Effectiveness Of Graded Levels Of Amino Acids On Hematological Parameters And Serum Biochemical Indices Of Broilers Chickens Fed Sprouted (Germinated Masakwa) Sorghum (Sorghum Bicolor (L.) Moench) Based Diets

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Abstract- The research was carried out at Department of Animal Science and Range Management Teaching and Research Farm Modibbo Adama University of Technology, Yola, Nigeria.
The study was conducted to determine the Effectiveness Level of Amino Acid Inclusion in Sprouted (germinated Masakwa Sorghum, Sorghum bicolor (l.) moench) Based Diet on the Performance of Broiler Chickens. One hundred and fifty day-old (Anak 200) broiler chickens were used for the study and were randomly allocated to five dietary treatments of thirty broiler chicks per treatment. Each treatment was replicated three times with ten birds per replicate in a completely randomized design (CRD). Five experimental diets were formulated at varying supplementation levels of methionine at 0.20%, 0.25%, 0.30%, 0.35%, and 0.40% of the diets with maize used as a control diet. No significant ($p > 0.05$) difference were observed for the hematological. The WBC ranged from 231.33 to 253.20, RBC ranged from 1.93 to 260, Hb ranged from 8.36 to 10.03, MCV ranged 1.33.13 to 139.73, MCH ranged 39.70 to 42.30 and MCHC ranged 29.76 to 30.50 and serum biochemical indices of the birds includes Glucose ranged from 12.00 to 13.46, Urea ranged from 0.59 to 1.07, Creatinine ranged from 19.33 25.20, protein ranged from 27.33 to 37.00, Albumin 16.18 to 19.25, Triglyceride ranged 33.33 to 116.66, Cholesterol ranged from 111.06 to 133.83 and Globulin ranged 10.69 to 16.20. Therefore, it was concluded from the result of the study that methionine could be a good supplement for a sprouted germinated masakwa in boiler diets and can be included in their diets at 0.30%, 0.35% and 0.40% levels for improved performance of bird on hematological and biochemical indices.

I. INTRODUCTION

Many feed stuff contain anti nutritional factors that may affect the health status of the animals. Therefore, hematology should be a routine procedure in nutrition studies to provide quick assessment of the health of the animals (Akinmutini, 2004). Apart from toxicants that could affect hematological values, diets also have strong influence on blood parameters (Harcbarth, *et al.,* 1983). Diets with toxic agents may also elicit response to body organs especially those that are directly involved in detoxification process; these responses may include; increase or decrease in organ size and secretion of cellular metabolite (Alumot and Nitzan, 1961). Blood examination is performed for several reasons such as screening procedure to assess general health of the animal (Jain, 1993). Glucose, cholesterol, calcium, protein, alkaline, phosphorus, uric acid, sodium was carried out to determine the biochemical indices. Maize contributes approximately 65% of the metabolisable energy and 20% of the protein in the broiler starter and is by far the most commonly used cereal grain in the diet of intensively reared poultry. One reason for the wide spread use of maize in the diets of livestock is that, there is a perception that maize is of a consistent and high nutritional values. The nutritional value of maize is a function of the content of starch, or protein and anti-nutrients (primarily phyate, enzyme inhibitors and resistant starch). In comparison with maize sorghum has similar average content of lysine, methionine fiber, crude extract, ash and phosphorus, but almost twice as much calcium.

One characteristic of sorghum is its content of tannins, which are polyphenols formed in the testa; they act as anti-nutritional factors with effects varying widely according to their composition and extent of polymerization. For non-ruminants they are known to lower nutritive value, palatability and protein utilization of sorghum based diets, In the gastro-intestinal tract, tannins are hydrolyzed to Gallic acid and sprouted extracted in the form of 4-O – methyl-gallic acid utilizing the methionine and choline of the feed as a source of methyl groups for the O – methylation (Potter and Fuller, 1968). According to Rostagno, *et al.,* (1973b) sorghum amino acid availability is strictly related to its tannin content. The apparent amino acid digestibility of sorghum with low, intermediate and high tannin content is 73, 41

and 22% respectively (Rostagno, *et al.*, 1973b). Digestibility is the principal problem with sorghum grains, Sorghum grain needs to be processed to utilize most of the feeding value. This can improve the starch digestibility rending the grain equivalent to barley, although, protein digestibility is not changed except by reconstitution.

