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	<p>per kg weight gain was reduced at the 10% SBH inclusion level as compared to other SBH diets. Dietary treatments did not have adverse effect on the haematology of pigs. Pigs fed the control diet (0% SBH) and those fed soybean hull based diets had comparable performance. It was concluded that soybean hull can be included in the diet of weaner pigs at 20% level without adverse effects on the growth performance and haematological values of the animals.</p> <p><b>Key words:</b> Soybean Hull, Diets, Growth Performance, Weaner Pigs</p>	<p>Watch Online</p>
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# MINERALS PROFILE IN PRE-AND POST FED DESERT SHEEP IN THE SUDAN

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**Abstract:** The objective of this study was to assess the changes in serum minerals profile in desert sheep in Sudan in relation to feed interval; pre feeding (fasting overnight), post feeding (3hrs after feeding). Twenty one yearling unsaturated males of Sudan desert sheep with an average body weight of 31.11kg were used in this study. The serum level of (Cu and Mn) was significantly high ( $P < 0.05$ ) in post feeding than pre feeding, while serum level of (Zn) was high in pre feeding when compared with the post feeding with percentage of changes amounting for (15%). However the serum level of (Na) was significantly ( $P < 0.05$ ) higher during pre feeding than post feeding whereas serum level of (Mg) was higher, while serum level of (K) was lower during pre feeding than post feeding with percentage of changes (8%) and (10%), respectively.

**Key words:** Mineral Profile, Feed Interval, Micro mineral, Macro mineral, Mineral requirements, Desert sheep.

## INTRODUCTION

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life, minerals are chemical constituents used by the body in many ways, although they yield no energy, they have important roles to play in many activities in the body (Malhotra, 1998; Eruvbetine, 2003). Every form of living matter requires these inorganic elements or minerals for their normal life processes (Hays and Swenson, 1985; Ozcan, 2003). The basic functions performed by the minerals are: they are structural components of body tissues, are involved in the maintenance of acid-base balance and in the regulation of body fluids, in transport of gases and in muscle contractions (Malhotra, 1998; Murray et al., 2000).

Animals of similar age, breed and physiological state in a common environment can show marked differences in their efficiency of mineral utilizations. For example, the fractional absorption of copper can vary from 0.042 to 0.112 (Suttle, 1984). Age can affect requirement through changes in absorption efficiency, for example, pre ruminating lambs have an 80% absorption efficiency for copper, whereas lambs with a functional rumen have only 3-5% (Underwood, 1981). The form of mineral in the diet and the presence or absence of synergistic and antagonistic compounds and elements are of prime importance in determining whether or not the sheep meets its mineral requirements, perhaps the best known interaction is between copper, molybdenum and sulfur as reported by researchers, showed that copper functions in the utilization of iron in an early stage of haemopoiesis. Copper deficiency results in an increase in iron in the liver, whereas an excess of copper results in a decrease in iron content of the liver, thus reflecting the role of copper in iron utilization. Copper is present in blood plasma as a copper-carrying plasma protein called erythrocuprin. Erythrocuprin provides a link between copper and iron metabolism and mediates the release of iron from ferritin and haemosiderin (Hays and Swenson, 1985). The dietary requirement of copper is affected by the level of some other minerals in the diet, and is increased in ruminants by excessive molybdenum. Treatment of copper poisoning is based on the rationale that excess molybdenum may cause copper deficiency and molybdenum in conjunction with the sulfate ion has been used in treating copper poisoning in ruminants (Pierson and Aenes, 1958). The Cu requirement varies among animal species to some extent but is influenced to a large degree by its relationship with and the intake of other mineral elements such as iron, molybdenum and sulfate.

Animal fluid levels of minerals, in addition to concentrations of particular enzymes, metabolites or organic compounds with which the minerals in question associated functionally, are also important indicators of minerals status (McDowell, 1987; Puls, 1994; Judson and McFarlane, 1998). As minerals form a crucial part in the nutrition of ruminants and are often the limiting factors in their diets, particularly in tropical regions (McDowell, 1976 and McDowell, 1985a). Minerals concentrations of plasma provide an indication of the complete mineral uptake of

ORIGINAL ARTICLE





grazing animals, also reflecting water, soil and other non-forge sources, with exception of reserves mobilized from bone. Furthermore, this analysis of mineral concentrations can provide an indication of the sub clinical presence of deficiencies (Underwood, 1981) impacting optimum production. Deviations from these normal limits, which are now well defined for most elements, therefore, constitute useful diagnosis indicators. A further valuable aspect of such fluid composition changes is that they frequently arise prior to the appearance of adverse clinical signs (Underwood, 1979 and McDowell, 1987). Certain plasma minerals are greatly in animals fed a severely deficient diet (Miller and Stake, 1974; Sutherland, 1980; McDowell, 1985a and Minson, 1990). Assessment of mineral status on the bases of plasma of grazing animals has been considered an important strategy to increase animal productivity, especially in those countries are commonly found.

Ideally, animal scientist would like to determine the minerals status of an animal by measuring the minerals content of one tissue that is readily a valuable from a live animal. Although unfortunately, no minerals concentrations of all minerals, the blood plasma is considered very useful tissue fluid as indicate the animal status of most the minerals with low concentrations indicative of dietary deficiency or excess. Plasma minerals after absorption immediately reflect the dietary intake, absorption and availability through gastrointestinal tract. Whole blood or blood plasma or serum is widely used for studies in mineral nutrition. Values significantly and consistently above or below " normal " concentrations or range provide suggestive but no conclusive evidence of dietary excess or deficiency of particular mineral.

The aim of the present study was to assess the changes in minerals profile during pre feeding and post feeding and to evaluate mineral requirements and appraise the concentrations of critical micro minerals and macro minerals in addition to define normal limit of these elements copper, Manganese, Zinc, Sodium, Magnesium and Potassium in the serum of desert sheep in Sudan.

## **MATERIALS AND METHODS**

### **Experimental animals**

Twenty one yearling unsaturated males of Sudan desert sheep with an average body weight of 31.11kg were used in this study. The animals were purchased from the local market; they bear the characteristic of the indigenous desert sheep breed. They have large and flabby ears, long tapering tail, and long -legged. The coat color was brown. The animals were housed in an un-shaded sheep's pen; at Halat Kuku in Khartoum north, Sudan. Prior to commencement of the experiment the animals were dewormed with antihelmintic (Ivermectin 0.5 ml per 25 kg body weight) they were also given long acting Oxtetracycline at a dose rate of 1 ml per 10 kg body weight, the animals were then allowed to adapt for approximately three weeks, the duration of the intervention was one week.

### **Feeding rations**

The animals were fed according to relevant standards for the group. The rations were offered ad libitum through out the experimental period. The ingredients for all diets were mainly grounded Bagasse 35%, Groundnut Hay 20%, molasses 20%, ground nut cakes 10%, wheat bran 10%, sorghum Hay 9% and salt 1%. The chemical composition of ingredients were calculated according to the Nutrient composition of Sudanese animals feeds Bulletin (3), 1999: Dry matter 89.82%, Crude Protein 6.66%, Fiber 28.27, Ash 7.11%, Nitrogen Free Extractive (NFE) 45.23% and Metabolisable Energy (ME) 0.74%. The Minerals contents of ingredients are derived from FAO's Animal Feed Resources Information System (1991-2002) and from Bo Gohl's Tropical Feeds (1976-1982) are shown in table (3).

### **Blood samples**

Daily Blood samples of 5.0 ml were collected from the jugular vein from each animal into plain vacuoners one sample in the morning (pre-feeding samples) the other was collected after 3hour (post- feeding samples) the blood was then allowed to clot, then centrifuged at 3000rpm for five minutes and the serum was removed and stored at - 20 °C into sealed plastic containers until analyzed.

### **Biochemical analysis**

**Determination of serum (Na<sup>+</sup>) and (K<sup>+</sup>):** Serum sodium and potassium were determined by flame photometer (Jenway PFP, England) as described by Varly (1967).

**Determination of serum Mg<sup>+2</sup>, Cu<sup>+2</sup>, Mn<sup>+2</sup>, and Zn<sup>+2</sup>:** These elements were determined using the atomic absorption spectrophotometer model (Unicam-929, England) at the department of biochemistry, central veterinary research laboratories.

**Statistical analysis:** Data were analyzed with the SPSS 10.0 statistical package program (SPSS Inc, Chicago, Illinois USA). Student test (T-test) was performed for the statistical analysis of biochemical results. Statistical significance was considered when P<0.05.

## **RESULTS**

Effects of deficiency and accesses of minerals, their critical or average values in different samples, requirements and tolerance for minerals in dietary components and their concentrations in pre-Feeding and post-feeding taken from the animals are presented in Tables; 1 to 6 and Figures; 1 and 2.



### Serum micro minerals

Table 4 and Figure 1 shows the status of serum micro minerals of (Cu, Mn and Zn) concentrations of the desert sheep the Pre- feeding and post- feeding. Serum (Cu) mean was (1.41 ± 0.05) mg/l and Serum (Mn) mean (0.30 ± 0.04) mg/l however, the level in post feeding were significantly (P<0.05) higher than pre feeding, (0.98 ± 0.04) mg/l, (0.30 ± 0.04) mg/l; for Cu and Mn respectively. Whereas the Serum (Zn) was lower in post feeding (1.32 ± 0.11) mg/l than pre feeding (1.12 ± 0.08) mg/l, the difference between them is amounted for (-15%). However, the mean (±Std) for the overall serum (Zn) was (1.22 ± 0.07) mg/l and (min - median - max) values were (0.6 - 1.2 - 2.2) mg/l, respectively.

### Serum macro minerals

The status on serum macro minerals (Na, Mg and K) concentrations of the desert sheep pre feeding and post feeding are shown in table 5 and figure 2. Mineral (Na) decreased significantly (P< 0.05) in post feeding than pre feeding. Mean level of (Mg) in pre feeding was (19.86 ± 2.41) mg/l and in post feeding was (18.23 ± 1.51) mg/l with percentage of change (-8%), while serum (K) increased in post feeding (141.7 ± 7.2) mg/l than pre feeding (128.5 ± 9.3) mg/l with a difference amounting for (10%).

