide range of diseases (Osowe *et al*., 2021). These compounds known as phytochemicals are naturally produced by the tree as protection against biotic and abiotic stresses (Dangarembizi*et al*., 2013). The anti-inflammatory properties of F. thonningii are probably a result of the action of stilbenes and flavonoids, and there is a likelihood of synergistic interaction between the flavonoids and stilbenes (Wu *et al*., 2009). As a result of the antioxidant activities of F. thonningii, it has been reported that there is a protective effect on erythrocyte membrane from acetaminophen induced membrane peroxidation (Ahur*et al*., 2010). The antihaemolytic and haematinic potential is possibly due to its antagonistic action against the depletion of glutathione and hence prevention of the generation of free radicals which result in oxidative stress (Ahur*et al*., 2010). Njoronge and Kabunga, (2009) reported on the extensive use of *F. thonningii* for the treatment of diarrhea in both livestock and humans. These properties are the consequence of the anti-secretory, anti-inflammatory and anti-bacterial effects of tannins and astringent phenolics such as Triterpenoids and saponins. Plants with anti-diarrhoeal properties act by decreasing intestinal motility, stimulating water absorption and reducing electrolyte secretion (Njoronge and Bussman, 2006).

1.3 Role of Ascorbic acid in mitigating impact of oxidative stress

Ascorbic acid or vitamin C is effective in poultry production to mitigate oxidative stress, infection and inflammation (El-Senousey*et al*., 2018). It is added to the diet of broiler chicken as an additional nutrient to enhance poultry performance by improving body weight and reducing mortality (Shewita*et al.*, 2019). In meat products, vitamin C is widely used as a natural agent for color retention, which assists in inhibiting lipid oxidation and maintaining of color stability (Caiyun*et al*., 2021). In comparison with other organic acids, vitamin C had the best protective effect on the quality of cured meat and a suitable ingredient for cured meat products (kim*et al*., 2019). The purpose of this study was to see how dietary supplementation with vitamin C and *Ficusthonningii* leaf powder affected the performance and meat quality of broiler chickens.

2 Materials and Method

2.1 Collection, processing, and analysis of phytogens after ethical approval

The Research and Ethics Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria, accepted the experiment's requirements and criteria for animal and animal protocol. *Ficusthonningii* leaves were freshly plucked and washed in clear running water before being drained and spread lightly on polythene in the shade for two weeks. FTLP was made from dried *Ficusthonningii*leaves.Osowe*et al.* (2021) investigated the proximate, phytochemical, antioxidant and mineral components of FTLP.

2.2 Experimental birds, preparation of experimental diets, living conditions, and experimental design

Arbor Acre (AA) and Cobb 500 (CO) were the two breeds of broiler chicken used for this study. Basal diet was compounded to meet the nutritional needs of the birds according to NRC (1994), for the starter phase (0 to 21 days) and finisher phase (21-42 days). The basal diet was divided into eight equal portions and named diets 1 to 8 for each of the phase. Diet 1 and 2 which had no supplement (negative control) were fed to AA and CO respectively; diets 3 and 4 supplemented with vitamin C at the rate of 200mg/kg of basal diet were fed to AA and CO respectively; diets 5 and 6, received 1g FTLP/Kg of basal diet supplementation and fed to AA and CO respectively while diets 7 and 8 had 1g FTLP/kg of basal diet + 200mg of vitamin C and were fed to AA and CO respectively. The feeding trial was carried out the Federal University of Technology's Teaching and Research Farm in Akure, Nigeria.

Table 1: Composition of the experimental diets

Ingredients (g/kg)	**Starter feed (%)**	**Finisher diet (%)**
Maize	52.35	59.35
Rice bran	7	0
Maize bran	0	6
Soybean meal	30	24
Soy oil	3	3
Fish meal	3	3
Limestone	0.5	0.5
Bone meal	3	3
Salt	0.3	0.3
Premix	0.3	0.3
Methionine	0.3	0.3
Lysine	0.25	0.25
Nutrient composition (g/kg)	100	100
*Crude protein	22.18	20.03
Metabolizable energy (Kcal/kg)	3018	3108.1

Two hundred and forty (240) day-old chicks, each of AA and CO breeds of broiler chicks with average weight of 37.54±0.45g were randomly assigned to all the eight experimental diets in a completely randomized design. AA breed were randomly allotted to diets 1, 3, 5 and 7 while CO breed were randomly allotted to diets 2, 4, 6 and 8, in six replicates per diet with ten birds (10 birds/replicate). Wood shavings were used to cover the floor of the experimental pen (2m x 1m) that housed each replicate to a depth of 3cm. The experimental house was kept at 31±2 degrees Celsius for the first week and then reduced by 2 degrees Celsius each week after that until the temperature reached 26±2 degrees Celsius. The birds were fed ad-libitum.