II. Materials and Methods

Study Area

The research was carried out at the Poultry Teaching and Research Farm of Animal Science and Range Management Department, Modibbo Adama University of Technology, Yola. It is located in Girei Local Government Area of Adamawa State. Girei is located in the Guinea Savannah zone of Nigeria and lies between latitude 90 and 11ºN of the equator and longitude 10 and 14ºE of the Greenwich meridian. Adamawa state shares its boundaries with Taraba State to the south and west, Gombe State to the North-west and Borno State to the North. Adamawa state has an international boundary with the Cameroon Republic along its eastern boarder (Adebayo 1999). The state has tropical climate with distinct dry and wet seasons. The rainfall begins in April and ends in the late October, while the dry season commence in October or November and ends in April. It has an average minimum and maximum temperature of 18 and 40ºC and relative humidity of 20 and 80%.

Procedure for Sprouting or Partial Germination

The procedure of sprouting or partial germination was adopted from the methods described by Yakubu, *et al.*, (2009b). The sorghum grain was soaked for 12 hours in water after which it was; drained and spread on a concrete floor. A polythene material was used to cover the grain to preserve moisture. Germination was then arrested after 12 hours by spreading the grain in the sun to dry. This was then incorporated into the treatment diets after grading.

Animal Management

One hundred and fifty (150) day-old (Anak, 2000) broiler chicks were used and were obtained from Avian Farm in Jos, Plateau State. The chicks were brooded for seven days. During the brooding state the chicks were fed on commercial starter mash and clean drinking water was provided ad-libitum. Light were provided by electric bulb. After brooding period necessary vaccination was administered and wood shavings were used as a litter material during the period of experiment. The birds were given experimental diet and water ad libitum.

Experimental Diet and Design

Maize and *Masakwa* sorghum was purchased from Yola town market. Dietary treatment was formulated, Maize based diet was served as the control while the partially sprouted *Masakwa* was supplemented with methionine at 0.25%, 0.3%, 0.35% and 0.4% which were served as treatments 2,3,4 and 5 respectively.

The dietary composition of the broiler starter and finisher are presented on tables 1 and 2 respectively. The chicks were removed from the brooder unit after 7 days and allocated into five treatments. Each treatment was replicated three times with (10) ten chicks per replicate in a completely randomized design (CRD).

Table 1: Broiler Starter Containing Methionine Supplementation Levels in Sprouted Masakwa based Diet.

	Supplementation levels of methionine (%)				
Ingredients %	T_1	T_2	T_3	T_4	T_5
Maize	48.39	0.00	0.00	0.00	0.00
PGMSC	0.00	48.32	48.25	48.19	48.11
Maize offal	10.00	10.00	10.00	10.00	10.00
G/nut cake	36.31	36.32	36.35	36.36	36.38
Bone meal	2.50	2.50	2.50	2.50	2.50
*premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.25	0.30	0.35	0.40
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Calculated Analysis					
Crude Protein	23.00	23.95	23.95	23.94	23.93
Crude fibre	4.69	4.58	4.31	3.95	3.56
Calcium	1.13	1.11	1.09	1.10	1.07
Phosphorus	0.78	0.81	0.79	0.80	0.82
Methionine	0.43	0.45	0.48	0.53	0.57
Lysine	1.12	1.10	1.10	1.10	1.10
ME (Kcal/kg)	3018.49	2965.44	2908.16	2892.10	2867.23

* Vitamin-mineral premix provides per kg.
The following: vit A, 1500 IU; vit D_3 3000 IU, vit E; 30 IU; vit K 2.5mg; Thiamine B_1 3mg; Riboflavin B_2 6MG; Pyridoxine B_6 4mg; Niacin 40kg; vit B_{12} 0.02mg; panthothenic acid 10mg; Folic acid 1mg; Biotin 0.08g; Chloride 0.125g; M_n 0.096g; Antioxidant 0.125g;

Cu 0.006g; I 0.0014g; Se 24g; Co 0.240g. SMSC – Sprouted *Masakwa* Sorghum Cultivar, PGMSC = Partially Germinated *Masakwa* Sorghum Cultivar.

Table 2: Composition of Broiler Finisher Containing Different Methionine Supplementation Levels in Sprouted Masakwa based Diet.

	Supplementation levels of methionine (%)				
Ingredients %	T_1	T_2	T_3	T_4	T_5
Maize	56.70	0.00	0.00	0.00	0.00
PGMSC	0.00	56.64	56.59	56.53	56.50
Maize offal	10.00	10.00	10.00	10.00	10.00
G/nut cake	28.00	28.01	28.01	28.02	28.02
Bone meal	2.50	2.50	2.50	2.50	2.50
*premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.25	0.30	0.35	0.40
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Calculated Analysis					
Crude Protein	20.67	20.62	20.61	20.61	20.59
Crude fibre	4.54	4.48	4.48	4.47	4.46
Calcium	1.10	1.12	1.12	1.11	1.11
Phosphorus	0.78	0.77	0.77	0.76	0.78
Methionine	0.42	0.46	0.49	0.52	0.54
Lysine	1.21	1.11	1.11	1.10	1.12
ME (Kcal/kg)	3189.62	3188.78	3032.56	3048.44	3121.45

* Vitamin-mineral premix provides per kg.

