Title: Varying inclusions of *Piper guineense, Aframomum melegueta* and their composite in the diets of broiler chickens: effects on growth and antioxidative status

Running Title: Effects of Phytogenic Peppers on Broiler Chickens' Brain

Key Words: Broilers, Brain, Alligator Pepper, Black Pepper, Protein

Abstract

Aims: To study the effects of varied levels of *Piper guineense* seed powder (PGSP), *Aframomum melegueta* seed powder (AMSP) and their composite mix on the growth and antioxidative status of broilers.

Study design: The experiment was a completely randomized design.

Place and Duration of Study: Department of Animal Production Health, Federal University of Technology Akure, between September 2020 and October 2020 (6 weeks).

Methodology: This study was a completely randomized design with seven diets which were: A (control/basal), B (A + 0.25 g PGSP/kg), C (A + 0.50 g PGSP/kg), D (A+ 0.25 g AMSP/kg), E (A + 0.50 g AMSP/kg), F (A+ 0.25 g {PGSP + AMSP[1:1]/kg) and G (A + 0.50 g {AMSP + PGSP[1:1]/kg). Two hundred and ten (210) day – old unsexed Cobb 500 chicks were randomly allotted to diets: Each treatment was replicated 3 times with 10 birds per replicate. The birds were fed *ad – libitum* and provided with clean water. Growth data were collected weekly and at the end of the study, 5 birds per replicates were selected for blood collection to determine the antioxidative status of the birds.

Results: There was a significant ($P < 0.05$) increase in the weekly weight gain of the birds fed 0.50 g/kg of the PGSP, AMSP and their composite. The relative growth rate was not statistically ($P > 0.05$) affected across all the experimental diets. The protein intakes of the birds were significantly ($P < 0.05$) enhanced across all the treatment diets. All the antioxidant ezymes studied (catalase, superoxide dismutase, glutathione peroxidase and total antioxidant capacity) were significantly ($P < 0.05$) increased by the varied inclusion of the additives while the serum malondialdehyde concentarion was significantly ($P < 0.05$) reduced.

Conclusion: It can be concluded that adding PGSP, AMSP and their composite mix up to 0.50 g/kg diet improved growth performance and enhanced the antioxidant status of the broilers.

Keywords: Black pepper; alligator pepper; relative growth rate; antioxidative enzymes; broilers

1. Introduction

Poultry industry, especially in the developing economies of the world, plays a very crucial role in food security as well as the development of such countries [1]. A main goal of the United Nation’s Sustainable Development Goals (SDGs) is ending poverty and hunger with improved well-being of the citizenry [2]. To achieve this lofty goal, production of healthy foods, especially of animal origin, should not be compromised. Over dependency on the use of conventional antibiotics and other feed additives in poultry production cannot be said to be sustainable in producing healthy poultry products consumers. The negative impact of the extensive use of antibiotics in poultry nutrition was also highlighted by Adu and Olarotimi [3] to include proliferation of antibiotic-resistant strains of microbes and resultant antibiotics residues in the poultry products which are a source of health concerns to the consumer. The drive to produce poultry products (meats and eggs) that is healthy and free from any form of antimicrobial contamination was reason behind the ban on the use of all antibiotic feed additives by the European Union in 2006 and recent campaign for safe, cheap and efficient alternatives to the traditional antibiotic feed additives [4, 5].

In recent times, research attention has been directed towards the use of phytogenic feed additives. The phytogenic feed additives are also known as phytobiotics or botanicals and are sourced from medicinal plants and could be presented in the form of leaf meals, liquid extracts and essential oils [6]. These plants are of great benefits to the animals because they are rich in bioactive components which play antioxidant, antimicrobial, antiviral, and immuno-modulatory roles in livestocks' system [7]. The medicinal plants of interest in the present study are African black pepper (*Piper guineense*) and alligator pepper (*Aframomum melegueta*).