**Table 1 - Minerals included in study for sheep, with their functions and effects of deficiency or toxicity**

Elements	Function	Deficiency	Toxicity
<b>Copper</b>	Many enzyme system, haemoglobin formation, cartilage/bone formation	Poor or faded hair, reduced growth, lameness	Anorexia, jaundice, abdominal pain, haemolytic crisis
<b>Manganese</b>	Growth, skeleton reproduction	Impaired reproduction, skeletal abnormalities, abortion, reduced growth	Disruption of rumen flora, reduced growth, anaemia
<b>Zinc</b>	Epidermal tissue, skeletal formation wound healing	Poor reproduction, rough skin, poor immune function, reduced intake and growth	Uncommon: anaemia, reduced bone formation, reduced weight gain
<b>Sodium</b>	Electrolyte, nerve impulse transmission	Common in grazing cattle, depressed appetite	Diarrhoea, anorexia thirst, salivation abdominal pain, convulsions, muscular spasms
<b>Magnesium</b>	Energy, fat and protein metabolism	Loss appetite, reduced gain, hyper excitability, "grass tetany" in coordination, convulsions	Reduced intake, diarrhoea
<b>Potassium</b>	Electrolyte, nerve impulse transmission	Rapid decline in feed and water intake, loss of vigour, pica	Unlikely to occur, cardiac problems, oedema

Sources: National Research Council, (1980). Mineral Tolerance of Domestic Animals, Washington, D.C: National Academy of Sciences

**Table 2 - Minerals requirements and tolerances for sheep**

Mineral mg/Kg DM	Minimum concentrations	Maximum tolerable concentration
<b>Cu<sup>2+</sup></b>	5	25
<b>Mn<sup>2+</sup></b>	15 - 25	1000
<b>Zn<sup>2+</sup></b>	20 - 30	300
<b>Na<sup>+1</sup></b>	700 - 900	35000
<b>Mg<sup>2+</sup></b>	1200	5000
<b>K<sup>+1</sup></b>	5000	30000

Where range is given, the lower value is for maintenance and higher value is for growing animals. (NRC, 1985; Reuter and Robinson, 1997)

**Table 3 - Minerals contents of ingredients in study for sheep**

Ingredients	Minerals					
	Cu mg/kg DM	Mn mg/kg DM	Zn mg/kg DM	Na g/kg DM	Mg g/kg DM	K g/kg DM
<b>Wheat bran</b>	14	113	89	0.1	4.6	13.7
<b>Baggasse</b>	12	-	103	0.1	0.8	1.3
<b>Molasses</b>	5	152	36	0.3	2.2	14.1
<b>Sorghum hay</b>	-	-	-	-	2.2	30.6
<b>Groundnut hay</b>	-	-	-	-	-	-
<b>Groundnut cake</b>	6	127	25	0.4	6.1	11.2
<b>Total</b>	37	392	253	0.9	15.9	70.9

The contents of this table are currently derived from FAO's Animal Feed Resources Information System (1991-2002) and from Bo Gohl's Tropical Feeds (1976-1982); Last updated on 24/10/2012; From (<http://www.feedipedia.org/content/feeds>).



**Table 4 - Pre feeding and post feeding status on serum micro minerals (Cu, Mn and Zn) concentrations of the desert sheep**

Statistics										
Elements	Status	N	Mean ± Std	SD	Min	Max	Med.	CV	Change	Average value*
Cu Mg/L	Pre feeding	21	0.98 ± 0.04 <sup>a</sup>	0.14	0.65	1.17	1.01	15%	44%	>0.65
	Post feeding	21	1.41 ± 0.05 <sup>b</sup>	0.16	1.13	1.67	1.44	11%		
	Overall	42	1.20 ± 0.05	0.26	0.65	1.67	1.15	22%		
Mn Mg/L	Pre feeding	21	0.14 ± 0.02 <sup>a</sup>	0.07	0.06	0.30	0.15	47%	113%	>0.015 – 0.5
	Post feeding	21	0.30 ± 0.04 <sup>b</sup>	0.12	0.17	0.51	0.23	42%		
	Overall	42	0.22 ± 0.03	0.13	0.06	0.51	0.18	58%		
Zn Mg/L	Pre feeding	21	1.32 ± 0.11	0.38	0.60	2.20	1.28	29%	-15%	>0.6
	Post feeding	21	1.12 ± 0.08	0.26	0.75	1.50	1.08	23%		
	Overall	42	1.22 ± 0.07	0.34	0.60	2.20	1.20	28%		

N: number, Std: Standard error mean, SD: Standard deviation, Min: Minimum value, Max: Maximum value, Med.: Median, CV: coefficient of variance and Aver.: Average. <sup>a, b</sup> Means with different superscripts in the same column are significantly different at (P <0.05). \*Minerals concentrations in plasma are higher than the above given values. Pamela et al., 2001).

**Table 5: Pre feeding and post feeding status on serum macro minerals (Na, Mg and K) concentrations of the desert sheep**

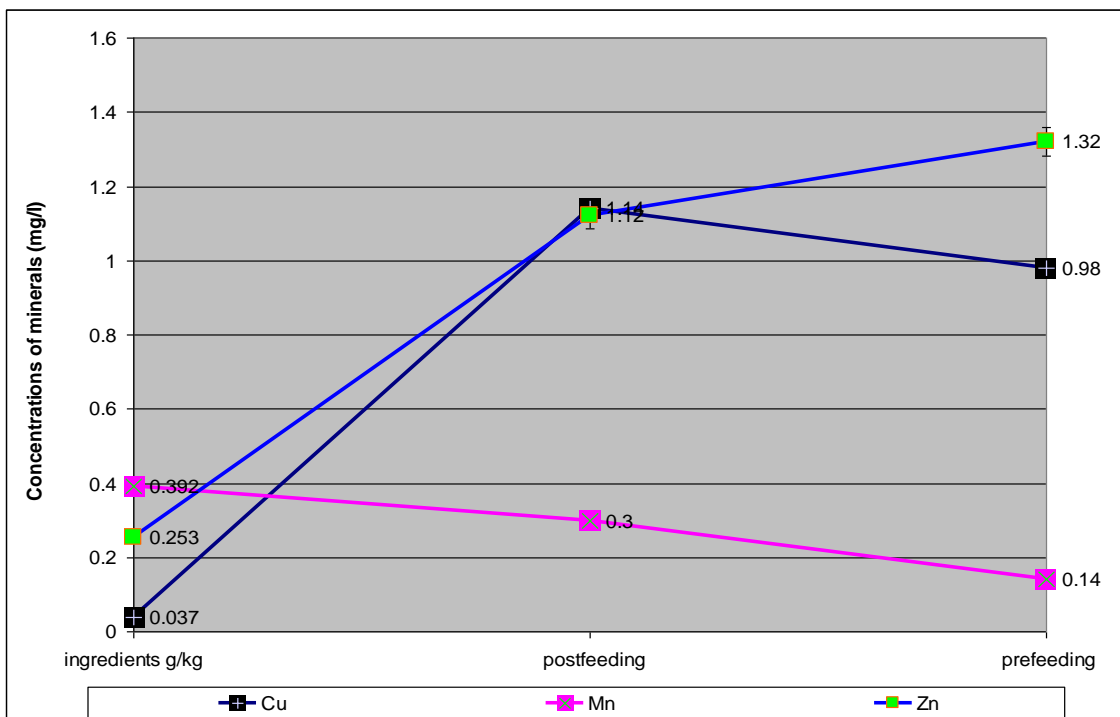
Statistics										
Elements	Status	N	Mean ± Std	SD	Min	Max	Med.	CV	Change	Aver. value*
Na Mg/L	Pre feeding	21	1069.3 ± 129.6 <sup>a</sup>	448.9	535	1860	930	42%	-47%	>3000
	Post feeding	21	571.3 ± 15.9 <sup>b</sup>	55.1	470	680	555	10%		
	Overall	42	820.3 ± 82.3	403.2	470	1860	625	49%		
Mg Mg/L	Pre feeding	21	19.86 ± 2.41	8.29	8.51	36.46	18.84	42%	-8%	>20
	Post feeding	21	18.23 ± 1.51	5.18	9.72	25.52	18.84	28%		
	Overall	41	19.03 ± 1.39	6.81	8.51	36.46	18.84	36%		
K Mg/L	Pre feeding	21	128.5 ± 9.3	32.1	80	190	133	25%	10%	>200
	Post feeding	21	141.7 ± 7.2	24.8	105	190	137.5	18%		
	Overall	42	135.1 ± 5.9	28.8	80	190	135.5	21%		

N: number, Std: Standard error mean, SD: Standard deviation, Min: Minimum value, Max: Maximum value, Med.: Median, CV: coefficient of variance and Aver.: Average. <sup>a, b</sup> Means with different superscripts in the same column are significantly different at (P <0.05). \*Minerals concentrations in plasma are higher than the above given values (Pamela et al., 2001).

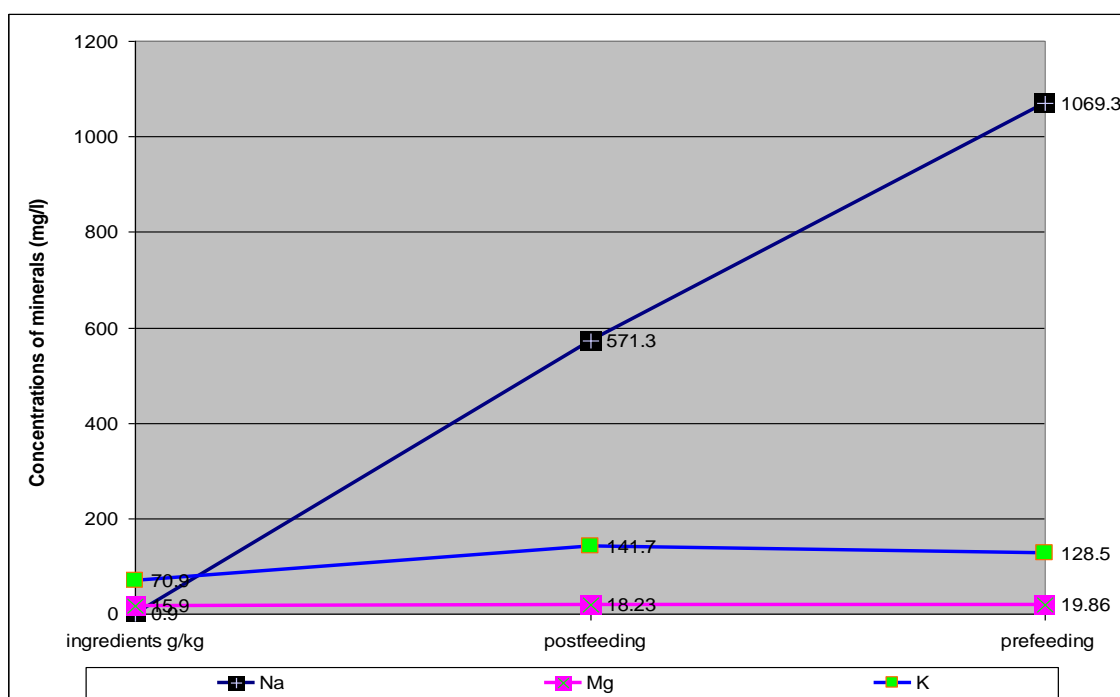
**Table 6 - Minerals contents of ingredients and mineral requirements of desert sheep in current study**

Ingredients	Cu	Mn	Zn	Na	Mg	K
Wheat bran	↓	↓	↓	↓	↓	↓
Baggasse	↓	-	↓	↓	↓	↓
Molasses	↓	↓	↓	↓	↓	↓
Sorghum hay	-	-	-	-	↓	↑
Groundnut cake	↓	↓	↓	↓	↑	↓
Total	↑	↓	↓	↓	↑	↑

↓: lower than requirements.  
↑: maximum than requirements.



**Figure 1 - Mean micro minerals (Cu, Mn and Zn) concentrations in ingredients, post feeding and pre feeding of desert sheep**



**Figure 1 - Mean macro minerals (Na, Mg and K) concentrations in ingredients, post feeding and pre feeding of desert sheep.**

## DISCUSSION

In this study, the serum mineral profile of desert sheep was investigated systematically during normal cycling, pre feeding (fasting overnight) and post feeding (3hrs after feeding). The analysis of the minerals content in the feed offered to the animals revealed that it extend the recommended values of NRC standards for sheep however, The extended improvement of the plan of nutrition of supplemented sheep and the monitoring of physiological responses provided a detail account, which facilitated critical evaluation of the changes that occurred in mineral profile in desert sheep. The results indicate that pre feeding and post feeding were associated with changes in the profiles of macro and micro minerals in desert sheep.

