



Tissue Biochemical Assessment of Broiler Chicks Fed Diets Containing Varying Concentrations of Watermelon Seed Meal (WMSM) as a Replacement for Soyabean

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Abstract

Watermelon and its seeds possess antioxidants that help fight reactive oxygen species. This research examined the biochemical parameters such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) and reduced glutathione (GSH) in the liver and kidney of broiler chicks fed diets containing varying levels of watermelon seed meal (WMSM). One hundred newly hatched unsexed broiler chicks were divided into five dietary treatments, four replicates of five birds per replicate in a completely randomized experimental design. Treatment 1 is the control diet (T₁. 0% watermelon seed meal), while Treatments 2 to 5 (T₂-T₅) represented inclusion levels of the seeds at 10%, 20%, 30% and 40% respectively. The experiment lasted for three weeks Birds were sacrificed by severing the jugular vein. Both the liver and kidney aspartate aminotransferase activities were significantly ($p < 0.05$) higher at 10% WMSM (3.68 and 3.56 UI/mg protein, respectively) compared with other diets. No significant ($p > 0.05$) differences amongst the kidney gamma-glutamyl transferase activities of birds fed 20, 30 and 40% WMSM (1.856, 1.969 and 1.950 U/mg protein) respectively. The superoxide dismutase activity in liver (30% WMSM) and kidney (20% WMSM) (2.78 and 3.02 U/mg protein) respectively of birds on the experimental diets were significantly ($p < 0.05$) different amongst other treatments. Overall, the results of liver/kidney enzymes and antioxidant parameters revealed that birds can effectively cope with watermelon seed meal up to 30% without any detrimental effect.

1.0 Introduction

The high financial cost incurred in feeding broiler birds has necessitated the search for non-conventional feed stuffs that will not only reduce cost but will meet up with the nutritional requirement of the broiler birds as recommended by [1]. Broiler birds require a balanced intake of energy, protein, minerals and vitamins.

The impact of the non-conventional feed stuffs must not only be assessed physically by performance characteristics, but the interest must transcend to checking the biochemical implication of the feed stuff at cellular level by assessing the enzyme activities of organs and tissues of the experimental birds, in order to ascertain and guarantee its usage in feed composition, as well as being able to recommend its' meat for consumers without posing any adverse effect on their health.

Watermelon (*Citrullus lanatus*), which is of tropical and subtropical region [2], [3], [4] has been proven to be a good source of antioxidant and flavonoids [5], [6], [7], [8]. The seeds are seen as wastes but have been reported to be good sources of proteins and hence amino acids, especially glutamate [9]. This is believed to augment proteins from soy bean meal, which is always expensive due to keen competition between man and animals for its source of protein. The seeds have also been reported to help people with constipation problem because of its high fibre content [10]. The seeds also have the ability to enhance the immune system because of its contents of iron and B vitamins [11].

This study examines the biochemical parameters of broiler chicks fed watermelon seed meal by monitoring activities of liver

and kidney enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST). Reduced glutathione (GSH) and weight gain were also assessed.



Figure 1: Watermelon seeds

2. Materials and methods

2.1. Experimental seeds, design and management of experimental animals

Citrullus lanatus was bought from Dan lemo market, Naibawa, Kano State, Nigeria. The seeds were shade dried for a period of four days to avoid moisture accumulation and subsequently milled to a particle size (0.1 mm) that the birds can pick. The seeds were then used to compound diets for the experimental birds following a standard procedure of birds' nutritional requirements as outlined by [1].

2.2. Chemicals and reagents

Assay kits for the enzymes were products of Randox Laboratories, County Antrim, UK. Other reagents were of analytical grade and

were prepared according to the outlined standard methods.