2.3 Data Collection

Data were recorded on feed intake. The birds were weighed weekly and weight changes recorded. The feed intake and weight changes were used to calculate the feed conversion ratio. At the end of the feeding trial 3 birds were randomly selected from each replicate and starved overnight, weighed after the fasting, slaughtered by severing the jugular vein, scalded in warm water for about a minute, de-feathered manually, eviscerated and dressed to determine carcass characteristics. Dressed weight and internal organs were expressed as percentage live weight (Ndelekwute*et al.*,2013). Lipid peroxidation was determined using the method of Botsoglou*et al*. (1994), meat cholesterol (Allain *et al.*, 1974), catalase activity (Hadwan and Khabt 2018), and glutathione peroxidase activity (Cichoski*et al.*, 2012). The total protein in the brain was determined as outlined by Boehringer Mannheim Diagnostica)1979) as reported by Bitto*et al*. (2000)

2.4 Data Analysis

Data collected on body weight, weight gain, feed intake, FCR, carcass and internal organs characteristics were subjected to Analysis of Variance (ANOVA) using procedure of SPSS version 20 (SPSS, 2010). Significant means were separated using Duncan Multiple Range Test

3 Result

3.1Carcass and organ characteristics

The carcass and organ characteristics of broiler chicken fed *Ficus thonningii* and vitamin C supplemented diet are presented in Table 2. Significant differences (P<0.05) were observed in dressing percentage, gizzard, heart and proventriculus. The Control diets recorded the highest (P<0.05) dressing percentage for AA but not significantly different from diets 3 (200mg of vitamin C), 6 (1% FTLP) and 8 (200mg of vitamin C + 1% FTLP). Diet 4 recorded the most significant values (P<0.05) for the gizzard. For the relative weight of the heart, diet 7 was significantly higher (P<0.05) though not significantly different (P>0.05) from diets 2 and 5. The relative weight of the proventriculus for birds fed diets 5, 6 and 7 were not significantly different (P>0.05) from one another. The CO breed of broiler chicken showed significant difference (P<0.05) from the AA breed in the weight of the heart and proventriculus while the inclusion of vitamin C recorded significance (P<0.05) for the gizzard and proventriculus. The inclusion of FTLP was significant (P<0.05) for the liver, spleen, lungs and proventriculus. For the interaction, breed and vitamin C showed significance (P<0.05) on the relative weight of the heart and proventriculus. The interactive effect between breed and FTLP was significant (P<0.05) for dressing percentage while the interaction between vitamin C and FTLP showed significance (P<0.05) for the relative weight of the gizzard. The interactive effect between breed, vitamin C and FTLP had no significance for all the parameters.

Table 2 The carcass trait and relative organ weight of different breeds of broiler chicken fed *Ficusthonningii* leaf powder and vitamin c supplemented diets