The health enhancing effects of *P. guineense* as well as its safety for human consumption has stressed [8]. *Piper guineense* was also noted to have stimulating effects on the digestive enzymes, lowered lipid peroxidation, and protected against oxidative damage [9, 10]. In another study, female albino rats administered aqueous extract of *P. guineense* at 25 – 75 mg/kg BW exhibited a significant increase in serum superoxide dismutase and glutathione-s-transferase, while a significant decrease in lipid peroxidation was recorded [11]. The hepatoprotective property of *P. guineense* was equally reported by Oyinloye et al. [12]. Apart from the antioxidant activities of *P. guineense*, its dietary supplementation at 1% reportedly resulted in significant body weight gain of broiler chickens [13].

On the other hand, the antioxidant properties of *A. melegueta* were also highlighted in an in vitro study where the extract showed a significant increase in serum catalase and superoxide dismutase activity when compared with the control group. The extract equally showed a significant decrease in the serum level of malondialdehyde in the same study [14]. Adigun et al. [15] also elucidated on the antioxidants and antihyperlipidemic properties of *A. melegueta* seed extract. In another development, Nwozo et al. [16] documented the excellent radioprotective potentials of *A. melegueta* on γ-radiation-induced liver damage in male Wistar rats where serum elevated lipid peroxidation and reduced hepatic enzymes activities were restored. Despite the well documented potentials of these medicinal plants, there is still paucity of information on the application of *A. melegueta* and *P. guineense* as nutritional additives in the diets of the domestic chickens. Therefore, the present study seeks to examine the effects of dietary *A. melegueta*, *P. guineense* and their composite on the growth and antioxidant status of broiler chickens.

2. Materials and Methods

2.1 Preparation of the Phyto-Additives

The seeds of *A. melegueta* and *P. guineense* were used for the study. They were procured from the local market, removed carefully from the twig and separated from all extraneous materials. The seeds were placed in a screen under a well aerated building till a moisture content of 10 -11 % was achieved. Thereafter, the dried seeds were grounded into powder to make *A. melegueta* seed powder (AMSP) and *P. guineense* (PGSP), respectively, using an electric blender.

2.2 Experimental Design, Birds and Diets

A total of two hundred and ten (210) day-old unsexed and Cobb 500 broiler chicks were used for the experiment at the Poultry Unit of the Teaching and Research Farm, The Federal University of

Technology, Akure. The initial weights of the chicks were captured at the commencement of the experiment. They were randomly allotted to seven (7) treatments (diets): A (control/basal), B (basal + {0.25 g AMSM/kg}), C (basal + {0.50 g AMSM}/kg), D (basal + {0.25 g PGSM}/kg), E (basal + {0.50 g PGSM}/kg), F (basal + {0.125 g AMSM + 0.125 g PGSM}/kg) and G (basal + {0.25 g AMSM + 0.25 g PGSM}/kg). Each treatment was replicated 3 times with 10 birds per replicate in a completely randomized design. Broiler starter (Table 1) and finisher (Table 2) diets were fed to the birds at 0–3 weeks and 4-6 weeks, respectively.