Vit A, 1500 IU; Vit D_3 3000 IU, Vit E; 30 IU; Vit K 2.5mg; Thiamine B_1 3mg; Riboflavin B_2 6mg; Pyridoxine B_6 4mg; Niacin 40kg; vit B_{12} 0.02mg; pantothenic acid 10mg; Chloride 0.125g; M_n 0.096g; Antioxidant 0.125g; Cu 0.006g; I 0.0014g; Se 24g; Co 0.240g. PGMSC = Partially Germinated *Masakwa* Sorghum Cultivar

All the internal organs which include liver, lungs, kidney, small intestine large intestine, heart and caecum were weighed and expressed in grams. Similarly, the ceacal length and gastrointestinal tract length were measured in centimeter.

Haematological and biochemical indices

Blood samples were collected from 15 birds, from each treatment at 8 weeks of the research period. Samples were collected from wing vein using 5mls syringes and to gauge needle. Portion of the whole blood was transferred to tubes without anticoagulant: then serum was collected by configuration; (10, 000G for 5 minutes) for biochemical and protein electrophoresis analysis. Blood samples were analyzed in the laboratory within 24hrs and hematological analyses of Red blood count cell (RBC), Packed Cell Volume (PCV) were determined by centrifugation at 10,000G for 5 minutes. Hemoglobin (Hb) content was determined by cyanide free hemoglobin determination. The erythrocytic indices MCV, MCH and MCHC was determined using the formula below as described by Sirios (1995).

MCV = (PCV/RBC) 100
MCH = (Hb/RBC) 100
MCHC (Hb/PCV) 100

Proximate Analysis

All the treatments diets were subjected to proximate analysis as described by AOAC (1999). The proximate analysis of the *Masakwa* was done before the germination.

Quantitative Determination of Tannin

The quantitative determination of Tannin was adopted by the procedure described by Darwa (1988).

Statistical Analysis

Data generated from the equipment was subject to one-way analysis of variance. Steel and Torrie (1980) and means were subjected using Ducan Multiple Rate Test.

III. Results

Proximate Composition of Raw and Sprouted Germinated *Masakwa* Sorghum Cultivar

The result of the proximate composition of raw and sprouted or germinated *Masakwa* sorghum cultivar are presented in Table 3 and 4 respectively. The result for the proximate composition of the raw *Masakwa* showed 94%, 11.60%, 7.85%, 2.65%, 73.55% and 4.35% for dry matter (DM) crude protein (CP) crude fibre (CF) ether extract (EE) nitrogen free extract (NFE) and ash respectively while tannin and metabolizable energy were 41 gce/kg and 3227.54 kcal/kg respectively.Similarly for the Sprouted or germinated *Masakwa*, values were 96%, 11.80%, 7.75%, 2.25%, 73.33% and 4.30% for dry matter (DM) crude protein (CP) crude fibre (CF) either extract (EE), nitrogen free extract (NFE) and ash

respectively while the tannin content and metabolizable energy were 19.20 gce/kg and 3235.25 kcal/kg respectively.

Table 3: Proximate Composition of Raw *Masakwa* Sorghum Cultivar

Nutrient	Percentage (%) composition
Dry matter (DM)	94
Crude protein (CP)	11.60
Crude fibre (CF)	7.85
Ether Extract (EE)	2.65
Ash	4.35
Nitrogen free extract (NFE)	73.55
Tannins	41.00
MECKcal/kg	3227.54

Metabolizable energy (Kcal/kg) of practically germinated sorghum cultivar was estimated using the methods of Pausenga (1985) as follows:
ME = 37 x CP% + 81.8 x EE% + 35.5% x NFE%
Tannin was expressed in gce/Kg

Table 4: Proximate Composition of Sprouted *Masakwa* Sorghum Cultivar

Nutrient	Percentage (%) composition
Dry matter (DM)	96
Crude protein (CP)	11.80
Crude fibre (CF)	7.75
Ether Extract (EE)	2.85
Ash	4.30
Nitrogen free extract (NFE)	73.30
**Tannins	19.20
MECKcal/kg	3235.23

*Metabolizable energy (Kcal/kg) of practically germinated sorghum cultivar were estimated using the methods of Pausenga (1985) as follows:
ME = 37 x CP% + 81.8 x EE% + 35.5% x NFE%
**Tannin was expressed in gce/Kg

Hematological and Biochemical Indices of broilers chickens feed Sprouted germinated *Masakwa* sorghum cultivar supplemented different levels of methionine. The results of the hematological and biochemical parameters are shown on Table 9. The hematological parameter considered for the study includes pack cell volume (PVC) red blood cell (RBC), white blood cells (WBC), Hemoglobin (Hb) concentration (MCHC). Amongst the hematological parameter, there was no significant difference ($p > 0.05$) across the treatment group. Similarly, the biochemical index which includes the protein, glucose, urea, creatinme, albumin, triglycerides, cholesterol, and globulin were not significantly different ($p > 0.05$) across all the treatment groups.