Diet	BRD	VC mg/kg	FTLP %	DP %DW	LVR %DW	GZD %DW	SPLN %DW	PNCRS %DW	HRT %DW	BILE %DW	LUNG %DW	PRVTLS %DW
1	AB	0	0	78.34^{a}	2.59	2.05^{bc}	0.11	0.24	0.55^{c}	0.12	0.48	0.32^{d}
2	CO	0	0	74.69^{bc}	2.86	1.96^{c}	0.14	0.30	0.61^{abc}	0.16	0.39	0.41^{bc}
3	AB	200	0	76.49^{ab}	2.85	2.13^{bc}	0.12	0.28	0.73^{bc}	0.17	0.45	0.36^{cd}
4	CO	200	0	74.93^{bc}	2.71	2.60^{a}	0.15	0.31	0.56^{cd}	0.16	0.59	0.36^{bcd}
5	AB	0	1	73.39^{c}	2.50	2.24^{b}	0.22	0.27	0.63^{abc}	0.17	0.55	0.42^{ab}
6	CO	0	1	77.37^{a}	2.15	2.17^{bc}	0.14	0.27	0.55^{c}	0.17	0.55	0.47^{a}
7	AB	200	1	74.82^{bc}	2.59	$2,16^{bc}$	0.14	0.26	0.78^{a}	0.16	0.58	0.41^{abc}
8	CO	200	1	77.47^{a}	2.45	2.25^{b}	0.20	0.24	0.57^{bc}	0.20	0.60	0.38^{bc}
SEM				0.38	0.06	0.04	0.01	0.01	0.02	0.01	0.02	0.01
P-value				0.01	0.11	0.01	0.09	0.10	0.05	0.29	0.16	0.01
	AB			75.86	2.54	2.06	0.17	0.25	0.59^{b}	0.14	0.47	0.37^{b}
	CO			75.65	2.72	2.42	0.13	0.27	0.75^{a}	0.16	0.59	0.38^{a}
	SEM			0.46	0.11	0.05	0.01	0.01	0.03	0.01	0.04	0.01
	P-value			0.45	0.45	0.07	0.59	0.16	0.02	0.24	0.63	0.04
		0		77.41	2.72	2.09^{b}	0.11	0.26	0.64	0.14	0.46	0.34^{b}
		200		74.11	2.54	2.20^{a}	0.18	0.27	0.70	0.16	0.56	0.41^{a}
		SEM		0.46	0.11	0.05	0.01	0.01	0.03	0.01	0.04	0.01
		P value		0.96	0.28	0.01	0.79	0.79	0.06	0.19	0.13	0.05
			0	76.51	2.72^{a}	2.00	0.13^{b}	0.29	0.58	0.14	0.43^{b}	0.36^{b}
			1	75.38	2.32^{b}	2.20	0.18^{a}	0.25	0.59	0.17	0.55^{a}	0.44^{a}
			SEM	0.46	0.11	0.05	0.01	0.01	0.03	0.01	0.04	0.01
			P value	0.46	0.01	0.70	0.02	0.16	0.65	0.07	0.03	0.01
	Interactions *P-value*											
	BRD x VC			0.69	0.67	0.01	0.06	0.37	0.03	0.79	0.13	0.01
	BRD x FTLP			0.01	0.20	0.11	0.34	0.31	0.27	0.79	0.82	0.15
	VC x FTLP			0.11	0.56	0.01	0.59	0.67	0.78	0.69	0.58	0.12
	BRD x VC x FTLP			0.08	0.18	0.08	0.07	1.00	0.46	0.15	0.20	0.89

Means with a different superscript in the same column are significantly (P<0.05) different; BRD: Breeds; AB: Arbor acre; CO: Cobb 500; VC: Vitamin C; FTLP: *Ficusthonningii leaf powder*; DW; Dressed Weight; DP: Dressing Percentage; LVR: Liver; GIZD: Gizzard; SPLN: Spleen; PNCRS: Pancreas; HRT: Heart; PRVTLS: Proventriculus; SEM: Standard error of the means.

3.2 Brain antioxidant status

The result of the brain antioxidants analysis for the two breeds of broiler chicken fed FTLP and vitamin C supplemented diets is presented in Table 3. The value of the brain catalase and brain glutathione for birds fed diets 7 and 8 compared statistically and were higher than the values recorded for the other diets. The CO breed showed higher significance (P<0.05) for the brain catalase and brain glutathione than that of the AA breed. Inclusion of vitamin C and FTLP were significant (P<0.05) while there was no significance in the interactions.

Table 3: Brain analysis of two breeds of broiler chickens fed *Ficusthonningii* leaf powder and vitamin C supplemented diets

Diet	BRD	VC mg/kg	FTLP %	BTP	BCAT	BGSH
1	AB	0	0	0.63	27.35d	24.40f
2	CO	0	0	0.54	41.21c	43.10e
3	AB	200	0	0.64	47.68bc	45.63de
4	CO	200	0	0.65	53.77b	54.76cd
5	AB	0	1	0.63	52.11b	57.78bc
6	CO	0	1	0.58	65.02a	66.37ab
7	AB	200	1	0.65	68.52a	72.52a
8	CO	200	1	0.57	73.07a	76.60a
SEM				0.01	3.07	3.52
P-value				0.65	0.01	0.01
	AB			0.64	48.92b	50.08b
	CO			0.58	58.27a	60.21a
	SEM			0.02	1.42	1.79
	P-value			0.13	0.01	0.01
		0		0.59	46.42b	47.91b
		200		0.62	60.76a	62.38a
		SEM		0.02	1.42	1.79
		P value		0.38	0.01	0.01
			0	0.61	42.50b	41.97b
			1	0.60	64.68a	68.32a
			SEM	0.02	1.42	1.79
			P value	0.84	0.01	0.01
	Interactions P-value					
	BRD x VC			0.62	0.06	0.18
	BRD x FTLP			0.72	0.76	0.15
	VC x FTLP			0.40	0.31	0.44
	BRD x VC x FTLP			0.41	0.94	0.62