Table 1: Ingredient composition of the experimental starter diets

Ingredients	A	B	C	D	F	G	H
Maize	480	480	480	480	480	480	480
Soybean meal	290	290	290	290	290	290	290
Rice Bran	70	70	70	70	70	70	70
Fish Meal	50	50	50	50	50	50	50
Wheat Offal	80	80	80	80	80	80	80
Bone Meal	13	13	13	13	13	13	13
Limestone	10	10	10	10	10	10	10
Salt	2.4	2.4	2.4	2.4	2.4	2.4	2.4
AMSM	0	0.25	0.5	0	0	0.125	0.25
PGSM	0	0	0	0.25	0.5	0.125	0.25
Lysine	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Broiler Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total	1000	1000.25	1000.5	1000.25	1000.5	1000.25	1000.5
Calculated Nutrients							
ME (Kcal/Kg)	**2923.16**	**2923.16**	**2923.16**	**2923.16**	**2923.16**	**2923.16**	**2923.16**
Crude Protein (%)	**22.49**	**22.49**	**22.49**	**22.49**	**22.49**	**22.49**	**22.49**
Calcium (%)	**1.21**	**1.21**	**1.21**	**1.21**	**1.21**	**1.21**	**1.21**
Phosphorus (%)	**0.53**	**0.53**	**0.53**	**0.53**	**0.53**	**0.53**	**0.53**
Lysine	**1.3**	**1.3**	**1.3**	**1.3**	**1.3**	**1.3**	**1.3**
Methionine	**0.55**	**0.55**	**0.55**	**0.55**	**0.55**	**0.55**	**0.55**
Fat	**4.32**	**4.32**	**4.32**	**4.32**	**4.32**	**4.32**	**4.32**
Crude Fibre (%)	**4.79**	**4.79**	**4.79**	**4.79**	**4.79**	**4.79**	**4.79**

Table 2: Ingredient composition of the experimental starter diets

Ingredients	A	B	C	D	F	G	H
Maize	550	550	550	550	550	550	550
Soybean meal	220	220	220	220	220	220	220
Rice Bran	100	100	100	100	100	100	100
Fish Meal	50	50	50	50	50	50	50
Wheat Offal	50	50	50	50	50	50	50
Bone Meal	13	13	13	13	13	13	13
Limestone	10	10	10	10	10	10	10
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5
AMSM	0	0.25	0.5	0	0	0.125	0.25
PGSM	0	0	0	0.25	0.5	0.125	0.25
Lysine	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Broiler Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total	1000	1000.25	1000.5	1000.25	1000.5	1000.25	1000.5
Calculated Nutrients							
ME (Kcal/Kg)	**3004.10**	**3004.10**	**3004.10**	**3004.10**	**3004.10**	**3004.10**	**3004.10**
Crude Protein (%)	**20.02**	**20.02**	**20.02**	**20.02**	**20.02**	**20.02**	**20.02**
Calcium (%)	**1.20**	**1.20**	**1.20**	**1.20**	**1.20**	**1.20**	**1.20**
Phosphorus (%)	**0.52**	**0.52**	**0.52**	**0.52**	**0.52**	**0.52**	**0.52**
Lysine	**1.12**	**1.12**	**1.12**	**1.12**	**1.12**	**1.12**	**1.12**
Methionine	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
Fat	**4.62**	**4.62**	**4.62**	**4.62**	**4.62**	**4.62**	**4.62**
Crude Fibre (%)	**4.64**	**4.64**	**4.64**	**4.64**	**4.64**	**4.64**	**4.64**

2.3 Data Collection

- The relative growth rate (RGR) of the bird was determined as follows:

RGR = $\{(W_2 - W_1)/ 0.5 (W_2 + W_1)\}$ x 100

W_1= weight of the birds at the commencement of the experiment and W_2 = weight of the birds at the termination of the experiment.

- The weekly weight gains (WWG) of the birds were determined using a very sensitive weighing balance.

- The Protein intake (PI), protein utilization (PU), energy intake (EI) and energy utilization (EU) were also estimated as:

    - ✓ PI = FI x CP (%)/100

    - ✓ PU = CP/WWG

Where : FI = feed intake, CP = crude protein of the feed, WWG = weekly weight gain of the birds

  - ✓ EI = FI x ME/1000

  - ✓ EU = EI /WWG

Where: EI = energy intake, ME = metabolizable energy

On the day the experiment was terminated (42nd day); five (5) birds per replicate were randomly selected for blood collection. The blood was collected through the jugular veins using needle and syringe. The blood samples collected in plain bottles were centrifuged for 10 minutes at 3000 rpm to obtain a clear supernatant serum. The harvested serum samples were used for the determination of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) catalase (CAT), total antioxidant activity (T-AOC), and malondialdehyde (MDA) as previously described by Olarotimi [17] using commercially available assay kits.