2.3 Animal grouping

A total of 100 one-day old healthy broiler chicks (unsexed) obtained from TUNS Farms Osogbo, Nigeria, were used for the study. The birds were divided into five treatment groups, made up of four replicates of five birds per replicate and arranged in a completely randomized experimental design. The five different diets (T1 –T5) consisted of varying concentrations of watermelon-seed-meal replacing soy bean weight for weight (Table 1). Treatment 1 (T1) served as the control with 0% watermelon seed-meal. Treatment 2 to 5 (T2 to T5) contained watermelon-seed-meal as a replacement for soy bean at 10, 20, 30 and 40% respectively. The birds were allowed access to feed and water *ad-libitum* throughout the period of experiment. A standard cage system of management, well illuminated and well ventilated was used for the research. The research protocol complied with the guidelines set forth by the University of Ilorin's Animal Care and Use Committee in Nigeria. The birds were vaccinated according to standard procedure. Antistress were also served in water when necessary. The experiment lasted for three weeks. Weights were taken weekly to allow for calculation of final weight gain.

2.4 Sample collection and homogenization for analysis

At the end of the feeding trial, two birds per replicate were sacrificed by severing the jugular vein and blood samples were collected in clean and labelled test tubes. The blood samples were thereafter centrifuged at 5000 rpm for 5 min using a Bench Centrifuge 90-1, Gallenkomp, England. The clear serum

was then pipetted into eppendorf tubes using a Pasteur pipette, refrigerated and used within 72 h to ensure intact enzyme activities. Birds were then scalded, defeathered and dissected. Kidney and liver were excised, cleaned with tissue paper to remove attached debris after which 0.5 g of the tissue samples were then homogenized in 4.5 mL ice-cold sucrose solution (0.25 M; pH 7.2). The resulting supernatants were kept frozen and used within 48 h.

2.5 Measurements of enzyme activities

The method described by [12] was used to measure the activities of alkaline phosphatase, alanine and aspartate transaminases (ALP, ALT and AST). Gamma glutamyl transferase (GGT) activity was determined following the procedure of [13]. Specific activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) as well as concentrations of the reduced glutathione (GSH) were assayed as described by [14].

2.6. Gross composition and calculated nutrient composition of experimental diets

Table 1 represents the gross composition of experimental diets with varying watermelon seed meal concentrations as the only varied ingredient. Other diets were as recommended by [1] to meet the standard requirement of broiler chicks.

Table 2 depicts the calculated nutrient composition of experimental diets; it showed that the crude protein composition of the diets ranged from 20.25 to 22.00%. The fibre content for the starter phase ranged from 3.75 to 4.50%. The watermelon seeds were not so oily, with ether extract values ranging between 8.28 to 10.88%. The methionine/lysine ratio were 0.6/1.2 respectively.

Table 1: Gross composition (kg/100 kg) of experimental diets

Ingredients	% Replacement of soyabean (w/w)				
	0	10%	20%	30%	40%
Maize	52.11	52.11	52.11	52.11	52.11
Oil	0.25	0.25	0.25	0.25	0.75
SBM	32	28.8	25.6	22.4	19.2
WMS	-	3.2	6.4	9.6	12.8
Wheat offal	10.37	10	9	8.5	7
Fish meal	2.22	2.59	3.59	4.09	5.09
Limestone	1.45	1.45	1.45	1.45	1.45
Salt	0.28	0.28	0.28	0.28	0.28
Methionine	0.34	0.34	0.34	0.34	0.34
Lysine	0.31	0.31	0.31	0.31	0.31
Premix	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00

WMS- Watermelon seed

SBM – Soyabean meal

DCP – Dicalcium phosphate

Table 2: Calculated nutrient composition of experimental diets (%)

Ingredients	% Replacement levels soyabean meal with WMSM				
	0%	10%	20%	30%	40%
ME(kcal)	2883.0	2866.0	2851.0	2835.0	2850.0
Crude Protein	22.00	21.40	21.50	20.62	20.25
Crude Fibre	4.50	4.20	3.95	3.84	3.75
Ether Extract	8.28	8.94	9.60	10.34	10.88
Fat	6.00	6.20	6.35	6.39	6.45
Methionine	0.58	0.60	0.62	0.65	0.69
Lysine	1.25	1.22	1.20	1.18	1.15
Calcium	1.20	1.12	1.10	1.05	1.00
Phosphorus	0.460	0.445	0.430	0.425	0.422

ME – Metabolizable Energy

2.7. Statistical analysis

Values were analysed as means \pm standard error of means (n=3) and were subjected to analysis of variance (ANOVA) followed by Tukey-Kramer's test for nonconcurrences amongst means to confirm any significant differences ($p < 0.05$) between means.