Means with a different superscript in the same column are significantly (P<0.05) different; BRD: Breeds; VC: Vitamin C; FTLP: *Ficusthonningii leaf powder*; BTP: Brain Total Protein; BCAT: Brain Catalase; BGSH: Brain Glutathione; AB: Arbor acre; CO: Cobb 500; SEM: Standard error of the means.

3.3Meat antioxidant status

The result of the meat antioxidant status of the two breeds of broiler chicken fed FTLP and vitamin C supplemented diets is presented in Table 4. The lipid oxidation, meat glutathione and meat catalase were significant (P<0.05). Result of the lipid oxidation showed that the control diet 1 was significantly higher (P<0.05) than the other diets. The value for the meat glutathione showed that diets 5 and 6 were significantly higher (P<0.05) than the rest of the diets while the meat catalase showed that diets 3, 4, 5, 6, 7 and 8 were not significantly different (P>0.05) from one another but significantly higher (P<0.05) than diets 1 and 2. The interaction between vitamin C and FTLP were significant (P<0.05) for lipid oxidation and glutathione while the interactive effect of breed, vitamin C and FCLP showed significance on lipid oxidation.

Table 4: Meat analysis of two breeds of broiler chickens fed *Ficusthonningii* leaf powder and vitamin C supplemented diets

Diet	BRD	VC mg/kg	FCLP %	LPDOX	CHOL	GSH	CATLS
1	AB	0	0	5.03[a]	121.55	28.30[b]	34.75[b]
2	CO	0	0	4.09[b]	115.01	29.78[b]	38.71[b]
3	AB	200	0	3.26[bc]	118.63	49.25[a]	53.22[a]
4	CO	200	0	3.08[cd]	114.22	52.67[a]	53.71[a]
5	AB	0	1	2.15[e]	111.61	45.36[a]	52.37[a]
6	CO	0	1	2.67[cde]	110.73	53.20[a]	55.53[a]
7	AB	200	1	3.14[cd]	117.56	54.20[a]	55.60[a]
8	CO	200	1	2.30[de]	115.13	55.30[a]	50.02[a]
SEM				0.20	4.07	2.47	2.33
P-value				0.01	0.99	0.01	0.09
	AB			3.39	117.34	44.28	48.99
	CO			3.04	113.77	47.74	51.05
	SEM			0.14	6.80	2.12	2.81
	P-value			0.09	0.71	0.26	0.01
		0		3.49	114.73	39.16	45.34
		200		2.94	116.38	52.85	54.70
		SEM		0.14	6.80	2.12	2.81
		P value		0.01	0.86	0.01	0.03
			0	3.87	117.35	40.00	45.10
			1	2.56	113.76	52.01	54.94
			SEM	0.14	6.80	2.12	2.81
			P value	0.01	0.71	0.01	0.02
	Interactions *P-value*						
	BRD x VC			0.45	0.98	0.69	0.71
	BRD x FTLP			0.33	0.84	0.74	0.97
	VC x FTLP			0.01	0.72	0.01	0.08
	BRD x VC x FTLP			0.01	0.92	0.48	0.95

Means with a different superscript in the same column are significantly (P<0.05) different; BRD: Breeds; VC: Vitamin C; FTLP: *Ficusthonningii leaf powder*; LPDOX: Lipid oxidation; CHOL: Cholesterol; GSH: Glutathione; CATLS: Catalase; AB: Arbor acre; CO: Cobb 500; SEM: Standard error of the means.