2.4 Statistical analysis

All experimental data obtained were subjected to One-Way Analysis of Variance (ANOVA). Significant differences between the treatment means were compared using the Tukey's Honestly Significant Difference (HSD) at 5% level of significance.

3. Results

3.1 Effects of PGSP, AMSP and PGSP-AMSP Mix on the Broilers' Growth Indicators

The results the growth indicators of broilers fed diets supplemented with PGSP, AMSP and their composite is in Table 3. Inclusion of 0.25 g/kg of the two phyto-additves and their composite mix respectively caused a slight ($P > 0.05$) increase in the weekly weight gain of the broiler chickens. However, there was a significant ($P < 0.05$) increase in the same weekly weight gain when the inclusion levels of the additives were doubled. The relative growth rate did not show any statistical ($P > 0.05$) difference across all the experimental diets. The protein intake (PI) of the broiler chickens were significantly ($P < 0.05$) influenced across all the treatment diets with birds on 0.50 g/kg PGSP-AMSP mix having the higher significant ($P < 0.05$) PI when compared with the broilers on diets D, E, F and the control respectively. However, the protein utilization (PU), energy intake (EI) and energy utilization (EU) were not significantly ($P > 0.05$) across all the treatment diets.

3.2 Effects of PGSP, AMSP and PGSP-AMSP Mix on the Antioxidant Status of the Broilers

The effects of the phyto-additves and their composite mix on serum catalase of the experimental birds were shown in Figure 1. Though the inclusion of 0.25 g/kg PGSP in the diet of the broilers enhanced serum catalase (CAT) concentration, it was not significantly ($P > 0.05$) affected unlike the inclusion 0.50 g/kg PGSP and 0.25 g/kg AMSP that significantly ($P < 0.05$) increased the serum CAT when compared with birds on the control diet. The inclusion of 0.50 g/kg AMSP, 0.25 and 0.50 g/kg PGSP-AMSP composite presented the higher significant ($P < 0.05$) means of serum CAT when compared with the values recorded by birds on all other diets.

