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Effects of Heat Treated Plant Proteins on Hatchability of Fertilized Eggs from Exotic Laying Hens in Potiskum, Yobe State -Nigeria

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ABSTRACT
One hundred and twenty (120) breeder hens comprising of 105 hens and 15 cocks were randomly selected and allocated to five treatment groups to evaluate the effects of aqueous-and dry-heated bambara groundnuts and Benniseeds on egg production, fertility and hatchability. Results indicated that egg weight and hen- day production were significantly (P <0.05) reduced in birds fed dry- heated bambara groundnuts and aqueous dry heated benniseeds. Regression analysis conducted revealed significant correlations (P<0.05) between egg weight and Tannin (r= -0.93), hen day production and Tannin (r= -0.82) as well as egg weight and oxalate (r= -0.89). Lower statistical values were obtained for yolk and albumen protein in birds fed with aqueous and dry heated benniseed. Significant reductions were obtained in percentages for hatchability of fertile eggs and weights of hatched chicks in birds fed with dry-heated bambara groundnut and aqueous dry heated benniseed respectively. Birds fed with aqueous -heated bambara groundnuts performed significantly better when compared with those fed with aqueous dry heated benniseed.
Keywords – Benniseed, Heat treatment, Layers, hatchability, aqueous and bambara groundnut

INTRODUCTION

Problems facing the production of animal protein in sub Saharan Africa are very numerous and depending on so many factors including locations and environment. Disease like trypanosomiasis limits the large scale production of cattle, sheep and goat to the drier Sudan and the Sahel savanna zones, where uncertain water supplies interface with the growth and productivity of these animals. The Guinea savanna with adequate rainfall and good pasture growth is infested with Tsetse flies that is, a vector for trypanosomiasis. Although seasonal migration to guinea savanna by the nomadic cattle men occurs, the stress of the long trek by the animal results in loss of body weight indeed. Future projecting of animal protein supply indicates a shortfall of about 500,000 tonnes of meat by 2030 and suggestions have been made that efforts be increased to ensure increased production of meat and meat products from other kind of livestock. According to Damon *et al.* (2019), this has led to the intensification of poultry production with emphasis on the production of chicken for protein. However feed has been a major limiting factor affecting many farmers. Cereal are needed as food by humans and for industrial uses and soybean which is the major source of Nitrogen (protein) for the birds is in limited supply due to the dwindling local production and lack of foreign exchange to support importation from abroad. To this extent, there is need for investigation of alternatives for soya bean. The critical situation of animal feeds and feeding has called for more judicious and economic use of available materials. This has necessitated pressure on some unconventional sources of plant proteins such as under-utilized indigenous legumes which abounds in Nigeria.

It is important to know that the inclusion of unprocessed plant proteins in diets of growing animals leads to a significant impairment of growth and other undesirable physiological and biochemical

alternation, which have been attributed to the occurrence of toxic and ant nutritional factors (ANFS). According to Longstafferal (2017), the intake of poisonous fecal can impede the synthesis of egg yolk and albumen thereby reducing the chances of hatchability of fertile eggs in laying hens. Many efforts have been made to detoxify ANFS through the applications of heat and other processes that only a few permanent breakthroughs have been made. There are at present no precise figures relating to safe levels of residual ANFs in processed plant seeds, although complete removal or inactivation often results in better feed utilization (Slager *et al.,* 2017). This is considered to be of particular importance if they are present at levels high enough to produce negative effects in the consuming animals. The nutritional significance of residual ANFS in plant seeds need to be properly assessed in view of their various practical applications in animal feeds. This study was therefore designed to investigate the effects of dry and aqueous heat treatment on ANFS in Bambara groundnut and Benniseed in Potiskum North eastern Nigeria.