4. Discussion

Herbs, spices and other phytogens has been utilized as feed additives in broiler production with good result as growth enhancement and carcass traits qualities improvement (Adu *et al*., 2020). Results on dressing percentage of broiler chicken fed FTLP and vitamin C supplemented diets were not significantly higher than the control diets. This indicates that FTLP and vitamin C did not affect the development of muscle when compared to the control. This agrees with the findings of Adu *et al*. (2020) who reported no significant effect of the supplementation of SLM, MSM and MSCM on carcass traits and internal organ weights of broiler chicken. The significant increase recorded in the internal organs without effect on the dressing percentage is likely an indication of their utilization as means of adaptation which agrees with the findings of Miya *et al*. (2019). The significant increase in the weight of the gizzard due to the interactive effect of FTLP and vitamin C could be as a result of increase in the efficiency of digestion and metabolism. This agrees with the results of Miya *et al*. (2019) who reported increase in gizzard weight as a result of the supplementation with *Vachelliatortilis* leaf meal. Maidala*et al*. (2016) also reported increase in the weight of gizzard in birds supplemented with neem leaf extract while no changes were observed in birds fed with diets supplemented with lemon peel extractor orange peel extract or *Curcuma xanthorrhiza*(Akbarian*et al*., 2013)**.**

Results on relative internal organ weight were significantly affected by the treatment which indicate that supplementation of FTLP influences the development of the organs when compared to the control diet. A similar result has been reported by Al-Mufarrej*et al.* (2019a, 2019b) who reported higher liver percentage in broiler chicken fed diet containing clove powder. However, Tariq *et al.* (2015) reported no significant effect of supplementation of clove powder at 0.5% to the relative organ weight of quail birds.

Catalase as an antioxidant enzyme found in all living organism exposed to oxygen, assist in catalysing the reduction of H_2O_2 to water, removing organic hydroperoxides and uses H_2O_2 to oxidize toxins including phenols, formic acid formaldehyde and alcohols (Mustafa (2012).Dringen (2005) reported that the microglial cells of the brain contain high concentration of glutathione and substantial activities of catalase which confers good antioxidative potentials that protects the microglial cells against oxidative damage that could impair important functions of these cells in defence and repairs of the brain. The significant levels of these antioxidants in the birds that are fed with diets supplemented with FTLP and vitamin C is an indication of the good antioxidant status of the leaf powder as reported by Osowe*et al*. (2021).

Antioxidants in herbs has been reported to promote the oxidative stability of poultry meat [Saleh, et al., 2018; Hashemipour*et al.*, 2013; Rostami *et al*., 2017; Kasapidou*et al.*, 2014]. Herbs and herbal products contain antioxidants that can prevent the oxidation of UFA and cholesterol present in animal-origin products and increase the antioxidant potential of the animal's body (Jachimowicz*et al.*, 2022).

Recent studies have shown that the fatty acids composition of meat can be altered by modifying the diet during animal production to improve the conformity of meat with nutritional guidelines (Jachimowicz*et al*., 2022). The significant low level of the value of lipid peroxidation of the supplemented diets compared to the control diet indicates that the antioxidant status of the FTLP as reported by Osowe*et al* (2021) could be useful in improving the quality status of broiler meat. Oxidation rate of post mortem muscle depends on the anti-oxidative capacity in the animal and oxidation of muscle component can be retarded by the action of endogenous antioxidative enzymes, especially SOD, CAT and GPx. SOD, CAT and GPx have been reported as effective antioxidant enzymes which prevent oxidation reactions to improve meat quality and extend shelf-life of animal products (Chen *et al*. 2012). The significant high levels of these antioxidants as a result of inclusion of FTLP in the diet of broiler chicken in this study is an indication that the leaf powder could enhance the endogenous antioxidative status of broiler chicken, thus improving the quality of muscle and enhancing the shelf-life. This confirms the findings of Osowe*et al.* (2021) who reported on the antioxidant status of*Ficusthonningii*.

Conclusion

From the study, it was observed that FTLP and vitamin C have no significance on the dressing percentage but on the relative weight of some internal organs. Lipid oxidation was significantly reduced by the inclusion of FTLP and vitamin C. Catalase and glutathione peroxidase of the brain and meat were positively influenced by FTLP, vitamin C and breed. Thus, the supplementation of broiler diets with 1% FTLP and 200mg/kg of vitamin C could significantly enhance meat quality as well as boost the brain's antioxidant status of broiler chicken.

Conflict of Interest

There was no conflict of interest in the course of carrying out this study.

Compliance with ethical standard

The study was carried out with the approval of the committee on ethics for care and use of animal for research of The Federal University of Technology, Akure, Nigeria.

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