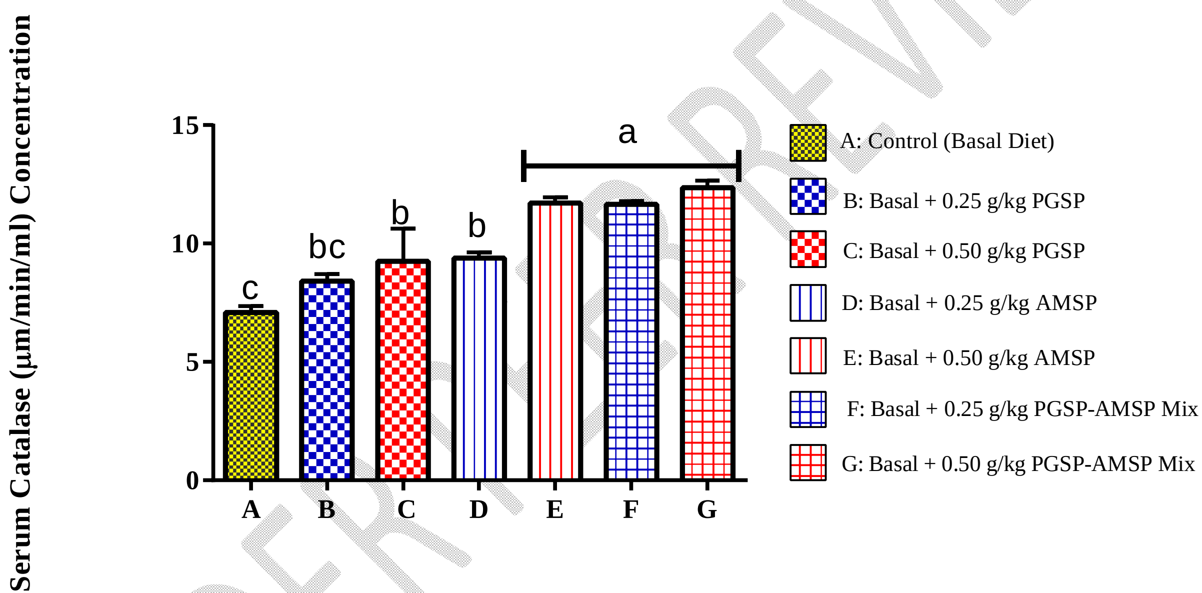

Figure 1: Effects of PGSP, AMSP and PGSP-AMSP Mix on Serum Catalase

Furthermore, the inclusions of 0.25 and 0.50 g/kg PGSP did not significantly (P $P > 0.05$) affect the serum glutathione peroxidase (GSH-Px) concentration (Figure 2) of the broiler chickens.However, the inclusions of 0.25 and 0.50 g/kg AMSP and PGSP-AMSP Mix significantly ($P < 0.05$) increased the serum GSH-Px concentrations of the birds.

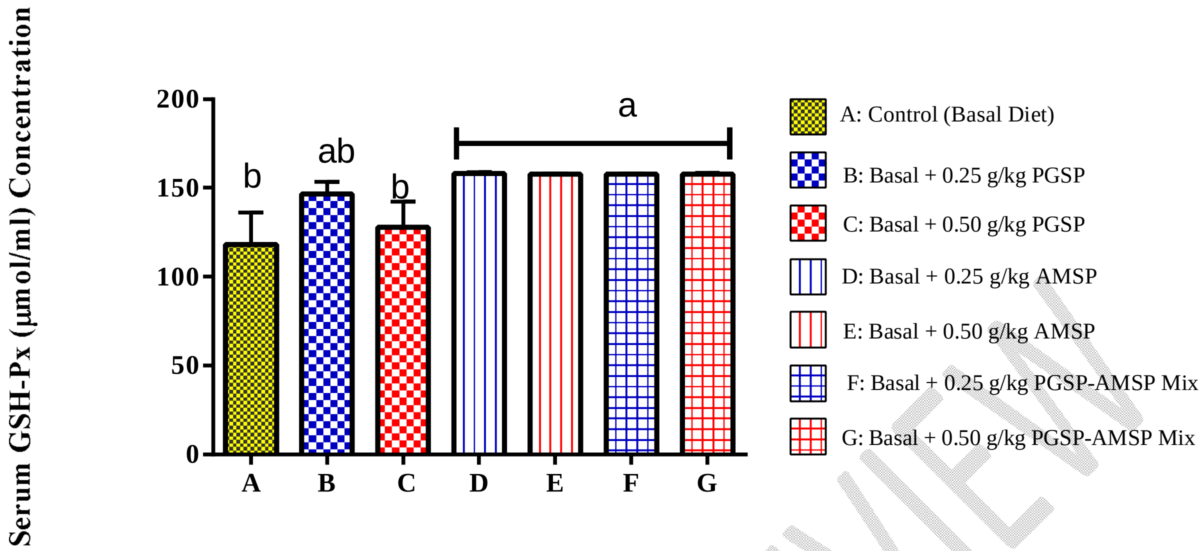

Figure 2: Effects of PGSP, AMSP and PGSP-AMSP Mix on Serum Glutathione Peroxidase

The additions of the two phyto-additives and their composite mix, also, significantly ($P < 0.05$) affect the superoxide dismutase (SOD) concentrations (Figure 3) of the birds across all the treatment diets. The inclusions of 0.25 and 0.50 g/kg of PGSP and AMSP respectively significantly ($P < 0.05$) increased the serum SOD when compared with the control at the same rate ($P > 0.05$). However, the varied inclusions of PGSP-AMSP mix provided a better result than the individual phyto-additive.