The level of micro minerals (Cu, Mn and Zn) concentrations in the blood serum of the desert sheep in this study were within the limits of the normal values (NRC standards, 1985; Pamela et al., 2001; Simon and Gasmir, 2001), but the level of macro minerals (Na, Mg and K) concentrations were slightly lower than the above normal



values, and had not shown any important deviation during the changes of the physiological states of the desert sheep, which is its characteristic.

### **Serum micro minerals**

The status on serum micro minerals (Cu, Mn and Zn) concentrations in the desert sheep Pre feeding and post feeding shown in Table 4 and Figure 1. Revealed significant ( $P < 0.05$ ) increase in serum (Cu) and (Mn) level during post feeding compared to the values measured during pre feeding in desert sheep this could be related to the major way of homeostatic control of trace elements for certain essential divalent cations is modification of the percentage of intestinal absorption in response to physiological need and dietary intake (Miller, 1973). According to Underwood, 1981; who showed variation of absorption efficiency for copper in pre ruminating lambs and lambs with a functional rumen. In this study the higher ( $P < 0.05$ ) concentrations of (Mn) during post feeding could be to the migration of (Mn) to the other tissues (red blood cells, liver, bones, kidney etc), furthermore tag along to decline pre feeding. In support of our finding, Hidioglou et al. (1978) reported that cows fed diets with 8 ppm Mn had 130 ng of Mn/mL of whole blood, compared to 210 ng of Mn/mL of whole blood in cows supplemented with 60 ppm Mn; hence the concentrations of (Mn) moved from plasma to the red blood cells and have been used to assess status. Similar pronouncement of some researchers who reported that some tissues remove (Mn) from plasma to liver, heart and bones (Bentley and Phillips, 1951; Masters et al., 1988). However, the serum (Zn) level during post feeding was lower (-15%) than pre feeding which could be attributed to the absorption from the gut (Kirchgesner, 1976; Suttle, 1988); the concentrations of Zn in plasma fluctuate with age, stress, infections, and feed restriction (Wegner et al., 1973; Kincaid and Hodgson, 1989; Wellinghausen and Rink, 1998; Kincaid, 1999). Additionally, Davies, 1984 indicated that (Zn) is bound primarily to albumin; the changes in albumin concentration may have a significant effect on (Zn) level. The author added that the level of circulating (Zn) reflects both serum albumin level and the affinity of albumin for (Zn).

### **Serum macro minerals**

The status on serum macro minerals (Na, Mg and K) concentrations of the desert sheep Pre feeding and post feeding represented in Table 5 and Figure 2. revealed that the serum (Na) concentration increased significantly ( $p < 0.05$ ) in pre feeding than post feeding state, while serum (k) was lower during pre feeding than post feeding (128.5 mg/L to 141.7 mg/L) with percentage difference of (10%), this is in the main line of a high level of potassium appears to increase the requirement for sodium and vice versa (Merck, 1986). The fluctuation of serum (Na) in pre feeding and post feeding and interrelationships with (k) could be due to the absorption and excretion from body tissues to circulate through the body to organize osmotic pressure or losses in perspiration and stress conditions; similar finding was reported by (Hays and Swenson, 1985). The mean value of serum (Mg) during pre feeding was (19.86 mg/L) higher than that of post feeding (18.23 mg/L) with percentage difference amounting for (-8%), could be due to the mechanism reaction of the magnesium through the blood to cells and tissues, as reported by (Murray et al. (2000) showed that Mg is an essential activator for the phosphate-transferring enzymes myokinase, diphosphopyridinenucleotide kinase, and creatine kinase. It also activates pyruvic acid carboxylase, pyruvic acid oxidase, and the condensing enzyme for the reactions in the citric acid cycle. It is also a constituent of bones, teeth, enzyme cofactor, (kinases, etc).

### **Minerals of ingredients and requirements in desert sheep**

As shown in table 6, the contents of minerals in ingredients fairly changed among species which had been analyzed from FAO's Animal Feed Resources Information System (1991-2002), and this could be mainly due to the difference in mineral contents of the soil on which the herbage were grown, concerning the minerals contents of ingredients, it will better to discuss and/or compare the contents of minerals in the ingredients with the amounts required generally in the feed (such as feeding standard). The amounts (or extent) of some minerals required in the feed for sheep (NRC, 1985) were extracted in table 2. The magnesium content of groundnut cake and potassium content of Sorghum hay were quite high as compared with that of standard, and this could be due to the characteristic of sugar cane plant and legume plant accepted generally (Cullison, 1979), and this may explain the increase of these minerals in this study. However, the obtained copper values listed in table 3 are obviously higher than that required for feeding of sheep, although the values of (Mg, K and Cu) in the ingredients were higher than the required for sheep feeding recommended by (NRC, 1985), it is worth to mention that the sheep were appeared healthy during the period of the study (1 month), this could be due to the sufficient amount of minerals offered in the ration and also the absorption and excretion through the body tissues and interrelationships among the minerals and minerals metabolic (Pierson and Aenes, 1958; Hays and Swenson, 1985).

### **Implications**

In general, the requirement of minerals for animals nutrition will differ from that of other major nutrients, such as protein or carbohydrates, which could be needed daily in relatively large amounts at a time, than they will be needed to constantly maintain a normal condition in the physiology of the animal. Therefore, some minerals will be excreted, in principles, into the urine or into the gut. when Mg, K and Cu were absorbed in an excess amount (greater than the upper limits), as shown in table 6, then the toxic symptoms seems to appear for all the minerals (McDowell, 1985b). Also there are also many metabolic and absorption interrelationships among the mineral elements which contribute to variations in the degree of physiological responses to deficient or toxic levels. These relationships make



it difficult to determine the optimum dietary level for the individual elements required for domestic animals. As a result of this, the recommended dietary level of any element should rarely be considered independent of the level of other essential nutrients (Hays and Swenson, 1985). The functions of minerals in animals are interrelated; therefore, there is a certain limit to the use of plasma levels of some minerals as an index for checking the conditions for minerals nutrition (Gibbons et al., 1976; Kincaid, 1999). To offer a more pertinent criterion for judging the nutritional status of mineral in desert sheep of the study, it will be better to discuss the mineral contents of main organs (liver, kidney etc) together with the concentrations of minerals in blood plasma.

## CONCLUSION

The study indicates that the mineral profile in desert sheep is affected by physiological states including feed interval. The pattern changes were influenced by dietary minerals content. The results were obtained pre feeding (fasting overnight) and post feeding (3hrs after feeding). Serum level of (Cu and Mn) increased significantly post feeding than pre feeding, while Serum level of (Zn) was higher pre feeding when compare with the post feeding with percentage of changes (15%). Serum level of (Na) was significantly higher during pre feeding than post feeding. Serum level of (Mg) was higher, while Serum level of (K) was lower during pre feeding than post feeding with percentage of changes (8%) and (10%), respectively. Also critical investigations should provide information regarding to actual mineral requirements of sheep so that appropriate nutritional strategies can be managed.

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# PROTEIN FRACTIONATION AND *IN VITRO* DIGESTIBILITY OF AZOLLA IN RUMINANTS

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**ABSTRACT:** A study was undertaken to evaluate the nutritive value and digestibility of Azolla in ruminants by *in vitro* techniques. The crude protein, crude fibre and ether extract contents were at a level of 21.37%, 12.5% and 2.3%, respectively. The neutral and acid detergent fibre levels were about 35.4 and 23.9%, respectively. The average *in vitro* dry matter digestibility, *in vitro* organic matter digestibility and metabolizable energy contents were 79.5%, 63.8 mg/200mg and 7.36 MJ/kg DM (1759 kcal/kg), respectively. The various protein fractions A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and C estimated by Cornell net crude protein solubility system were 18.22, 42.56, 15.15, 7.47 and 16.61% of total protein, respectively. The Azolla contained significantly higher B<sub>1</sub> fraction followed by A, B<sub>2</sub> and C and lowest fraction of C. Thus in view of above, present study indicated Azolla to be a good source protein supplement with 21.37% crude protein with highest B protein fractions, moderate source of energy (1759 kcal ME/kg), high dry matter and organic matter digestibilities and rich in trace minerals thus could be used as an alternate protein supplement or as supplementary protein supplement to ruminants.

**Key words:** Azolla, Digestibilities, *In Vitro*, Protein Fractions, Proximates

## INTRODUCTION

In India, the cost of conventionally used protein supplements in livestock diets like ground nut cake and soya bean have more than doubled over the past few years due to their demand, export policy coupled with low production and more cultivation of other cash crops. The high cost of feed is largely due to the exorbitant price and scarcity of conventional feed ingredients. Thus, depending on groundnut cake and soybean meal as the sole source of protein in livestock diet is gradually becoming economically impracticable in India. Since the cost of feeding is a significant factor dictating the economic viability of livestock industry, it must be reduced by adopting new measures in the ration formulation. Hence to make livestock production as a lucrative enterprise, there is a great need to use alternate feedstuffs replacing the traditional sources. Azolla is a free-floating water fern that floats in water containing 28% crude protein and has a potential to be used as a protein supplement in ruminants (Ahirwar and Leela, 2012). Azolla fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*, making it an excellent source of protein for livestock. The present study was undertaken to evaluate Azolla (*Azolla pinnata*) as protein supplement in terms of chemical composition and nutritive value by *in vitro* techniques. Moreover, the data regarding nutritive value, protein fractionations and digestibility of Azolla appears to be scanty. Hence an attempt was made by applying different *in vitro* techniques to explore Azolla as an alternate protein supplement for livestock.

## MATERIALS AND METHODS

The samples of Azolla (*Azolla pinnata*), harvested on 10-15 days of cultivation were procured from different localities in and around the Hyderabad, Andhra Pradesh. The fresh samples of Azolla were collected in two sets, one set for dry matter (DM) estimation and other for sun drying. The dried samples were ground separately to get 1 mm size. Later the ground samples were mixed to get homogeneous sample before subjecting to analysis.

The mixed samples were analyzed in triplicate for proximate principles (AOAC, 1997) and fibre fractions analysis (Van Soest et al., 1991). The calcium (Ca) and phosphorus (P) contents were estimated as per Talapatra method (Talapatra et al., 1940), while the trace minerals (Cu, Fe, Zn and Mn) were estimated using atomic absorption spectrometry (Arenza et al., 1977).

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The samples were screened for *in vitro* DM digestibility (IVDMD) (Goering and Van Soest 1970) and *in vitro* gas production techniques (Menke et al., 1979) using buffalo rumen liquor. The *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) was estimated as per the formulas suggested by Krishnamoorthy et al. (2005) and Menke and Steingass (1988), respectively.

$$\text{IVOMD (mg)} = \text{Gv} \times 2.2$$

$$\text{ME (MJ/kg DM)} = 2.2 + 0.136 \times \text{Gv} + 0.0057 \times \text{CPDM/kg}$$

Where, Gv = Net gas volume at 24 hours incubation (ml/200 mg DM)

CPDM = Crude protein on dry matter basis

The protein fractionation was done according CNCP system (Licitra et al., 1996), where in the protein was sub divided in to 5 divisions (Fraction A, B1, B2, B3 and C) according to their degradabilities and passage rate in gastro intestinal tract (Pichard and Van Soest, 1977 and Van Soest, 1994). Fraction A (PA) constitutes non-protein nitrogen (NPN), was separated by using trichloroacetic acid (TCA) according to the method described by Licitra et al (1996). The fraction B of azolla protein considered as true protein (Pichard and Van Soest, 1977) was further divided into 3 parts namely, fraction B1 (rapidly degraded true protein), B2 (intermediately degraded true protein), B3 (slowly degraded true protein) (Van Soest et al., 1981 and Krishnamoorthy et al., 1983). The fraction B1 (PB<sub>1</sub>) was expressed by estimating the true protein soluble in a borate-phosphate buffer at pH 6.7-6.8 (Krishnamoorthy et al., 1982) and the fraction B2 (PB<sub>2</sub>) known as neutral detergent soluble protein, was estimated as the difference between buffer insoluble protein (IP) and protein insoluble in neutral detergent (NDICP), and the latter was expressed by estimating the amount of protein recovered in the neutral detergent residue obtained upon standard fibre fraction analysis (Van Soest et al., 1991).