3.0. Results

3.1 Nutritional analysis of experimental diets

The calculated nutritional composition in Table 2 were supported by the proximate analysis findings (Table 3). The percentage crude protein was within the range of 20.75 to 22.15%. This was not much different from the calculated nutrient composition. Ditto for crude fiber (4.02 to 4.70%) and ether extract (8.34 to 11.00%). It is crucial to make sure there is no appreciable variation between the calculated and tested nutritional contents of a specific diet in order to guarantee the correctness of the results.

Table 3: Average Proximate composition (g/100 g DM) of experimental diets

Analysis (%)	% Replacement of soyabean with WMSM				
	(0%)	(10%)	(20%)	(30%)	(40%)
DM	91.56	90.00	84.90	84.20	92.50
CP	22.15	21.63	21.57	20.71	20.75
C F	4.70	4.40	4.11	4.21	4.02
E E	8.34	9.01	9.75	10.56	11.00
Ash	2.00	2.00	2.00	3.00	2.00
NFE	62.81	62.96	63.57	61.52	62.23

DIET 1 = 0%, DIET 2 = 10%, DIET 3 = 20%, DIET 4 = 30%, DIET 5 = 40% Watermelon seed meals, NFE = Nitrogen Free Extract, DM = Dry Matter, CP = Crude Protein, CF = Crude fibre, EE = Ether extract

3.2. Liver and kidney enzymes

Serum ALP (Table 4) revealed a statistical significant ($p < 0.05$) values across board, with birds on the control diets having the highest significant value. Birds placed on 20% watermelon seed meal (WMSM) supplemented diets had the highest significant ($p < 0.05$) liver ALP activity, while birds on the 40% had the least significant ($p < 0.05$) activity of same enzyme. Ditto for kidney ALP activity.

Serum ALT activities were statistically significantly ($p < 0.05$) different amongst all treatments. Birds placed on the 0% WMSM supplemented diets had the highest significant ($p < 0.05$) activity of the enzyme. Liver ALT activities was significantly ($p < 0.05$) different at 20% inclusion level. The kidney activity of the same enzyme was significantly ($p < 0.05$) highest for birds on 40% WMSM supplemented diets.

Serum AST was significantly ($p < 0.05$) highest for birds on 0% WMSM supplemented diets. The analysis revealed a statistical significant ($p < 0.05$) liver AST activity at 10% inclusion level. Ditto for the kidney enzyme activity

Serum GGT enzyme activity was significantly ($p < 0.05$) highest for birds on the 10% inclusion level. The liver activity of the enzyme was significantly ($p < 0.05$) highest at 40%. There were no significant differences ($p > 0.05$) in the kidney GGT activities of the birds placed on 20 to 40% WMSM supplemented diets.

Table 4 Selected tissues enzymes activities of broiler birds fed varying concentrations of watermelon seed meal (WMSM) supplemented diets