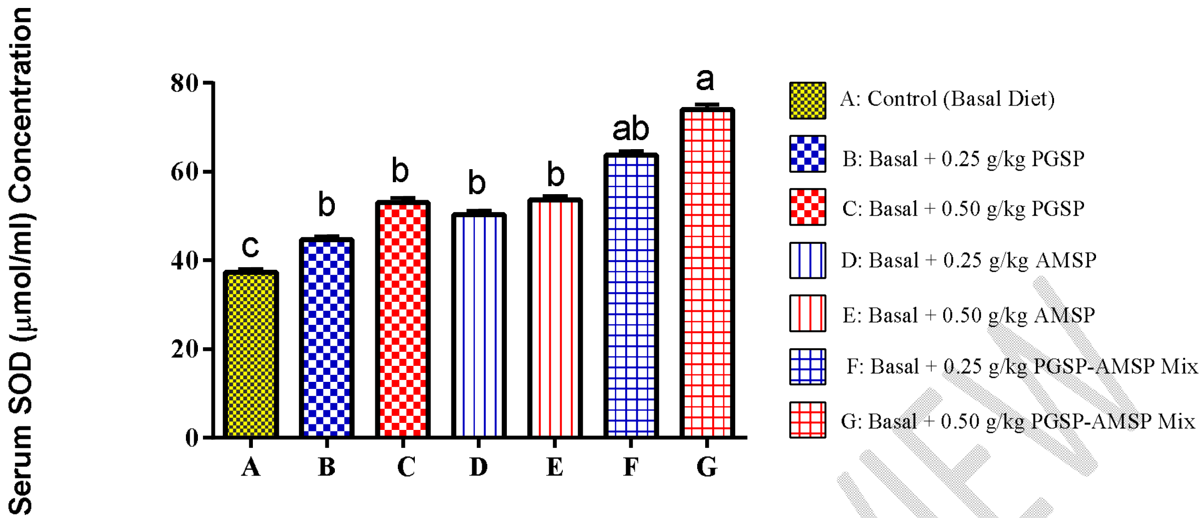

Figure 3: Effects of PGSP, AMSP and PGSP-AMSP Mix on Serum Superoxide Dismutase

More so, the inclusions of the phyto-additives and their composite significantly ($P < 0.05$) influenced the serum total antioxidant activities (T-AOC) of the birds (Figure 4) except among the broilers on the diet containing 0.25 g/kg PGSP where there was no statistical ($P > 0.05$) difference in the serum T-AOC when compared with the control. The birds on the diet containing 0.50 g/kg PGSP-AMSP Mix recorded the higher significant ($P < 0.05$) serum T-AOC when compared with those on other diets except birds on 0.50 and 0.25 g/kg AMSP and PGSP-AMSP Mix respectively.