The fraction C (PC) referred as acid detergent insoluble protein (ADIP), measured by estimating nitrogen in ADF residue. The amount of soluble fibre-bound CP (Fraction B<sub>3</sub>; PB<sub>3</sub>) was calculated as CP in NDF minus ADIP. The data was subjected to one way analysis of variance as per the procedures of Snedecor and Cochran (1980) by using SPSS 17. The differences between the means were tested by significance using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

Azolla contained 8.7% dry matter (DM). The proximate constituents and fibre fractions Azolla is given in Table 1. Azolla contained 21.37% crude protein (CP), 35.40% neutral detergent fibre (NDF) and 23.97% acid detergent fibre (ADF) on dry matter basis. The proximate composition of Azolla obtained in the present study was in similar range to values obtained by Ahirwar and Leela (2012). The concentration of calcium, phosphorus, copper, iron, zinc and manganese in Azolla were 0.58%, 0.44%, 17.15 ppm, 710.65 ppm, 77.30 ppm and 207.87 ppm, respectively, indicating to be a rich source of micro nutrients. The CP was comparable, while crude fibre (CF) content was lower in Azolla in comparison to Lucerne (16-25% CP and 20-30% CF, ICAR, 1998). Thus it indicates that Azolla could be good source of protein having low fibre content compared to legume forages.

The *in vitro* dry matter digestibility, *in vitro* organic matter digestibility and metabolizable energy contents were 79.5%, 63.8 mg/200mg and 7.36 MJ/kg DM (1759 kcal/kg), respectively (Table 2). Several *in vivo* experiments indicated improvement in DM digestibility with replacement of 50 % of ground nut nitrogen in diets of buffalos (Indira et al., 2009) and 30 parts of ground nut in concentrate diet of Nellore Sheep (Ravidra reddy et al., 2011).

**Table 1 - Nutrient composition of Azolla (*Azolla pinnata*)**

Nutrient	% on DMB
Dry matter	8.70±0.49
Crude protein	21.37±0.91
Crude fibre	12.73±0.50
Ether extract	2.36±0.21
Total ash	16.23±0.52
Nitrogen free extract	47.30±1.61
Neutral detergent fibre	35.40±0.64
Acid detergent fibre	23.97±1.01
Cellulose	12.15±1.47
Hemicellulose	11.43±1.47
Lignin	12.57±0.81
Calcium	0.58±0.07
Phosphorus	0.44±0.08
<b>Trace minerals</b>	<b>ppm</b>
Copper	17.15± 0.25
Iron	710.65± 23.31
Zinc	77.30± 2.95
Manganese	207.87± 22.46

Values are average of triplicate



**Table 2 - In vitro dry matter digestibility and gas production**

Attribute	Content
<i>In vitro</i> dry matter digestibility (%)	79.55±0.26
<i>In vitro</i> organic matter digestibility (mg/200mg)	63.8±3.36
Metabolizable energy (MJ/Kg)	7.36±0.21

Values are average of triplicate

**Table 3 - Protein fractions of Azolla analyzed as per CNCPS system**

	a	b <sub>1</sub>	b <sub>2</sub>	b <sub>3</sub>	c	P value
% Protein	18.22 <sup>b</sup> ±4.30	42.56 <sup>a</sup> ±2.54	15.15 <sup>b</sup> ±1.04	7.47 <sup>c</sup> ±0.48	16.61 <sup>b</sup> ±2.32	0.001

Values are average of triplicate

Azolla being evaluated as protein supplement, its protein fractions were evaluated under CNCPS system (Table 3). The fraction A of protein (PA) in Azolla (18.22±4.30 % CP DMB) signifies the instantaneously degradable protein in the ruminant digestive system (amino acids, peptides) i.e. non protein nitrogen (NPN) (Pichard and Van Soest, 1977). Most of the reported concentrations of NPN in grasses and legume forages are having the ranges as fresh material (10-15%), hay (15-25%) and silage (30-65%) (Hughes, 1970; Krishnamoorthy et al., 1982; Xu et al., 1996). This indicated Azolla had rumen degradability similar to grasses and legumes and their hays. The Fraction B<sub>1</sub> (PB<sub>1</sub>) referred as true soluble protein (globulins and some albumins) with Rumen degradation of 200 – 300 %/hr, was found to be 42.56±2.54 % CP and was higher (P<0.001) than other protein fractions. Elizalde et al. (1999) reported 17.1% CP of PB<sub>1</sub> in alfalfa which was much lower than that observed in Azolla. The PB<sub>1</sub> in oil seed cakes ranged from 13.22% to 49.37% (Kamble et al., 2010). The PB<sub>2</sub> with Rumen degradation of 5 – 15 %/hr was found to be 15.15 ±1.04 % CP in Azolla which was higher (P<0.001) than PB<sub>3</sub>. The fractions PB<sub>1</sub> and PB<sub>2</sub> (approx. 58% for Azolla) have 100% Intestinal degradability which signifies the potency of Azolla as a protein supplement. The protein fraction B<sub>3</sub> (PB<sub>3</sub>) having 80% Intestinal degradability in ruminants was 7.47% for Azolla. This finding is in accordance with Krishnamoorthy et al. (1982) who reported that protein supplements contain a small amount of PB<sub>3</sub> which mainly included prolamine proteins such as zein protein in corn (Van Soest et al., 1981). According to Van Soest (1994), metabolizable protein is defined as the amount of true protein or amino acids absorbed in the small intestine and specifically in ruminants, are represented by the amount of amino acids or protein of microbial or dietary origin absorbed from the intestine. In this study, the metabolizable protein in Azolla was approx 84% of CP (PA+PB<sub>1</sub>+PB<sub>2</sub>+PB<sub>3</sub>) which implies the capability of Azolla as a protein supplement.

The fraction C of protein (PC) varied significantly (P<0.001) with other fractions and was found to be 16.61 ±2.32 % CP, which contains protein associated with lignin, tannin-protein complexes, and maillard products that are highly resistant to microbial and mammalian enzymes and does not provide amino acids post ruminally to the ruminants (Krishnamoorthy et al., 1982). So PC is considered as undegradable protein fraction i.e. PC is resistant ruminant degradation and digestion. Sniffen et al. (1992) reported wide variability in PC content in protein supplements i.e 0 to 20%. The present finding falls in the range that reported by Sniffen et al. (1992) for protein supplements.

## CONCLUSION

The study indicated Azolla to be a good source protein supplement with 21.37% crude protein with highest B protein fractions, moderate source of energy (1759 kcal ME/kg), high digestibility of dry matter and organic matter (79.55%) and rich in trace minerals thus could be used as an alternate protein supplement or as supplementary protein supplement to ruminants.

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# ASSESSMENT OF PALATABILITY ATTRIBUTE OF *Gluteus Medius* STEAKS (BEEF TOP SIRLOIN BUTT)

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**ABSTRACT:** Beef top sirloin butts ( $n = 48$ ) were selected on the bases of USDA quality grade (USDA Choice or Select) and USDA yield grade category (yield grades 1 and 2 or 4 and 5) to measure Warner-Bratzler shear force (WBSF) variation within the *gluteus medius* (GM). Eight 2.54-cm-thick steaks were cut from the GM, with 2 steaks removed from the anterior (ANT), middle (MID) and posterior (POST) sections of the GM. One steak cut into 3 equal length steaks designated as lateral (LAT), central (CENT), and medial (MED) portions. The second steak of each pair was subsequently cut from each location pair and cooked to 71°C in an air-impingement oven for WBSF determinations. Cooking losses were not ( $P > 0.05$ ) affected by yield grade or steak location; however, top Choice steaks had lower ( $P < 0.01$ ) cooking loss percentages than Select steaks and cooking losses were the greatest ( $P < 0.05$ ) and least ( $P < 0.05$ ) in the medial and central portions of the GM steaks, respectively. Neither quality grade category (PO.133) nor yield grade category ( $P = 0.485$ ) affected the WBSF values of GM steaks, but the central portion of anterior GM steaks received the lowest ( $P < 0.05$ ) WBSF values, whereas the medial portion of middle steaks received the greatest ( $P < 0.05$ ) WBSF values (steak location  $\times$  within-steak position,  $P < 0.001$ ). This study indicated that central portion of anterior steaks was less tough portion.

**Key words:** USDA Quality & Yield Grades, Instrumental Tenderness, Beef, *Gluteus medius* Steaks

## INTRODUCTION

The meat industry is still trying hard to produce beef in order to satisfy consumers' palatability needs at lower costs. Tenderness, juiciness and flavour are evaluated through palatability, and consumers consider tenderness as the most liked attribute (Huffman et al., 1996). The most valuable attribute of palatability of meat is tenderness, because it is the primary measure of meat quality (Dikeman, 1987). Therefore, the consumers' overall eating experience is determined by tenderness as an important attribute of palatability (Dikeman, 1987). Some other researchers have found that the extent of modification of the muscle structural and associated proteins determines the ultimate tenderness of meat (Hopkins and Taylor, 2002). Furthermore, tenderness had been valued as one of the top 10 concerns by the USA retailers and restaurateurs (Smith et al., 1992). A typical character of tenderness is designated by the substantial difference among muscles, carcasses, cuts of meat and animals (Searls et al., 2005). Research findings by (Reuter et al., 2002) revealed that tenderness in a cut of meat differ within its own borders.

The objective of this study was to assess the interactive effect of USDA quality and yield grades on palatability of beef top sirloin butts.

## MATERIALS AND METHODS

### Top sirloin butt selection and fabrication

Beef top sirloin butts selection was based on USDA quality grade (USDA Choice [modest and moderate degrees of marbling] or USDA Select [slight degree of marbling]) and USDA yield grade category (yield grades 1 and 2 or 4 and 5). Yield grade data were obtained via the facility's video-image analysis, and the plant also supplied the USDA quality grade data for each selected carcass. Individually-identified top sirloin butts ( $n = 48$ ) from left carcass sides were captured during carcass fabrication, vacuum-packaged, and transported under refrigeration to the University of Arkansas Red Meat Abattoir for further processing. Top sirloin butts were allowed to age at 2°C for 14 days from the box date before removal from vacuum-sealed packages. Beginning at the posterior end of the resulting *gluteus medius* (GM), eight 2.54-cm-thick steaks were cut: 1) first and second steaks designated as

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posterior (POST) steaks; 2) third steak cut and discarded; 3) fourth and fifth steaks designated as middle (MID) steaks; 4) sixth steak cut and discarded; and 5) seventh and eighth steaks designated as anterior (ANT) steaks. One steak was randomly chosen from each location pair, individually identified, vacuum-packaged, and frozen at -20 °C for Warner-Bratzler shear force (WBSF) determination.