MATERIALS AND METHODS

Dry seeds of Bambara groundnut (*Voandzela substerranean*) and benniseed (*Sessamum indicum* L.) were obtained from local market in Potiskum, Yobe State, North Eastern Nigeria. Potiskum falls within the Sudan Savanna Zone of Nigeria with mean rainfall of about 800 mm per annum and temperature of $39 – 42^0$C. It is located between latitude $11^0$42' N to $11^0$43' N and longitude $11^0$04' E to $11^0$06' E. The two vegetation zones in Yobe State are Sahel in the North and the Sudan Savanna in the Southern part of the state where Potiskum is located.

Sample Preparation

Aqueous heating: The seeds were soaked in water for two (2) hours at room temperature and heated in fresh water for one hour at 100 ^{O}C. The seeds were then sun-dried, stored in plastic bottles and labeled as aqueous heated bambara groundnut (AHBG) and aqueous heated Benniseed (AHBS) respectively.

Dry Heating

Dry Bambara groundnut and Benniseed were heated in the over at 120 ^{O}C for 15-20 mins until they changed colour and became crispy to touch. The toasted seeds were air-dried, stored in plastic bottles and labelled dry – heated Bambara groundnut (DHBG) and Dry-heated Benniseed (DHBS) respectively.

Experimental Diets

The experimental diets comprised of five breeder rations formulated on least – cost basis, mainly from maize – soyabean meal (SBM) basal diet (Table 1). The aqueous and dry – heated Bambara groundnut and Benniseed were included in the basal diet at the expense of SBM – on this basis, some adjustment were made in the level of maize, fish meal, palm kernel meal and wheat bran in order to make the diets Isonitrogenous (one source of protein). Each diet was supplemented with meth wrine, lysine to ensure that amino acid were not limiting for egg production.

Laying Hen Management

A total of 120 breeder birds, comprising one hundred and five (105) laying hens of 52 weeks old and 15 cocks of 48 weeks old were randomly distributed into five dietary groups at the rate of 21 hens and 3 cocks per groups. Each group was further divided into 7 hens to 1 cock per replicate group. The birds were allowed to run together in each replicate group in order to allow for free pen – mating for 8 weeks feed and water were supplied ad-libitum and uniform light was provided 24 hours daily.

Incubation and Hatching of Eggs

Eggs were collected from each pen daily and weighed. The eggs collected at the end of each week were incubated in an incubator for a period of 21 days. Fertility test was carried out on the fifth day of incubation using the candling method. Non fertile eggs were discarded and the eggs were again tested for the presence of living or dead embryos on the 18^{th} day of incubation. Eggs with living embryos were subsequently transferred into the hatchery unit where chicks were hatched on the 21^{st} day.

Chemical Analysis

Proximate composition of the raw and processed seeds were analysed by AOAC (2006) method. Concentration of lectins and trypsin inhibitors were determined using the haemagglutination assay (valde-bouze et al, 2019). Tannin, oxalate and phytate content were determined by method described by Hofford singleton 1980, wheeler and Ferrel (1983) and Apata (1996) respectively.

Statistical Analysis

The results were subjected to analysis of variance. Means with significant differences were compared using Duncan's multiple range test (Steel and Torrie). Regression analysis was carried out to assess the relationship between anti nutritional factors and the response induces.

Table 1. Gross and Chemical Composition of Experimental Diets (g/kg DM)

Ingredients	Control	AHGB	DHBG	AHBS	DHBS
Maize	550.0	395.0	400.0	412.7	416.1
Soyabean meal	175.5	-	-	-	-
Bambara G/nut	-	323.6	320.6	-	-
Benniseed	-	-	-	299.3	296.5
Fish meal	25.0	40.0	40.0	40.0	40.0
Palm kernel meal	76.0	79.0	78.0	84.5	85.4
Wheat bran	58.5	47.4	46.0	46.5	45.0
Oyestex shell	30.0	30.0	30.0	30.0	30.0
Bone meal	75.0	75.0	75.0	75.0	75.0
Methionine	3.0	3.0	3.0	3.0	3.0
Lysine	2.0	2.0	2.0	4.0	4.0
Premix	3.0	3.0	3.0	3.0	3.0
Salt	2.0	2.0	2.0	2.0	2.0
Determined chemical composition					
Crude protein (%DM)	16.41	16.38	16.44	16.34	16.29
Melabolesable energy (MJ/kg)	11.80	11.00	12.10	11.60	11.00

DHGB – Dry heated Bambara nut, AHBS = Aqueous heated Benniseeds, AHBG= Aqueous heated Bambara nut and DHBS = Dry heated Benniseed.