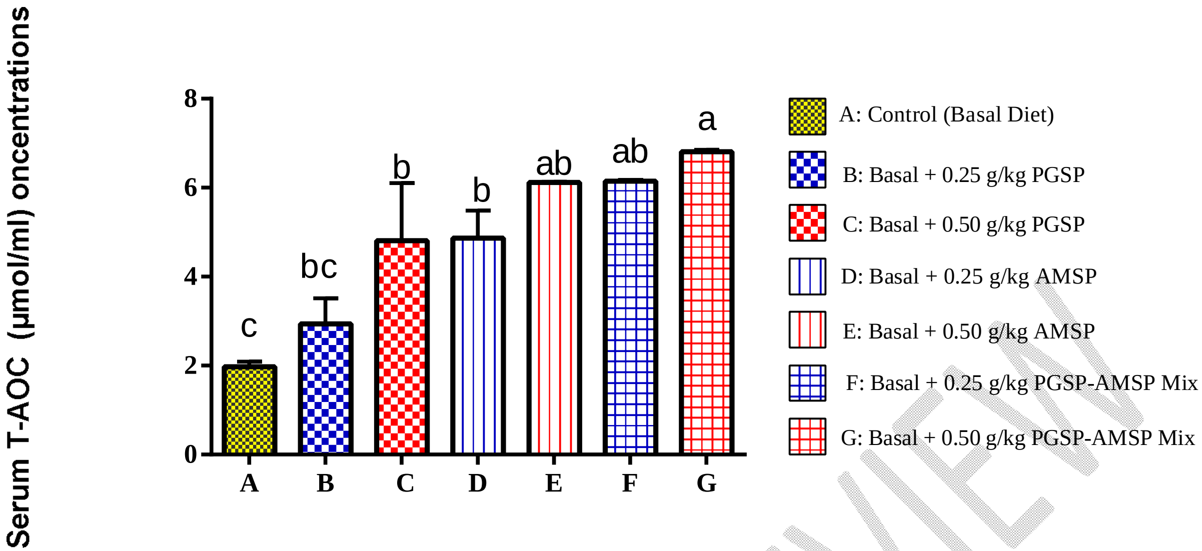

Figure 4: Effects of PGSP, AMSP and PGSP-AMSP Mix on Serum Total Antioxidant Capacity

For the serum malondialdehyde (MDA) concentration (Figure 5), the broilers on the control diets recorded the higher significant ($P < 0.05$) values when compared with those on other diets. Though the inclusions of 0.25 PGSP and AMSP did not significantly ($P > 0.05$) reduce the MDA concentration, a slight reduction was, however, observed. The inclusions of 0.50 g/kg PGSP and AMSP as well as 0.25 and 0.50 g/kg PGSP-AMSP Mix significantly ($P < 0.05$) caused a reduction in the serum MDA of the broiler chickens.