### Warner-Bratzler shear force analysis

Steaks were allowed to thaw for 16 hours in a 4 °C commercial refrigerator before removal from packages and identified with heat-resistant tags. Thereafter, steaks were weighed and oriented with the medial side to the left side on the belt of a gas-fired, air-impingement oven (Lincoln Impinger; Food Service Products, Inc., Ft. Wayne, IN, USA). The oven was preheated to 165 °C, to produce a desired endpoint temperature of 71 °C, and endpoint temperature of each cooked steak was confirmed at the completion of cooking with a hand-held thermometer (model KM28; Co-mark Instruments Inc., Beaverton, OR, USA). Cooked steaks were allowed to cool to room temperature, weighed, and the difference between the pre-cooked and cooked steak weights was used to calculate cooking loss percentage. Cooked steaks were then wrapped in an oxygen-permeable, PVC film and chilled overnight in a 4 °C commercial refrigerator before 1.27-cm-diameter cores were removed parallel to the muscle fibre orientation from the LAT, CENT and MED areas (6 cores / area) of steak. Each core was sheared once through the center with a WBSF device attached to an Instron Universal Testing Machine (Instron Corp., Canton, MA, USA) equipped with a 981-N load cell and set at a crosshead speed of 250 mm/min. The peak WBSF of the 6 cores/within steak location was averaged before statistical analyses.

### Statistical analyses

The general carcass data were analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC, USA), with quality grade (QG) and yield grade (YG) categories, as well as the QG × YG interaction, included in the model as the fixed effects. The experiment was conducted as a split-split plot design, with QG and YG as the whole plot, steak location within the GM (POST, MID, or ANT) as the sub-plot, and the within steak position (LAT, CENT, and MED) as the sub-sub-plot. Cooked steak data were generated with PROC MIXED, and the fixed effects included in the statistical model included QG, YG, steak location (STK), within-steak position (WSP), whereas the random effects were QG × YG × top sirloin butt, and STK × WSP × top sirloin butt. Least squares means calculated for all main and interactive effects, and when significant (P<0.05) F values were observed, least squares means were statistically separated with pair-wise t-tests PDIFF option).

## RESULTS

### Cooking loss

Even though steaks from top Choice carcasses had lower (P<0.01) cooking loss percentages than steaks from Select carcasses, cooking losses were similar (P>0.53) between steaks of YG 1 and 2 and YG 4 and 5 carcasses (Table 1). Furthermore, the interactions between quality and yield grades were similar in terms of cooking loss percentage. However, the percentage losses for quality grades were significantly different (P< 0.05) (Table 2). The interaction between quality grade and yield grade showed no significant difference in cooking loss percentage. Cooking losses did not (P>0.53) differ among anterior-, middle- and posterior-located steaks, but cooking loss percentages were greatest (P<0.05) in the medial portion and least (P<0.05) in the central portion of the GM steaks (Table 2). Nevertheless, the cooking loss percentage within steak position showed a great significant difference (P<0.001). However, the interaction between steak location and within steak position revealed no significant difference.

### Warner-Bratzler shear force (WBSF)

Neither quality grade category (P=0.133) nor yield grade category (P=0.485) affected the WBSF values of GM steaks. Although there were main effect differences associated with steak location and within-steak position, the central portion of anterior GM steaks received the lowest (P<0.05) WBSF values, (Figure 1). This indicated that less force was used to shear that particular steak portion. On the other hand, the medial portion of middle steaks received the greatest (P<0.05) WBSF values (steak location × within-steak position, (P<0.001); (Figure 1). Within anterior steaks, the lateral position had greater (P<0.05) WBSF values than either the central or medial positions, but the medial position had greater (P<0.05) WBSF values than the lateral position within middle steaks. Findings showed that there was little to no variation (P>0.05) among the lateral, central and medial portions of steaks originating from the posterior of the GM.

**Table 1 - Effects of USDA quality grade (QG) and yield grade (YG) categories on shear force and cooking characteristics of *gluteus medius* steaks**

Variable	USDA Top Choice		USDA select		P > F		QG xYG
	1 & 2	4 & 5	1 & 2	4 & 5	QG	YG	
Cooking Loss, %	29.5 ± 0.65	30.1 ± 0.65	31.8 ± 0.68	32.1 ± 0.65	0.003	0.533	0.496
Shear Force, N	34.04 ± 2.57	34.43 ± 2.56	40.22 ± 2.69	36.19 ± 2.55	0.133	0.485	396

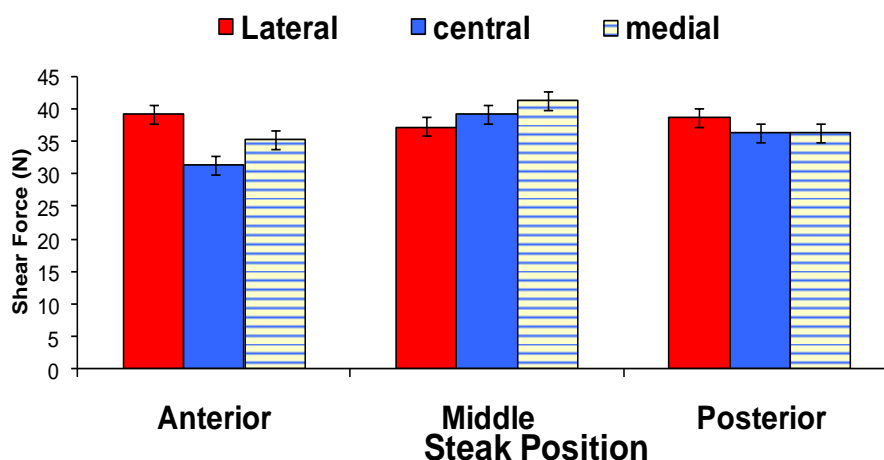
Probability value of the main and interactive effects included in the statistical model



**Table 2 - Main effects of steak location (S) and within steak position (P) on shear force and cooking characteristics of *gluteus medius* steaks.**

Variable	Steak location <sup>1</sup>				Within steak position <sup>2</sup>				P > F <sup>3</sup>		S × P
	ANT	MIDD	POST	SE	LAT	CENT	MED	SE	S	P	
Cook loss, %	31.2	30.8	30.6	0.44	32.0 <sup>x</sup>	29.5 <sup>z</sup>	31.1 <sup>y</sup>	0.4	0.536	<0.001	0.425
Shear force, N	34.64 <sup>y</sup>	38.36 <sup>x</sup>	35.61 <sup>y</sup>	1.52	37.47	34.82	36.39	1.45	0.025	0.06	<0.001

<sup>x,y,z</sup> Within a row and main effect, least squares means lacking common superscript letters differ ( $P < 0.05$ ). <sup>1</sup> Steak location: ANT = anterior; MIDD = middle; and POST = posterior; <sup>2</sup> Within steak position: LAT = lateral; CENT = central; and MED = medial; <sup>3</sup> Probability value of the main and interactive effects included in the statistical model.



**Figure 1 - Interactive effect of steak location and within steak position ( $P < 0.001$ ) on Warner-Bratzler shear force values of *gluteus medius*. <sup>a-e</sup> Bars lacking common letters are different ( $P < 0.05$ ).**

## DISCUSSION

### Cooking loss and Warner-Bratzler shear force

The cook loss was found to have significant difference within the steak positions on quality grades: Choice and Select grades estimates. High cooking loss may result in low water holding capacity. For any muscle, water holding capacity is minimal at low ultimate pH. The variations in cooking loss were attributed to specific species. This study revealed that cooking losses were the same between steaks of YG 1 and 2 and YG 4 and 5 carcasses, although top Choice had lower cooking loss percentage than Select carcasses.

GM muscle was observed not to be uniform regarding instrumental tenderness in relation to within steak location and position. The less tougher, anterior-central steak was measured with force of 30.71 N, and middle-medial being the toughest steak, needed more force to shear and measured 40.12 N. (Figure 1). This supports the study in which textural properties differed to a greater extent particularly from lateral to medial than origin to insertion (Segars et al., 1974). The differences in instrumental tenderness within GM sectioned steaks might be due to the same interpretations made by Hannula and Puolanne, (2004) on *semi-membranosus* muscle who stated that the rate of muscle temperature effecting rigor development or muscle fiber may have an influence on the variations within the muscle. Dikeman and Tuma (1971) reported that the palatability of beef is affected by various factors; for instance, intramuscular collagen solubility reduces as cattle age, developing into a tougher beef. It was noticed that shear force measurement and taste panel tenderness of beef steaks were greatly related to collagen solubility. Finally, the central portion of anterior was noted to be most tender part of GM muscle because the results in fig.1 showed that less force was used to shear the central portion.

## CONCLUSION

The study focused on assessment of tenderness areas within the *gluteus medius* steaks. The results indicated that Warner-Bratzler shear force values can be utilised as criteria for establishing steaks which will meet the satisfying consideration in tenderness by consumers prior to dissemination to the retail of food service outlets. The results of the study could be used to add value to the beef top sirloin butts by utilising those muscles with uniform tender areas for fabrication and marketing them as single muscle steaks.

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# GROWTH PERFORMANCE OF WEANER PIGS FED SOYBEAN HULL BASED DIETS

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**ABSTRACT:** A study was conducted to evaluate the response of weaner pigs to diets containing graded levels of soybean hull (SBH). Twenty-four male hybrid (large white x landrace) weaner pigs, about 6 weeks old, weighing 5.92-6.85kg were randomly divided into four groups of six pigs each using a completely randomized design (CRD). Each group was randomly assigned to one of the four isocaloric (2800kcal ME/kg) and isonitrogenous (18% crude protein) diets containing 0% (control), 10, 15 and 20% SBH for 56 days. Each treatment was replicated 3 times with 2 pigs per replicate placed on a concrete-floored pen. Daily feed intake, body weight gain, feed conversion ratio, protein efficiency ratio and feed cost per kg weight gain were determined. During the 8<sup>th</sup> week of the experiment, blood samples were collected from two pigs per treatment for haematological evaluation. Results showed that pigs fed the 10% SBH diet had higher ( $P<0.05$ ) average final body weight, average weight gain and better efficiency of feed conversion than those fed 20% SBH diet. Increasing levels of SBH in the diets had no significant effect ( $P>0.05$ ) on the PER values. Differences between the treatments in total digestible nutrients (TDN) were significant ( $P<0.05$ ). Feed cost per kg weight gain was reduced at the 10% SBH inclusion level as compared to other SBH diets. Dietary treatments did not have adverse effect on the haematology of pigs. Pigs fed the control diet (0% SBH) and those fed soybean hull based diets had comparable performance. It was concluded that soybean hull can be included in the diet of weaner pigs at 20% level without adverse effects on the growth performance and haematological values of the animals.

**Key words:** Soybean Hull, Diets, Growth Performance, Weaner Pigs

## INTRODUCTION

The scarcity of conventional feeds has hindered the growth of the livestock industry in Nigeria. The food deficit problem is indeed more serious with protein supply when compared with the availability of calories. Shortage of protein, particularly those of animal origin is prevalent in most parts of Africa where it is estimated that on the average 10g of animal protein is consumed per day compared to a recommended daily intake of 35g (FAO, 1997). Therefore, there is the need, to increase the production of such domestic animals as pigs and poultry which are conventional sources of animal protein. Pig production in particular represents one of the fastest ways of increasing animal protein, since pigs grow at a faster rate and are highly more prolific than cattle, sheep and goats. In growth rate pig is only surpassed by broilers (Holness, 2005). Apart from their high rate of reproduction, pigs and poultry are characterized by the best efficiency of nutrient transformation into high quality animal protein (Smith, 2001; Holness, 2005). However, the high cost of the conventional feedstuff most especially the protein supplement, necessitated the quest for locally available alternatives that can substitute for the conventional feedstuffs economically by reducing feeding cost, thereby making the pig enterprise a more profitable one (EL-Sabben et al., 1970; Fontenot, 1971). The alternative cheap and available feedstuff to be considered in this study is Soy bean hull. Soybean hulls referred to as soy hull, soybean mill-run or soybean flakes are by-products of soybean milling industry which do not attract competition between man and animals. Soybean hull is readily available when compared to other alternative sources of feed ingredient. This study was therefore, conducted to investigate the effect of varying dietary levels of toasted soybean hull on growth performance of weaner pigs.