Alkaline phosphatase activities -ALP (UI/mg protein)					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Serum	1.20 ± 0.65 ^a	1.13 ± 1.03 ^b	0.87 ± 0.47 ^c	0.10 ± 0.73 ^{bc}	0.74 ± 0.70 ^d
Liver	129.34 ± 0.85 ^b	117.74 ± 0.95 ^c	268.90 ± 1.02 ^a	116.06 ± 0.43 ^c	80.94 ± 0.70 ^d
Kidney	144.18 ± 1.15 ^b	138.17 ± 0.52 ^c	177.53 ± 0.67 ^a	128.68 ± 1.61 ^d	100.62 ± 0.79 ^e
Alanine transaminase activities - ALT (UI/mg protein)					
Serum	1.27 ± 0.04 ^a	0.94 ± 0.02 ^b	1.23 ± 0.04 ^a	0.77 ± 0.02 ^c	0.59 ± 0.04 ^d
Liver	1.16 ± 0.26 ^e	1.78 ± 0.02 ^b	2.43 ± 0.05 ^a	1.32 ± 0.04 ^d	1.53 ± 0.05 ^c
Kidney	1.17 ± 0.04 ^b	1.35 ± 0.01 ^{ab}	1.25 ± 0.04 ^b	1.19 ± 0.05 ^b	1.66 ± 0.04 ^a
Aspartate transaminase activities (UI/mg protein)					
Serum	3.24 ± 0.03 ^a	2.26 ± 0.04 ^b	1.68 ± 0.03 ^c	2.18 ± 0.01 ^b	1.42 ± 0.10 ^d
Liver	2.64 ± 0.04 ^d	3.68 ± 0.01 ^a	2.41 ± 0.02 ^e	3.35 ± 0.03 ^b	3.12 ± 0.06 ^c
Kidney	2.75 ± 0.07 ^{bc}	3.56 ± 0.06 ^a	2.56 ± 0.06 ^c	2.29 ± 0.01 ^d	2.99 ± 0.01 ^b
Gamma glutamyl transferase activities (UI/mg protein)					
Serum	0.168 ± 0.01 ^b	0.239 ± 0.01 ^a	0.152 ± 0.01 ^b	0.146 ± 0.01 ^c	0.096 ± 0.01 ^d
Liver	0.355 ± 0.01 ^c	0.305 ± 0.01 ^d	0.340 ± 0.01 ^c	0.491 ± 0.01 ^b	0.518 ± 0.01 ^a
Kidney	1.577 ± 0.04 ^b	1.309 ± 0.02 ^c	1.856 ± 0.11 ^a	1.969 ± 0.03 ^a	1.950 ± 0.06 ^a

The results are mean ± SEM of 3 determinations. Diet 1= 0% Watermelon seed meal, Diet 2= 10% Watermelon seed meal, Diet 3= 20% Watermelon seed meal, Diet 4= 30% Watermelon seed meal, Diet 5= 40% Watermelon seed meal.

^{a, b, c, d and e} are means across the rows with different superscripts that are significantly ($p < 0.05$) different.

3.3. Selected tissues antioxidant enzyme parameters

Serum SOD (Table 5) concentration was statistically ($p < 0.05$) significantly highest for birds on the 40% inclusion level of the seeds. The liver activity of the enzyme was significantly ($p < 0.05$) different at 30% and the kidney at 20%.

CAT concentrations were significantly different ($p < 0.05$) amongst treatments. Serum CAT concentration was significantly ($p < 0.05$) different at 20% when compared with the control. Liver CAT was significantly ($p < 0.05$) highest at 20% and kidney at 10% respectively, when compared with those of the birds on the control diet.

GST concentrations of the birds were significantly ($p < 0.05$) different amongst all treatments. The serum GST concentration was significantly ($p < 0.05$) different at 0% inclusion level of the seed, while liver GST concentration was significantly ($p < 0.05$) different at 10% when compared with the control and other percentages inclusion levels. Ditto for kidney. There were no significant ($p > 0.05$) differences in the serum GSH concentrations of birds on the various inclusion levels of the seeds when compared with the control. Liver GSH concentration was significantly ($p < 0.05$) different at 20% when compared with the control and with every other diets inclusion level. Ditto for the kidney GSH.