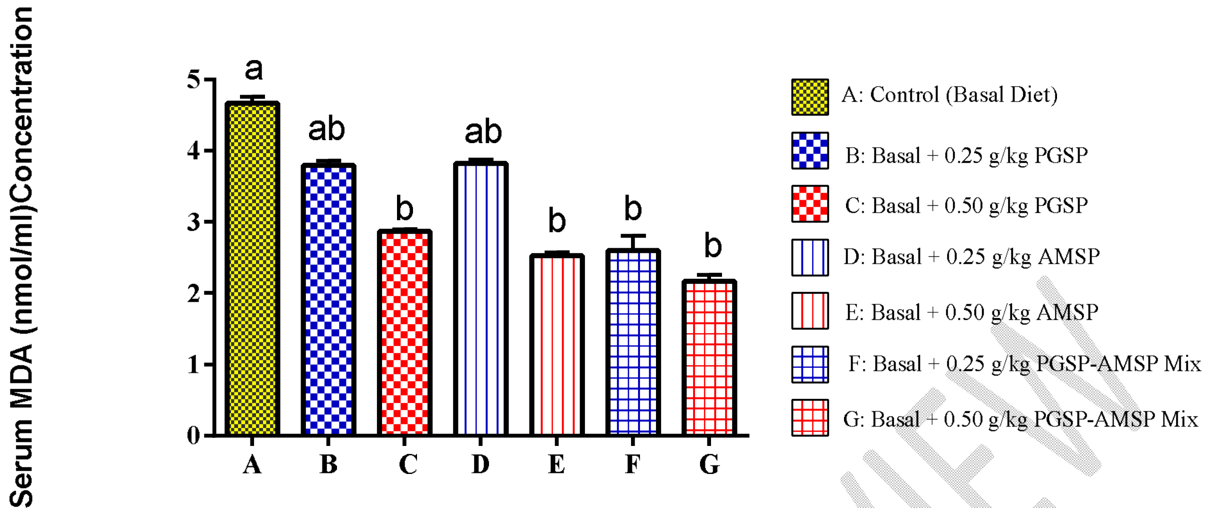

Figure 5: Effects of PGSP, AMSP and PGSP-AMSP Mix on Malondialdehyde

4. DISCUSSION

The significant increase observed in the WWG among the birds on diets C, E and G when compared with birds on diet A and the insignificant increase among the birds on diets B, D and F were suggestive of the abilities of the phyto-additives used in this study and their composite to enhance weight gain in broilers. This was in agreement with previous studies that highlighted the positive influence of PGSP on body weight gain of broiler chickens [13] and AMSP on the specific weight gain of catfish[18]. Our study has further strengthened that the composite mix of the phyto-additive provides a synergistic effects on the growth rate of the broiler chickens on 0.50 g/kg PGSP-AMSP Mix resulting in the highest WWG rate. Protein intake is one of the yardsticks used in measuring the performance poultry chickens. The significant increase in PI among the birds fed the varied inclusions of PGSP and AMSP and their composite indicated the contributory effects of the additives to protein contents of the diets, thereby, enhancing the productive performance of broiler chickens.

The results of the present study revealed the enhancing effects the two phyto-additives and their composites on the antioxidant status of the broiler chickens. Lipid peroxidation, measured as malondialdehyde (MDA) levels, was significantly higher among the control birds. In response to the varying inclusions of the PGSP, AMSP and PGSP-AMSP Mix, significant reductions were observed in the serum MDA concentrations. From this study, it could be opined that the inclusion of 0.50 g/kg PGSP and AMSP were more potent than 0.25 g/kg inclusions. This was highlighted by the non-significant reduction in MDA concentration observed among the birds fed 0.25 g/kg PGSP and AMSP respectively. However, the synergistic interactions of the bioactive components of the two phyto-additives in the composite mix might be responsible for the significant reduction in serum MDA observed among the birds fed 0.25 g/kg of the composite mix. This study agreed with the previous studies that outlined the protective roles of these additives against oxidative stress [9, 10].

In the present study, the SOD which is the first line of defense was also enhanced by the varied levels of the phyto-additive inclusions. Generally, SOD translates superoxides to hydrogen peroxides (H_2O_2) and GSH-Px turns the H_2O_2 to water and gaseous oxygen [17]. The significant increase observed in SOD activities among the birds fed varied inclusions of the two phyto-additives and their composite mix confirmed the enhancing effects of the additives in boosting the antioxidant status of the birds against possible oxidative stress. The higher levels of SOD among the birds fed the composite mix further proved that the synergistic effects of the additives were of great advantage to the birds. The significant increase in GSH-Px and CAT observed among the birds fed varied inclusions of AMSP and PGSP-AMSP mix was indicative of possible higher antioxidant contents of AMSP and PGSP-AMSP mix to that of PGSP. This will definitely impacts positively on the role of GSH-Px as a second line of antioxidant defense mechanism.

Increase in the levels of MDA normally causes oxidative stress while increase in T-AOC protects against free radicals and peroxides. Since there is always an inverse relationship between lipid peroxidation (MDA) and T-AOC [17], results clearly displayed an increasing level of T-AOC against a decreasing level of MDA. It is, therefore, suggestive that increased activities of these enzymes as observed among the broilers fed diets with the phyto-additives may protect the chickens against possible oxidative stress.

5. Conclusions

The results obtained from this study has demonstrated that PGSP and AMSP possess antioxidant and growth enhancing properties that could be utilized in nutritional fortification of broiler chickens against possible oxidative stress and enhance the growth potentials of the birds for productive purposes. It is also proposed that the possible mechanism by which PGSP-AMSP mix brought about the observed enhancement in the present study may be due to the synergistic interactions of its bioactive components. Therefore, PGSP, AMSP and PGSP-AMSP mix are a candidate with antioxidant and growth enhancing potentials in broiler production.

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