RESULTS

The chemical composition of the dry and heated Bambara nuts and Benniseed are shown in table 2 and 3. Both seeds showed similarities in the dry matter, crude proteins, crude fibre, and ash content but either extract was considerably different, i.e. higher in Benniseed than in Bambara nut. Haemagglutinnin and trypsin inhibitor activities in both seeds were destroyed in aqueous heating while dry heating eliminated haemagglutinin in Benniseed but retained some amount of trypsin inhibitor activity in Bambara nut. DHBG. Tannin, oxalate and phytate were stable to heat and were only marginally reduced by aqueous and dry – heating of the plant seeds.

Table 2 – Proximate composition of Raw and Heat treated Bambara nut and Benniseed %

	RGB	AHBG	DHBG	RBS	AHBS	DHBS
Dry matter	96.66	93.41	94.78	97.10	92.60	93.37
Crude protein	21.87	20.77	22.32	22.12	21.84	22.71
Crude fibre	5.65	5.40	5.60	6.95	6.77	6.88
Ether extract	6.67	6.43	6.64	52.75	50.48	51.66
Ash	5.50	5.48	5.47	5.32	5.27	5.30
Total Carbohydrate	61.31	58.59	59.15	10.86	14.64	12.55

RBG= Raw Bambara Groundnut, AHGB = Aqueous – Heated Bambara Groundnut, DHBG= Dry-Heated Bambara Groundnut. RBS = Raw Benniseed, AHBS = Aqueous – Heated Benniseed; DHBS = Dry – Heated Benniseeds.

Table 3: Anti-nutritional factors in raw and processed Bambara and benniseed seeds

	Haemaglutinin Hu/mg Protein	Trypsin inhibitor (Tiu/mg protein)^2	Tannin (g/100g DM)	Oxalate (g/100g DM)	Phytate (g/100g DM)
RBG	5.00	9.41	0.38	0.21	0.31
AHBG	-	-	0.24	0.08	0.22
DHBG	-	0.41	0.35	0.16	0.29
RBS	1.27	-	2.36	2.27	5.18
AHBS	-	-	1.71	1.56	3.79
DHBS	-	-	1.74	2.04	4.70

Hu = Haemagglutintinin unit
Tiu = Trypsin inhibitor units.

The performance of birds fed on diets containing Bambara ~~bamabara~~ nut benniseed are shown in table 4. Feed intake was not significant different in all groups but the least intake was obtained in birds fed dry-heated bambara nuts. All diets with the exception of aqueous-heated bambara nut (AHBG) significantly ($P<0.05$) reduce egg weights compared with the control. Hen-day production of birds on dry – heated bambara nut (DHBG), AHBS and DHBS were not significantly ($P>0.05$) lower than birds on AHBG and the control diet which were also statistically similar. Feed conversion ratio ranged between 2.06 and 2.27 for birds on the control and DHBS diets respectively. Regression analysis (Table 5) showed that egg weight and Hen-day production correlated significantly ($P<0.05$) with Tannin and oxalate.