Table 5: Selected antioxidant parameters in tissues of broiler birds fed varying concentrations of watermelon seed meal (WMSM) supplemented diets

Superoxide dismutase concentrations - SOD (UI/mg protein)					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Serum	1.21 ± 0.54 ^b	0.35 ± 0.36 ^d	0.59 ± 0.04 ^c	1.22 ± 0.01 ^b	1.86 ± 0.0023 ^a
Liver	1.41 ± 0.05 ^d	1.88 ± 0.01 ^c	2.38 ± 0.09 ^b	2.78 ± 0.04 ^a	2.37 ± 0.01 ^b
Kidney	1.47 ± 0.037 ^d	2.61 ± 0.058 ^b	3.02 ± 0.130 ^a	2.28 ± 0.026 ^c	2.18 ± 0.013 ^c
Catalase concentrations - CAT (UI/mg protein)					
Serum	0.353 ± 0.009 ^c	0.287 ± 0.008 ^b	0.333 ± 0.004 ^a	0.252 ± 0.008 ^c	0.251 ± 0.012 ^c
Liver	1.418 ± 0.088 ^c	2.728 ± 0.053 ^b	4.677 ± 0.023 ^a	1.690 ± 0.061 ^c	1.586 ± 0.106 ^c
Kidney	0.636 ± 0.004 ^d	2.413 ± 0.058 ^a	1.658 ± 0.064 ^b	1.443 ± 0.085 ^c	1.396 ± 0.071 ^c
Glutathione-S-transferase concentrations - GST (UI/mg protein)					
Serum	0.062 ± 0.008 ^a	0.035 ± 0.006 ^b	0.064 ± 0.011 ^c	0.026 ± 0.007 ^b	0.005 ± 0.006 ^c
Liver	1.05 ± 0.003 ^{ab}	1.19 ± 0.180 ^a	0.72 ± 0.190 ^b	0.42 ± 0.009 ^c	0.54 ± 0.003 ^b
Kidney	115.15 ± 0.15 ^c	128.90 ± 5.530 ^a	126.85 ± 0.50 ^b	113.12 ± 0.53 ^c	112.97 ± 0.26 ^c
Reduced glutathione concentrations - GSH (nmol/mg)					
Serum	21.622 ± 0.45 ^a	22.156 ± 0.62 ^a	21.709 ± 0.45 ^a	20.395 ± 0.35 ^a	20.707 ± 0.43 ^a
Liver	152.479 ± 1.22 ^b	143.426 ± 1.15 ^c	174.454 ± 0.37 ^a	101.822 ± 0.15 ^e	120.489 ± 0.51 ^d
Kidney	115.001 ± 0.42 ^b	106.552 ± 0.84 ^c	127.186 ± 0.45 ^a	113.605 ± 1.42 ^b	109.373 ± 1.29 ^c

The results are mean ± SEM of 3 determinations. Diet 1= 0% Watermelon seed meal, Diet 2= 10% Watermelon seed meal, Diet 3= 20% Watermelon seed meal, Diet 4= 30% Watermelon seed meal, Diet 5= 40% Watermelon seed meal.

a, b, c, d and e are means within the rows with different superscripts that are significantly ($p < 0.05$) different.

3.4. Performance index of broiler birds fed varying concentrations of watermelon seed meal (WMSM) supplemented diets

Weight gain analysis (Table 6) of birds (gain/bird/day as a percentage of initial weights) of birds fed different percentages of watermelon seed meals revealed that birds placed on diet 4 and 5 (30 and 40% WMSM supplemented diets) had a noticeable statistical significant ($p < 0.05$) higher weight gains when compared with the weight of birds on the control diet and those on other experimental diets. No statistical significant ($p > 0.05$) differences were observed in the final weight gain of birds placed on diet 2 and 3 (10 and 20% WMSM supplemented diets). Birds placed on the control diet (0% WMSM supplemented diet) had the least significant ($p < 0.05$) weight gain.