Table 5: Effect of Sprouted *Masakwa* Sorghum Cultivar Fortified with Different Levels of Methionine on Haematology and Serolocial Parameters of Broiler Chicks Fed.

	Supplementation levels of methionine					
Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Haematological indices						
WBC	249	245	231.33	253.20	250	6.72^{NS}
RBC	2.37	2.26	1.94	2.52	2.60	6.1^{NS}
HB	10.03	8.93	8.36	10.23	10.16	0.69^{NS}
MVC	139.73	136.80	141.66	134.70	133.13	3.22^{NS}
MCH	42.30	40.96	43.23	40.23	39.70	1.11^{NS}
MCHC	30.26	30.50	30.26	30.13	29.76	0.61^{NS}
PCV	27.03	34.66	34.33	33.66	33.33	2.25^{NS}
Biochemical indices						
Glucose	12	12.13	13.06	11.80	13.46	0.97^{NS}

Urea	0.78	1.07	0.88	0.78	0.59	0.15 NS
Creatinine	21.76	19.33	21.43	19.60	25.20	1.65 NS
Protein	35.50	27.33	37.00	34.00	34.76	2.57 NS
Albumin	19.25	16.18	18.84	17.80	18.84	1.22 NS
Triglyceride	116.66	33.33	96.66	80.00	80.00	18.97 NS
Cholesterol	133.83	119.43	111.06	122.20	116.63	7.85 NS
Globulin	16.20	10.69	17.51	16.20	15.25	2.35 NS

a, b, c = Means within the same raw bearing different superscripts differ significantly ($p < 0.05$)
*** = significant ($P < 0.001$)
NS = not significant
SEM = Standard error of means

Effect of Sprouted *Masakwa* Sorghum Cultivar Fortified with Difference Levels of Methionine on Hematological and Serum Biochemical Parameters of Birds

There was no significant ($P > 0.05$) difference observed among the treatment groups for all the hematological values. The PVC range (35-38%) reported by Etuk *et al.,* (2012) and 24-39% (Aiello and Michael, 1998). They appeared to be no effect of dietary treatments on the PVC values. This suggested that subjecting *Masakwa* Sorghum to the process of partial germination might have reduced the level of tannin to a considerable level as demonstrated or observed in the values of PCV recorded in the study. According to Oyawoye and Ogunkunle (1980), PCV is usually an index of toxicity reduction in the blood and also suggest presence of a toxic factor which has adverse effect on blood formation. The value for the hemoglobin (Hb) ranged (8.36-10.16g/bird) (Table 8) was also within the range of 9.40 – 12.50g/dl reported by Etuk *et al.,* (2012). In this study, the results in regard to the PCV and Hb are indications of the nutritional quality of the experimental diets in terms of proteins that met the nutritional needs of the experimental broiler birds. Adejumo (2004) reported that hematological traits especially PCV and Hb were correlated with the nutritional status of the animal.

There was also no significant difference ($p>0.05$) observed for the serum biochemical indices such as the creatine, protein, albumin triglyceride, cholesterol and globulin. The Serum biochemical indices observed were not within the range as reported by Kwari *et al.,* (2011). This was probably due to the differences in the concentration of tannin in the treatment diets. According to Hartis (1971) the tannin concentration of sorghum grain varies with the cultivar, this is an indication that residue tannin present in the treatment diets did not have much effect on the availability of methionine (Williams 1997).

IV. Conclusion

From the result of this study, it was an evident that fortified methionine of Sprouted *Masakwa* sorghum cultivar has been found to improve the performance of broiler chickens; it can be included in the diet of broiler chickens at 0.30% for optimum performance and without adverse effect on the growth performance, carcass yield, internal organs characteristics, hematological and serum biochemical indices. It was also evident from final weight (2535.009) T_1 (maize based control diet) and T_3 (0.30%) with final weight 2423.33) that sprouted or germinated *Masakwa* can replace maize by 95%.

V. Recommendations

According to the result of this study, it clearly shown that fortification of methionine on Sprouted germinated *Masakwa* Sorghum Cultivar can be included in broiler diets at 0.30%.

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