Table 4: Performance characteristics at breeder Hen fed processed bambara and benniseed – based diets

Parameters	Control	AHBG	DHBG	AHBS	DHBS	MSG
Feed intake g/day	125.61	124.42	121.90	124.71	123.45	124.01
Egg weight g/day	60.90^a	60.03^a	57.81^b	54.70^c	54.41^c	57.57
Hen-day product %	72.11^a	68.00^b	56.10^c	55.10^c	53.10^c	60.94
Feed conversion ratio	2.06	2.07	2.11	2.17	2.27	2.14

abc = Means with different superscript across the rows are significantly different ($P<0.05$)
AHBG

Table 5: Regression Analysis of performance of breeder birds fed processed bambara and benniseed based diets

Parameters	Anti-nutritional factor	Regression regulation	r	SEb	LsL
Egg weight	Tannin	60.25-0.257x	-8.93	0.003	*
	Oxalate	59.91-0.51x	-0.89	0.066	*
Hen day production	tannin	69.25-0.619x	-0.82	0.24	*
	Oxlata	66.11-0.941x	-0.70	0.007	*

x = inclusion rate of ANFs (g/kg)
r = coefficient of linearity
SEb = Standard error of b
LSL = Significance of linearity
* = significance at ($p<0.05$)

The biochemical composition of an egg is shown in table 6. Yolk protein was Significantly (P<0.05) elevated in birds fed AHBG (16.28G/dl) but lower (P<0.05) in birds fed DHBG, AHBS and DHBS respectively. Values for albumen – protein ranged between 9.36 – 9.82 g/dl and were not significantly different in all the birds.

Table 6: Biochemical Composition of Eggs

Parameters	Control	AHGB	DHBG	AHBS	DHBS	MSC
Yolk protein (g/dl)	16.58	16.28	15.91	15.33	15.15	15.85
Albumen protein (g/dl)	9.82	9.55	9.47	9.38	9.36	9.52

abc = Means with different superscript across the rows are significantly different (P<0.05) AHBG

Fertility and hatchability of eggs were significantly (P<0.05) reduced and the lowest values were obtained in birds fed AHBS and DHBS – based diets respectively as seen in table 7.

Table 7: Egg fertility and hatchability and weight of Hatched chicks

	Control	AHBG	DHBG	AHBS	DHBS	MSE
Fertility %	65.00	63.30	62.50	57.90	54.41	60.62
Hatchability of fertile eggs (%)	96.25[a]	94.78[a]	88.74[b]	87.18[b]	78.30[c]	89.05
Weight of hatched chicks (g)	35.51[a]	34.16[a]	31.14[b]	30.17[b]	30.11[b]	32.32

abc = Means with different superscript across the rows are significantly different (P<0.05) AHBG

Table 8: Regression Analysis of Egg Fertility Hatchability

	Anti-nutritional factor	Regression equation	R	SEb	LSL
Egg fertility	Tannin Oxalate	62.16-3.79x 61.49-2.01x	-0.70 -0.62	0.026 0.107	* *
Weight of hatched chicks	Tannin Oxalate	34.17-2.11x 33.94-1.10x	-0.75 -0.63	0.011 0.101	* *

x = inclusion rate of ANFs (g/kg)
r = coefficient of linearity
SEb = Standard error of b
LSL = Significance of linearity
* = significance at (p<0.05)

Other groups gave significantly (P<0.05) higher values which ranged between 62.50 and 65.00% for egg fertility and between 88.74 and 96.25% for egg hatchability. The weight of hatched chicks in the group fed AHGG compared with those on the control diets were significantly (P<0.05) reduced in groups fed DHBG, AHBS and DHBS respectively with values ranging between 30.11 and 31.14 g. Regression analysis reveals significant (P<0.05) Correlations between percentage fertility and Tannin (r = -0.70) weight of hatched chicks and tannin (r = -0.75), percentage fertility and oxalate (r = -0.62) and weight of hatched chicks and oxalate (r = -.0.63) respectively.

DISCUSSION

The result obtained in this research show that there were lots of variations in the concentration of ANFS between bambara nut and benniseed. Raw bambara nuts contained high levels of trypsin inhibitor (9.41 Tiu/mg Protein) and haemagglutinin (5.00Hu/mg protein), but exhibited negligible level of tannin, phytate and oxalate, marginal levels of haemagglutinin and none of trypsin inhibitor activity. These results confirm previous findings on different special and varieties of tropical legumes and oil seeds (Liener, 2016, Shehu, 2008).