## MATERIALS AND METHODS

The study was conducted at the Piggery Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Soybean hull and other feed ingredients used for the study were procured from Nsukka, Orba and Enugu in Enugu state, Nigeria.

ORIGINAL ARTICLE





### Formulation of experimental diets

Four diets were formulated to contain 0, 10, 15 and 20% soybean hull (SBH). The Composition of the diets is presented in Table 1.

### Management of experimental animals

Twenty-four male hybrid (large white x landrace) weaner pigs, about 6 weeks old, weighing 5.92-6.85kg were randomly divided into four groups of six pigs each using a completely randomized design (CRD). Each group was randomly assigned to one of the four isocaloric (2800kcal ME/kg) and isonitrogenous (18% crude protein) diets (1, 2, 3 and 4) containing 0% (control), 5, 10 and 20% SBH for 56 days.

**Table 1 - Percentage composition of weaner pigs' diets**

Ingredients (%)	0	10	15	20
Maize	49.8	47.2	45.2	44.2
Brewer's dried grain	7.00	5.00	4.00	4.00
Groundnut cake	18.4	16.4	15.2	14.00
Soybean hull	0	10	15	20
Palm Kernel meal	13.00	9.00	8.00	5.00
Fish waste (32%CP)	4.00	4.00	4.00	4.00
Palm oil	2.8	3.4	3.6	3.8
Bone meal	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Vitamin-mineral premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
<b>Calculated composition:</b>				
Crude protein (%)	18.06	18.07	18.09	18.05
Crude fibre (%)	5.00	5.80	6.28	6.74
Energy (MJ/ Kg ME)	12.26	12.26	12.22	2.22

Each treatment was replicated 3 times with 2 pigs per replicate placed on a concrete-floored pen with windows installed with wire nets to prevent flies from entering into the pens. Water was given *ad-libitum*. Pigs were fed 4% of their body weight in the first 2 weeks and later increased to 5% of their body weight as ration per replicate. The pigs were injected with Ivermectin (0.5ml per pig) subcutaneously against endo and ectoparasites. At the beginning and at the end of the experiment, pigs in each replicate were weighed individually to determine the initial body and final body weights of pigs, respectively. Live weights were recorded weekly for each replicate to determine the body weight gain. Feed intake was determined daily by the weigh-back technique. Feed conversion ratio was then calculated from these data as quantity (grams) of feed consumed per unit (grams) weight gained over the same period. Protein efficiency ratio and feed cost per kg weight gain were also determined. All measurements were taken between 8.00am and 12.00 noons.

### Hematological Evaluation

At the 8th week of the feeding trial, blood was sampled from three pigs per treatment by human puncture of the hind leg and ear vein. The blood samples were separately collected using sterile disposable syringes and needles into properly labeled sterilized bottles containing EDTA (Ethylene diamine tetra-acetic acid) for haematological analysis. Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined by the methods described by Lamb (1991). Red blood cell (RBC) and total white blood cell (WBC) counts were estimated using the haemocytometer, while mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were calculated according to Mitruka and Rawnsley (1977). The design and implementation of the study conformed to with the relevant provisions of the Animal Use Act of the University of Nigeria, Nsukka (2006).

### Proximate and Statistical Analyses

The proximate analysis of the diets was determined according to AOAC (1990) and the gross energy of each diet was also determined using the adiabatic bomb calorimeter. The data collected were subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980). Duncan's New Multiple Range Test was used in separating the significant means (Duncan, 1955).

## RESULTS

### Performance of weaner pigs

Table 2 shows the proximate composition of grower pigs' diets. Data on the performance of growing pigs fed diets containing graded levels of soybean hulls are presented in Table 3. The effect of the treatments on average final body weight was significant ( $P < 0.05$ ). The result shows that average final body weight (9.90kg) was highest at 15% SBH inclusion in the diet. However this did not differ significantly ( $P > 0.05$ ) from the final body weight of pigs fed 0% (control) and 20%SHB diets. Nevertheless it differed significantly ( $P < 0.05$ ) from the final body weight of pigs



fed 5% SBH diet. Results show that average daily feed intake and average daily protein intake followed the same trend as the average final body weight. The average daily weight gain (ADWG) of pigs fed 10 and 15% SBH diets was significantly ( $P < 0.05$ ) higher than that of pigs fed 20% SBH diet. However pigs fed 0% SBH diet (control) had similar ADWG with those fed 10, 15 and 20% SBH diets.

**Table 2 - Proximate composition of weaner pigs' diets (Experiment1)**

Components	Dietary SBH levels (%)			
	0	5	15	20
Dry matter%	93.7	92.75	92.3	92.75
Crude Protein %	18.08	18.04	18.07	18.03
Ether extract %	9.40	9.20	7.66	10.60
Crude fibre %	5.70	4.20	4.30	5.05
Ash %	17.35	11.85	10.40	11.25
N- Free extract %	43.17	49.46	51.87	47.82
Gross energy (Mj/kg)	15.03	15.06	15.04	15.02

**Table 3- Effect of graded level of soybean hull on performance of weaner pigs**

Parameters	Dietary levels of SBH (%)				SEM
	0	10	15	20	
Av. initial body weight gain (kg)	6.30	5.92	6.33	6.85	-
Av. final body weight (kg)	9.52 <sup>ab</sup>	9.90 <sup>a</sup>	9.43 <sup>ab</sup>	9.12 <sup>b</sup>	0.22
Av. daily feed intake (g/day/pig)	852.50 <sup>ab</sup>	802.07 <sup>b</sup>	911.60 <sup>a</sup>	854.17 <sup>ab</sup>	22.42
Av. daily weight gain (g/day/pig)	277.38 <sup>ab</sup>	290.86 <sup>a</sup>	290.52 <sup>a</sup>	252.64 <sup>b</sup>	11.48
Feed conversion ratio (feed: gain)	3.09 <sup>ab</sup>	2.75 <sup>b</sup>	3.19 <sup>ab</sup>	3.47 <sup>a</sup>	0.14
Protein efficiency ratio	1.80 <sup>b</sup>	2.02 <sup>a</sup>	1.75 <sup>b</sup>	1.64 <sup>b</sup>	0.07
Av. daily protein intake	154.13 <sup>ab</sup>	144.69 <sup>b</sup>	164.74 <sup>a</sup>	154.01 <sup>ab</sup>	4.06
Total Digestible Nutrient	171.79 <sup>b</sup>	182.03 <sup>a</sup>	184.27 <sup>a</sup>	183.38 <sup>a</sup>	1.51

a,b means with different superscripts across a given row differs significantly ( $P < 0.05$ )

The feed conversion ratio (FCR) of pigs fed 20% SBH diet was significantly ( $P < 0.05$ ) higher than that of pigs fed 10% SBH diet. Pigs fed 0% SBH diet had comparable ( $P > 0.05$ ) FCR with those fed 10, 15 and 20% SBH diets. The protein efficiency ratio (PER) value of pigs fed 10% SBH was significantly ( $P < 0.05$ ) higher than that of pigs fed 0, 15 and 20% SBH diets. Pigs fed 0, 15 and 20% SBH diets had comparable PER ( $P > 0.05$ ). The effect of treatments on the total digestible nutrient (TDN) was significant ( $P < 0.05$ ). Pigs fed 10, 15 and 20% SBH diets had comparable ( $P > 0.05$ ) TDN and this was significantly ( $P < 0.05$ ) higher than that of pigs fed the control diet.

#### Cost implication of feeding graded levels of soybean hull to weaner pigs

Table 4 shows data on cost implication observed in this experiment. Feed cost per kg weight gain did not follow any particular trend. Pigs on the control diet (0% SBH) had the same cost of per kg weight gain value with pigs fed other SBH diets. However, feed cost per kg weight of pigs fed 20% SBH diet differed significantly ( $P < 0.05$ ) from values observed in treatment 2 (10% SBH diet). The effect of treatment on total weight gain was significant ( $P < 0.05$ ). Pigs fed 10 and 15% SBH diets had significantly ( $P < 0.05$ ) higher value (3.57kg) than pigs fed 20% SBH diet. Pigs fed 0, 10, 15 and 20% SBH diets had comparable ( $P > 0.05$ ) total weight gain. There were significant ( $P < 0.05$ ) differences among treatments in total feed intake were. Pigs fed 15% SBH diet had total feed intake value of 25.53kg and this differed significantly ( $P < 0.05$ ) from the value (22.46kg) observed interatment 2 (10% SBH diet). Pigs fed the control diet (0% SBH) had total fed intake of 23.87kg and this did not differ significantly ( $P > 0.05$ ) from the value (23.92kg) observed in other treatments (10, 15 and 20% SBH diets). The cost of total feed intake and cost of daily feed consumed followed the same trend as total feed intake.

**Table 4 - Cost implication of feeding graded levels of soybean hull to weaner pigs**

Parameters	Dietary levels of SBH (%)				SEM
	0	10	15	20	
Cost of 1kg feed ₦	92.71	91.40	89.87	89.09	-
Feed cost per kg weight gain ₦	286.47 <sup>ab</sup>	251.65 <sup>b</sup>	286.99 <sup>a</sup>	309.44 <sup>a</sup>	11.72
Total feed intake (kg)	23.87 <sup>ab</sup>	22.46 <sup>b</sup>	25.53 <sup>a</sup>	23.92 <sup>ab</sup>	0.63
Total weight gain (kg)	3.18 <sup>ab</sup>	3.20 <sup>a</sup>	3.57 <sup>a</sup>	2.58 <sup>b</sup>	0.21
Cost of total feed intake ₦	2,212.99 <sup>ab</sup>	2,052.54 <sup>b</sup>	2,294.08 <sup>a</sup>	2,130.74 <sup>ab</sup>	55.50
Cost of daily feed consumed ₦	79.04 <sup>ab</sup>	73.31 <sup>b</sup>	81.93 <sup>a</sup>	76.10 <sup>ab</sup>	1.98

a,b - means with different superscript across a given row differ significant ( $P < 0.05$ )

#### Effect of graded levels of soybean hull on haematology of weaner pigs

The effects of treatments on haematological values of pigs are presented in Table 5. The results show that the effect of treatment on haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC)



and white blood cell count (WBC) were all significantly ( $P < 0.05$ ) influenced by the SBH levels in the diets. The Hb, PCV, RBC, and WBC values of pigs fed 15% SBH diet differed significantly ( $P < 0.05$ ) from the values observed in treatment 1 (0% SBH diet). However, pigs fed 10 and 20% SBH diets had comparable ( $P > 0.05$ ) Hb, PCV and RBC, and WBC values. There were no significant differences ( $P > 0.05$ ) among treatments in mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV).