Table 6: Weight gain (g) of broiler birds fed varying concentrations of watermelon seed meal-(WMSM) supplemented diets

Dietary treatment	Weight gain/bird/day (% of initial weights)
Diet 1	42.96±1.27 ^c
Diet 2	45.50±1.56 ^b
Diet 3	45.87±2.07 ^b
Diet 4	48.20±1.25 ^a
Diet 5	48.27±0.99 ^a

The results are mean ± SEM of 3 determinations. The weight gained are expressed as a percentage of birds' initial weight. Diet 1= 0% WMSM, Diet 2= 10% WMSM, Diet 3= 20% WMSM, Diet 4= 30% WMSM, Diet 5= 40% WMSM.

a, b, and c are means within same column with different superscripts that are significantly ($p < 0.05$) different.

4.0. Discussion

This study investigated the effects of introducing graded levels of watermelon seed meal-supplemented diets as an alternative and cheaper protein source into broiler feedstuff with a view to reducing cost and improving profits of poultry farmers [15]. In addition to performance index, biochemical parameters such as enzymes and antioxidants were monitored in serum, liver and kidney. These parameters will give an insight into the effects of this diets on the metabolism of the birds and potential toxicity, if any.

Proximate analysis is a way of partitioning nutrients in food into basic nutritional composition like carbohydrate, protein, limiting amino acids [1]. The obtained close values of the calculated and analyzed nutritional composition is a guaranty of the reliability of the result. An essential source of energy is carbohydrate. The soluble carbohydrates in the feed, such as starch and sugar, are represented by the nitrogen-free extract (NFE). Insoluble carbohydrates are represented as crude fiber [1]. Proteins have a significant role in food composition, provide a significant amount of energy, and contain key amino acids like lysine, tryptophan, methionine, leucine, isoleucine, and valine that are necessary for human health but that the body is unable to produce on its own [1].

Alkaline phosphatase, a marker enzyme of liver functionality, bone growth and protein metabolism, is used nutritionally to ascertain the effect of an experimental feed/diet on the integrity of the plasma membrane, as changes in the activity of this enzyme might signify that the membrane has been compromised [16]. The findings of [15] show that the inclusion of 26.6% seed cake of *C. lanatus* in

broiler birds diet could be an alternative strategy to improve feed and ensure income in the broilers' chicken production, this correlate with the highest liver and kidney ALP enzyme activities at 20% WMSM replacement level found in the current study. Alanine transaminase is an enzyme found in the liver and kidney, but more predominant in the liver as shown in the results obtained from this study. It is synthesized by the cells of the liver and aid in the digestion of food to yield energy [17]. Higher percentage of the enzyme should be find in the liver as against its concentration in the serum. Higher percentage in the serum is an indication of liver damage [17], [18], [19]. Lower serum concentration activity of this enzyme except in the control diets is in accord with the work of [20] that reported lower serum concentration of the enzyme when different processed watermelon seed were fed to broiler chickens. Significant ($p < 0.05$) values of the liver enzyme activity at 20% and kidney at 40% WMSM replacement levels negate the findings of [21]) which recommended melon seed cake of not more than 10% inclusion level in broiler diet. This might be as a result of different processing methods that the seeds were subjected to.

Aspartate transaminase (AST) is another liver/kidney enzyme [22] but more liver specific. It is usually measured alongside alanine transaminase to assess liver functionality. Nutritionally, AST activity should be retained within the tissue cum organ than in the serum. Higher quantity in the serum could signify plasma membrane compromise [17], [18], [19]. Outstanding values ($p < 0.05$) of both liver and kidney enzyme activities at 10% WMSM inclusion levels is in line with the results presented by [21]) which recommended melon seed cake

of 10% inclusion level in broiler diet. Lower levels of the enzyme in the serum for birds on experimental diets is in line with the findings of [23] when broiler birds were fed different processed watermelon seed meal diet. Birds fed the control diet (0% WMSM) had higher ($p < 0.05$) activities of the enzyme in the serum which indicate cellular malfunction [16].