Crude protein values were slightly increased as a result of dry heating possibly due to burning off of some organic compounds. They however dropped after aqueous heating, possibly as a result of solubilization and removal of some nitrogenous compound during heating in water. Aqueous heating completely eliminated trypsin inhibitor and haemagglutinin activities in Bambara nut and benniseed but dry heating did not have similar effect on trypsin inhibitor activity in bambara nut. The residual trypsin inhibitor (T_1) activity in DHBG must have originated from Boloman – Birk inhibitor, which together with Kunitz inhibitor constitute the main group of trypsin and chymotrypsin inhibitors in

plant seeds (Shehu, 2008). Liener (2016) also reported that Bowmen – Kirk inhibitors have a high proportion of discipline bonds which are resistant to dry heat and account for about 20% activities in plant seeds. Although the bulk of Table 1 activity in bambara nut was heat – labile, the residual activity adversely affected the utilization of DHBG compared to AHBG. Tannin, phytate and oxalate were mildly affected by heat treatment. There ANFS are generally heat-stable and severe processing conditions may be required to inactive them which inadvertently may cause irreversible damage to plant proteins (Louis et al., 2017).

From the results of the feeding trials with breeder birds, it was observed that birds fed with heat-treated bambara nut and benniseed diet showed significant performance than those fed with the control diet. The poor performances of birds fed AHBS and DHBS – based diets may be attributed to the marginal effects of heat on tannin, phytate and oxalate in DHBS and AHBS. Tannin has been linked with negative effect on feed intake, digestive enzymes the pancreas and on the digestibility of proteins, minerals and to a lesser extent carbohydrates (starch and cellulose) (Caldwell, 2016; Jansman and Longstaft, 2018).

The effect of dietary treatments on egg fertility revealed significant improvements in the percentages of fertilized eggs in birds fed AHBG and DHBG while those fed AHBS and DHBS gave significant reduction. The high levels of residual tannin in DHBS and AHBS might have affected the quality of spermatozoa in male fowls thereby decreasing their fertilizing ability. Mohammed et al. (2016) reported that a bereaved in the protein quality of feed decreases sperm concentration, motility and viability in composition of eggs. The significant reduction in egg hatchability suggests that the absorption of nutrients for embryonic growth and development was hindered by the residual ANFS. Oluyemi and Roberts (1979) reported that when egg fall below normal levels; the fertilized egg may not hatch, since the embryo can only develop into a viable chick when the fertilized egg contains the nutrients needed for embryonic growth and development. Birds fed benniseed diets gave a significantly lower percentage of hatchable eggs. Than birds fed bambara nuts and control diets. The concentrations of residual ANFS were higher in AHBS and DHBS than AHBG and DHBG and consistently lower values were obtained for the reproductive induces in the birds. According to Shehu, (2008) protein and other nutrients complexed to tannin adversely affect nutrient utilization for egg embryo development and hatchability in laying hens. This probably explains the significant reduction in the weights of fertile eggs and consequently low weights of hatched chicks obtained with feeding DHBS and AHBS – based diets.

In conclusion, the results presented in this study clearly demonstrate that dry and moist treatment were not effective methods for processing plant protein containing high concentration of non-thermolabile anti-nutrients while trypsin inhibitor and haemagglutinin were easily inactivated by dry and aqueous heating. Tannin, phytate and oxalate were only marginally reduced, the consumption of these feeds by poultry, especially dry and aqueous – heated benniseed diets may not be adequate in practical feeding situation when long – time feeding maybe required, because the birds, may develop metabolic disorders and other undesirable anomalies which may have serious consequences on egg, embryo development, hatchability and progeny growth. To this end, there is the need to investigate other detoxification methods that will be able to inactivate heat – stable ANFS in plant seeds without damaging their protein and other essential nutrients. The physico-chemical characteristics of heat stable ANFS in plant seeds may also have some influences on their thermo stability and their identification may be used to develop processing techniques that will eliminate levels that will not exert adverse effects when plant proteins are consumed by poultry and other livestock.

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