**Table 5 - Effect of graded levels of soybean hull on haematology of weaner pigs**

Parameters	Dietary levels of SBH (%)				SEM
	0	10	15	20	
Haemoglobin concentration (g/100ml)	8.10 <sup>b</sup>	9.57 <sup>ab</sup>	10.85 <sup>a</sup>	9.57 <sup>ab</sup>	0.37
Packed cell volume (%)	24.30 <sup>b</sup>	28.70 <sup>ab</sup>	32.55 <sup>a</sup>	28.70 <sup>ab</sup>	1.11
RBC ( $\times 10^6/\text{mm}^3$ )	4.05 <sup>b</sup>	4.78 <sup>ab</sup>	5.43 <sup>a</sup>	4.78 <sup>ab</sup>	0.18
WBC ( $\times 10^3/\text{mm}^3$ )	17,283.33 <sup>b</sup>	20,600.00 <sup>ab</sup>	26,450.00 <sup>a</sup>	18,966.67 <sup>b</sup>	1,292.69
MCH (%)	20.00	20.00	19.99	20.00	0.01
MCV ( $\mu\text{m}^3$ )	60.00	60.00	59.98	60.00	0.50
MCHC (%)	33.30	33.30	33.30	33.30	0.01

a,b - means with different superscript across a given row differ significant ( $P < 0.05$ )

## DISCUSSION

### Performance of pigs

It was observed (Table 3) that pigs fed the 10% SBH diet had higher average final body weight, average weight gain, protein efficiency ratio and better efficiency of feed conversion than those fed 20% SBH diet. However, the overall performance of pigs fed the soybean hull based diets was not inferior to that of pigs fed the control diet (0% SBH). This tends to suggest that growing pigs can tolerate 20% SBH in their diet. The 6.74% crude fibre in the 20% SBH diet is below the limit (20%) reported by MacDonald et al. (2002) as the highest level of crude fibre in the diet of simple stomached animals. Moreover, the positive effect of dietary fibre cannot be undermined. Dietary fibre had been found to activate the intestine, enhance peristaltic movement and ensure more enzyme production, thereby resulting in efficient digestion of nutrients (Esonu et al., 1997). The lack of increase in the feed intake of pigs on the diets containing soybean hull meal is quite remarkable. Although soybean hull meal contains high fibre, its inclusion in the diets at 20% level did not result in increased dietary fibre. Such a situation would have led to the dilution of other nutrients thereby leading to increased feed intake. Pigs as well as other farm animals eat to meet their energy requirements and to sustain rapid growth and development. The energy needs of the growing pigs were therefore satisfied even at 20% SBH inclusion, hence the pigs did not consume more feed than those on the control diet. Similar observations had been reported (Beynen, 1990; Esonu et al., 1997; Esonu, 1998; Anyanwu et al., 2003; Esonu et al., 2004). Generally, the comparable growth performance of pigs fed SBH diets and those fed the control diet is quite interesting in two ways. First, it showed that the utilization of soybean hull meal by pigs is relatively high at the level offered in this study. This observation contradicts earlier reports by Ash and Akoh -Petia (1992), Udedibie and Igwe (1989) and Cheeke et al. (1983). Secondly, it could be that the heat treatment applied to SBH before its inclusion in the diets helped to improve its texture, palatability and nutritive value by destroying or inactivating the heat - labile toxic compounds and anti-nutritional factors such as protease inhibitors, haemagglutinins, tannins, cyanogenic glycosides and flatulence factors in the raw soybean (Liener and Kakade, 1980; Ensminger, 1996; Enwere, 1998). This suggestion agrees with the findings of Khan et al. (1979) that heat treatment applied to legume foods improved their texture, palatability and nutritive value by destroying or inactivating heat - labile toxic compounds and other enzyme inhibitors. Palatability in particular had been shown to influence feed intake and hence the overall performance of animals (Holness, 2005; Jurgens, 2002). Perhaps, the palatability of the control diet was not superior to that of the test (SBH) diets.

### Cost Implication

As shown in Table 4, the dietary inclusion of soybean hull meal reduced the cost of producing one kilogramme of feed. This observation was in line with that of Esonu et al. (1997) and Anyanwu et al. (2003). Feed cost per kg weight gain was reduced at the 10% SBH inclusion level as compared with other SBH diets. This agrees with the reports of Phillips (1984) Sonaiya et al. (1986) and Ukachukwu and Anugwa (1995) that reduction in feed cost per kg gain is not only dependent on cheap feed but is also dependent on the production result obtained with this cheap feed. The efficiency with which the feed is utilized is of major importance.

### Haematological evaluation

As indicated in Table 5, dietary treatments did not have adverse effect on the haematology of weaner pigs. This could be attributed to the efficacy of toasting to completely remove or reduce the negative effect of the anti-nutritional factors (ANFs) and toxicants such as cyanogens, tannins and lectins in the raw bambara nut waste on the haematology of pigs. Liener (1986) and Ensminger et al. (1996) had shown that cyanogens, tannins and lectins in the raw bambara nut have the ability to destroy the red blood cells. The haematological values obtained in the present study are within the normal range as reported by Miller et al. (1961) and Schalm et al. (1975). Miller et al. (1961) reported a red blood cell count of 4.5 million/ $\text{mm}^3$  as the lowest value and 7.6 million/ $\text{mm}^3$  as the highest



value for matured pigs. Schalm et al. (1975) reported a range of 5.3 million/mm<sup>3</sup> to 7.3 million/mm<sup>3</sup> average values for red blood cells in their work with 20 lactating sows, 20 weaned piglets and 15 fattening pigs.

## CONCLUSION

It is evident from this study that soybean hull can be included in the diet of weaner pigs at 20% without adverse effects on the growth performance and haematological values of the animals.

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# EFFECT OF FEEDING TIME ON THE PERFORMANCE OF JUVENILE AFRICAN CATFISH (*Clarias gariepinus*, Burchell 1822)

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**ABSTRACT:** The experiment was conducted to investigate the effect of feeding time on the performance of juvenile African Catfish (*Clarias gariepinus*, Burchell 1822). The experimental fish were randomly assigned to four treatment groups (different feeding time intervals) of 60 fish each in a completely randomized design (CRD). Each treatment was replicated three times with 20 fish per replicate. The fish were fed with extruded fish feeds (Catco® fish concentrate) at 3% of the fish body weight. The four treatments (feeding time) were T1 - once a day feeding time of morning hours (07.30 to 08.30) only, T2- once a day feeding time of afternoon hours (12.30 to 13.30) only, T3- once a day feeding time of evening hours (17.00 to 18.00) only and T4- twice a day feeding time of morning hours (07.30 to 08.30am) and evening hours (17.00 to 18.00) only for twelve weeks. There were significant difference ( $P < 0.05$ ) among treatments in fish' final body weight (223.63g, 200.13g, 196.33g and 168.17g for T4, T1, T3 and T2, respectively), mean total body weight gain (208.97g, 184.83, 181.07g and 153.41g for T4, T1, T3 and T2, respectively), mean daily body weight gain (2.60g, 2.20g, 2.16g and 1.83g for T4, T1, T3 and T2, respectively), specific growth rate (SGR) of 1.41, 1.33, 1.32 and 1.26 for T4, T1, T3 and T2, respectively) and daily feed intake (3.27g, 3.09, 2.95g and 2.54g for T4, T1, T3 and T2, respectively). There were also significant differences ( $P < 0.05$ ) among treatments in water temperature (26.13 °C, 25.50°C, 26.43 °C and 28.10 °C for T4, T1, T3 and T2, respectively). However, there were no significant differences ( $P > 0.05$ ) among treatments in dissolved oxygen (7.1 mg/l, 6.8mg/l, 7.3 mg/l and 7.5 mg/l for T1, T2, T3 and T4, respectively), water pH (7.1), feed cost per kg weight gain (N390.00, N380.00, N379.00 and N368.00, for T1, T2, T3 and T4, respectively) and mortality rate of fish (13.38%, 11.67%, 10.00% and 13.3% for T1, T2, T3 and T4, respectively). It is evident from the result obtained in the present day study that the growth performance of African catfish (*Clarias gariepinus*, Burchell 1822) fed twice a day (in the morning and evening hours) was superior to the performance of those fed once a day especially those fed in the afternoon hours only.

**Key words:** Effect, Feeding Time, African Catfish, Growth Performance

## INTRODUCTION

Aquaculture, the farming of aquatic organisms including fish, molluscs, crustacean and aquatic plant is necessary to meet the protein need of Nigerians. Over time, there has been increase in fish production in Nigeria. Bello (2007) and FAO (2005) reported increase in fish production in 2005. According to him, the artisan fish production level grew by 5.4%, aquaculture fish production by 43% and industrial fishery through the use trawlers by 12% over the previous years. However, of this increase in fish production, the desired result has not been attained. Quantitatively, details of fish production as at 2005 stood at 490,600 tons from the artisan fishery, 56,300 tons from industrial fishery through the use of trawlers; while fish importation stood at 61,150 tons. In meeting up with the growing need for fish production, aquaculture practice has been identified as a possible alternative; the reasons being that the activities of artisans and industrial fishery in our natural waters have led to over exploitation and degradation due to human activities in our coastal water. To fully bring aquaculture to its desired level, four production challenges have been identified. These are the challenges of feeding the fish stock in the pond, management of pond water quality, fish seeds provision and pond construction/establishment. The first two challenges: fish feeding and water quality management affect each other. The level of feeding of the stocks affects the water quality and the level of water quality affect the feeding performance of fish in the pond (George, 2001).

Fish like other animals need food to be able to carry out their metabolic activities. In aquaculture, fish feeding is either supplemental or complete (total supply). Supplemental feeding is when feeds are given to the

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animal at a minimal level to add to the natural food available for the fish in the pond water. These natural foods are in the form of phytoplanktons and zooplanktons. The complete feeding is when the source of food fed to the fish is solely supplied by the farmer. In whichever case, the type of feeding practiced depends on the nature of the pond and the type of production the farmer is involved with (Michael, 1987; Michael et al., 2005). The most popular cultured fish in Nigeria is the catfish. It is naturally carnivorous, a bottom pond dweller, nocturnally very active and belongs the family of *Clariidae* (William, 1967; Idodo-Umeh, 2003). However, with the fish domestication, its modes of feeding and activities have been destabilized and modified. To this end, the feeding regime has become diverse but the thumb rule of feeding stock at optimum level should be very economical so as to have savings in feed cost and the overall economic justification. Webster et al. (1992) reported that catfish can be fed once or twice daily and rainbow trout at three times a day. In whichever case, the type of production, climatic condition and economic status of the farmer dictate the feeding requirement. According to Raven and Walker (1978), a major problem facing fish feed manufacturers and fish nutrition is the increasing competition for the same feeding stuff between man and the fish feed industry due to their conventional status. This has brought about the high price and scarcity of such feed stuffs. Various studies have been done in fish feeding (Collins and Delmendo, 1979; Sena and Brain, 1992) but much is still to be done in the area of the best time of the day to feed catfish so as to have good growth performance that will justify the high cost of feeds provided by the farmer. Determining the best time of the day to feed the catfish will therefore help to maximize performance, discourage waste and ensure the success of the enterprise. This will help to discourage the deterioration of water quality which may arise from the decomposition of feeds fed to the fish due to feeding at inappropriate time. This will help to minimize fish mortality due to pond water quality deterioration. The overall production of the stock will also be enhanced (Norm Meck, 2000).

This study was therefore conducted to determine the effect of feeding at different time intervals of the day on the growth performance of African catfish (*Clarias gariepinus*, Burchell 1822).