Higher level of gamma glutamyl transferase (GGT) in the blood is an indication of liver or bile duct damage. It has a high enzyme concentration in the cell membranes of the liver and kidney, but could also be found in the brain and other tissues [24], [25]. Lower levels of this enzyme in the serum across board is an indication that watermelon seed meal couldn't have had any detrimental effect on the tissues under investigation. A 20% Bambara groundnut meal as replacement for soybean was fed by [26] to broiler birds without any detrimental effect on the studied enzyme activities. Significant liver and Kidney enzyme activities at 40% WMSM replacement level indicates the safety of feeding birds with as high as 40% WMSM diet without any detrimental effect. The quantity in the serum was significantly ($p < 0.05$) low.

Superoxide dismutase (SOD) is an antioxidant enzyme that break down dismutase into a less reactive form [27]. It is an enzyme that should be more dominant in the tissues and organs than in the serum. Lower level of this enzyme in the serum than the quantity found in the studied tissues across experimental diets is an indication of the efficacy of the seed as a good antioxidant that could actually help in combatting or reducing superoxide dismutase to a lesser risky superoxide anion [28], [29]. It had been earlier reported by [30], [31] that water melon seed and rind possessed a higher reactive

oxygen scavenging abilities than the pulp extract, hence could avert series of degenerative diseases.

Catalase is an antioxidant enzyme. It fights free radicals in the body [32]. Nutritionally, the activity of the enzyme either in the tissues or serum could be used to predict the antioxidant ability of an experimental diet. The ability of the enzyme activities to be retained in the liver and kidney significantly ($p < 0.05$) at various concentrations than in the serum proves the efficacy of the antioxidant ability of the seed. A higher antioxidant ability of pumpkin seed oil have earlier been reported by [33], [34], [35].

Glutathione-S-transferase, a phase 11 detoxification enzyme is known to protect the cell against oxidative damage [36], [37]. Results of the present study showed that water-melon-seed-meal is an efficient antioxidant seed that is capable of guiding against cellular damage [37], [38]. It had earlier been reported by [31], [39] that watermelon seed exhibited higher antioxidant ability than the pulp, hence, the seed is capable of guiding against some nutritional related diseases.

Reduced glutathione, an antioxidant produced in the cell, is capable of preventing cellular damage caused by free radicals, peroxides and other forms of reactive oxygen species. It is naturally found in the liver and could be find in many other tissues [40], [41]. Malnutrition or undernutrition can reduce its amount in the body, hence, leading to oxidative stress. [40], [41], [42]. A higher GSH liver enzyme activity was reported by [43] when mice were administered with 50% watermelon juice after they had been previously induced with low dose X-Ray. This research on the other hand negate the findings of [44] which reported a surge in

serum GSH level of carbon tetrachloride (CCl₄) induced oxidative stressed rats treated with juice extracts of watermelon mesocarp rind probably because the birds were previously induced with carbon tetrachloride or because the rind was used as against the seed used in the current research.

Weight gain as an indices of performance mostly depict the influence of experimental diets on the birds' ability to convert feed to meat, when relatively compared with the performance of birds on the control diet in a given research. Water melon seed meal was incorporated in broiler diets up to 20% without any detrimental effect [26], [45] on the birds' performance. This study fed it to birds up to 40% without any detrimental effect. Differences in results could be attributed to differences in ration composition and methodology in processing techniques employed for the seeds.

4.1. Conclusion

Overall, the results of liver/kidney function tests revealed that birds can cope with watermelon-seed-meal effectively up to 40% without any detrimental effect on the studied tissues. Weight gain as performance indices were not also jeopardized as a result of feeding with watermelon-seed-meal based diets. In conclusion, it is safe to feed broiler chickens up to 40% watermelon supplementation level in the formulation of their ration.

Ethics approval and consent to participate

This research met the University of Ilorin ethical review committee with approval number UERC/ASN/2021/2278

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contribution

SMO, OHM and OOO conceived the research idea, SMO, HOA, FAO and RAB designed the experiment, while SMO, HOA and AMA executed the research. Interpretation of research results were handled by SMO, OHM and OOO. Writing of the manuscript was handled by SMO, ADF, RAB, FAO and HOA. All authors read and approved the final manuscript.

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