## MATERIALS AND METHODS

The experiment was carried out in the Fisheries Unit of the Teaching and Research Farm, Department of Animal Science, University of Nigeria, Nsukka. Two hundred and forty post juvenile African catfish fingerlings (*Clarias gariepinus* Burchell 1822) were used for the study which lasted for ten weeks. The post juvenile African catfish fingerlings were purchased from the local hatchery in Makurdi, Benue state, Nigeria.

### MANAGEMENT OF THE EXPERIMENTAL FISH

A total of two hundred post juvenile African Catfish fingerlings weighing  $15.0 \pm 0.26$  g on the average were randomly divided into four treatment groups (T1, T2, T3 and T4) of 60 fish per group using a completely randomized design (CRD). The treatment groups were designated as follows: T1 (Fish in this group were fed once daily in the morning at 07.30 hour to 08.30 hour at 3% of their body weight), T2 (Fish in this treatment were fed once daily in the afternoon at 12.30 hour to 13.30 hour at 3% of their body weight), T3 (Fish in this group were fed once daily in the evenings at 17.00hour to 18.00hour at 3 % of their body weight) and T4 (Fish in this treatment were fed twice a day in the morning and evening at 07.30 hour to 08.30 hour and at 17.00 hour to 18.00 hour, respectively at 3% of their body weight). The feed used for treatment 4 was divided into two so that the fish receive half of the ration in the morning and the remaining half in the evening. Each group was replicated three times with 20 fish per replicate placed in plastic tanks measuring 0.6m x 0.6m x 0.9m. The fish were fed with extruded commercial feeds of Catco® Fish Concentrate. The composition of the diet is presented in Table 1.

**Table 1 - Nutritional composition of the experimental diet**

Crude Protein	42.0
Crude Fat	13.0
Crude Fibre	1.9
Ash	9.5
Phosphorus	1.1
Vitamin A (I.U./kg)	15000
Vitamin D3 (I.U./kg)	2000
Vitamin E (mg/kg)	200
Vitamin C (Stable) (mg/kg)	150
Copper (mg/kg)	5

To mitigate the environment as a result of the exposure of the plastic materials to atmospheric temperature, and the volume of the water used for the experiment, an open shed was constructed with rough thatch over the water holding vessels with its sides rounded up with wire mesh up to three feet high to prevent the entrance of rodents and human factors.

The fish were fed daily with 1.5mm to 4.5mm feed size of the extruded commercial feeds at 3% body weight throughout the twelve weeks experimental period. The initial body weight (gm) and length (cm) of the fish were taken using sensitive scale and meter rule, respectively before they were stocked and subsequently at two weeks interval. The temperatures of the water were also measured daily using the thermometer and the pH using the pH meter before feeding the animals. The dissolved oxygen was monitored and measured weekly using the dissolved



oxygen meter. The volume of the water was maintained at 0.18m<sup>3</sup>. The top of the vessels was also covered with 5mm mesh size net to protect the stocks from jumping out while the water in the vessels was changed bi-weekly to avoid the buildup of nitrates and nitrites as effluent leaching was not possible due to the use of plastic materials.

### PARAMETERS MEASURED

Live weight (g) of the fish was measured using sensitive top loading scale. The length (cm) of the fish was measured using the rule meter. Feed in-take of the fish was measured using sensitive top loading scale. Dissolved oxygen was measured using the dissolved oxygen meter; water temperature was measured using the thermometer and water pH using the pH meter according to the various replicates and treatments. Some of the data generated were used to calculate weight gain, protein efficiency ratio and feed cost per kg weight gain. The specific growth rate was calculated as follows: Specific Growth Rate = Final body weight of fish – initial body weight of fish/No of Days the fish were reared. Mortality was monitored and records kept on daily basis.

### STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980) and Akindele (2004). Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955).

### RESULTS AND DISCUSSION

Data on the growth performance and body length of African catfish fed at different time intervals are presented in Tables 2 and 3, respectively. Significant differences (P<0.05) were noticed in the feed intake and weight gain of the fish in the various treatments. Final mean body weight was 223.63g (T4), 200.13g (T1), 196.33g (T3) and 168.17g (T2). The mean body weight gained was 208.97g (T4), 184.83g (T1), 181.07g (T3) and 153.41g (T2) and the mean daily weight gain for the period was 2.60g (T4), 2.20g (T1), 2.16g (T3) and 1.83g (T2). The specific growth rate (SGR) also showed significant difference at (P<0.05) with mean value as follows: T4 (1.41) T1 (1.33), T3 (1.32) and T2 (1.26). These results were due to the feed consumption rate of the fish that showed significant difference (P<0.05) in the same order. This shows that the feed fed to the fish impacted the fish positively at the various time intervals of feeding. This is in agreement with Davies et al. (2006) and Odeyemi (2007) of the high performance of the fish fed twice a day as there was efficient utilization of the feed. However, there were no significant differences (P>0.05) in the feed conversion ratio with a range of 1.23 - 1.30. This result agreed with the result of 0.98 -1.46 recorded by Hecht and Appelbaum (1988). The feed conversion ratio of 1.23 was observed in treatment T4; also support that of Mostafa et al. (2002). This high performance is attributed to the high quality of the extruded feed used for the experiment whose nutritional composition and form conformed to the prescription by ADCP (1980), Jan (1995) and Zulfiker (2001)

**Table 2 - Growth performance of African catfish fed at different time intervals**

Parameters/Treatments	T1	T2	T3	T4	SEM
Initial number stocked	60	60	60	60	-
Final stock density less mortality	52	53	54	52	0.41
Initial body weight (g)	15.1	14.5	15.2	14.5	0.26
Final body weight (g)	200.13 <sup>b</sup>	168.17 <sup>c</sup>	196.33 <sup>b</sup>	223.63 <sup>a</sup>	3.74
Total weight gain (g)	184.83 <sup>b</sup>	153.47 <sup>c</sup>	181.07 <sup>b</sup>	208.97 <sup>a</sup>	3.74
Average daily weight gain (g)	2.20 <sup>b</sup>	1.83 <sup>c</sup>	2.16 <sup>b</sup>	2.60 <sup>a</sup>	2.58
Specific growth rate (SGR)	1.33 <sup>b</sup>	1.26 <sup>c</sup>	1.32 <sup>b</sup>	1.41 <sup>a</sup>	0.02
Mortality	8.0	7.0	6.0	8.0	2.04
Mortality %	13.3	11.67	10.00	13.33	2.04
Total feed consumed (g)	13512.2 <sup>b</sup>	11297.5 <sup>c</sup>	13372.7 <sup>b</sup>	14268.3 <sup>a</sup>	0.57
Average daily feed intake (g)	3.09 <sup>b</sup>	2.54 <sup>c</sup>	2.95 <sup>b</sup>	3.27 <sup>a</sup>	0.03
Feed conversion ratio (FCR)	1.30	1.27	1.26	1.23	0.21
Protein efficiency ratio (PER)	1.96	1.94	1.93	2.09	0.04

<sup>abc</sup> = Means with different superscripts on the same row are significantly (P<0.05) different, SEM = Standard error of mean

Similarly, the time of feeding also supported the growth performance of the fish. Nutritionally, feed intake of fish is controlled by three factors which are the environmental factor, the fish physiological factor and the feed factors. So long the same feed was used in the various treatments, feed factors should not be considered to be the reason for the observed significant differences, Kasumya (1999) and NRC (2009) reported that environmental factors in relation to feeding time and water physico-chemical quality have a marked impact on the feed intake of the fish as they can affect the fish physiological endowment capable of creating all sort of stress and neuro-endocrinological imbalance (Wynne et al., 2003). Fish feeding is one of the enormous tasks the farmer is faced with if the fish must grow considering the aforementioned relationship between the feeding and the water quality as they affect each other during the cause of management. The practice of feeding is far from being an exact science. It is a highly subjective process. FAO (2005), Edwin and Meughe (2007) and Brown (2008) reported that though catfish has been cultured over the years and it ranks the most popular cultured fish in Nigeria, there is a





considerable alteration in the feeding behaviour of the fish. Recently, Edwin et al. (2009) argued that the best time of day to feed fish is still an object of debate. Nevertheless, they opined that the time of day to feed fish is largely dictated by the logistics of feeding practice. Thus the response of the fish to time of feeding and its acceptance is not static as it's nocturnal habit make up has been broken due to the practice of domestication.

As shown in Table 3, the length of the fish showed no significant difference ( $P>0.05$ ) in all the various treatments.

**Table 3 - Body length of African catfish fed at different time intervals**

Parameters/Treatments	T1	T2	T3	T4	SEM
Initial body length(cm)	10.5	9.8	10.0	10.0	0.28
Final body length(cm)	31.2	29.7	31.0	32.0	0.48
Gain in body length(cm)	20.7	19.9	21.0	22.0	0.18

<sup>abc</sup> = Means with different superscripts on the same row are significantly ( $P<0.05$ ) different, SEM = Standard error of mean

The final mean total body length (cm) of 31.2cm (T1), 29.7cm (T2), 31.0cm (T3), and 32.0cm (T4) and the total gain in length of 20.7cm (T1), 19.9cm (T2), 21.0cm (T3) and 22.0cm (T4) did not contradict the observed body weight gain of the fish within the said short period of the experiment. However, differences in total body length could have begun to appreciate with more time as observed by Marc and Jean (1991) and Hengsawat et al. (1997). There was no significant difference ( $P<0.05$ ) in the mortality rate in all the treatments. The mortality rate was observed as follows: 13.38% (T1), 11.67% (T2), 10.00% (T3) and 13.3% (T4). The observed mortality values in all the experimental treatments were traceable to handling stress during weighing and change of water. This same scenario was also recorded by Davies et al. (2006). The recorded mortality was within the production range of 10-20% reported by Graaf et al. (1995) and was not due to any pathological disease conditions.

As shown in Table 4, there were significant differences ( $P<0.05$ ) among treatments in water temperature. The difference may be due to environmental condition vis-à-vis the degree of exposure to sunlight and heat absorption. The water temperature was highest in treatment T2 followed by T3 and T4. The least value was recorded in treatment T1.

**Table 4 - Physicochemical properties of pond water**

Parameters/Treatments	T1	T2	T3	T4	SEM
Water temperature(°C)	25.50 <sup>c</sup>	28.10 <sup>a</sup>	26.43 <sup>b</sup>	26.13 <sup>b</sup>	0.14
Dissolved oxygen (mg/l)	7.1	6.8	7.3	7.5	0.10
Water pH	7.1	7.1	7.1	7.1	0.17

<sup>abc</sup> = Means with different superscripts on the same row are significantly ( $P<0.05$ ) different, SEM = Standard error of mean

These observed water temperature value also were in line with the findings of Odeyemi (2007) and was attributed to the level of the water in the experimental tanks which might affected the assimilation of heat since the tanks were exposed to atmospheric sunlight and heat (Boyd, 1979; Boyd, 1995; Boyd, 2002). However, the recorded temperature range of 25.5°C to 28.10°C is within the tolerable range for catfish production in the tropics but with minimal metabolic effect fluctuation (Boyd, 1982; Boyd, 2002; Yeamni et al., 2007). Although the temperature range (25.5°C to 28.10°C) recorded in the present study is within the tolerable range for catfish production in the tropics, the differences in temperatures being quite significant ( $P<0.05$ ) might have fluctuating effect on fish metabolism. Thus the high water temperature (28.10°C) of treatment 2 could have altered the metabolic activities within the fish due to heat-induced stress. This would have affected the growth of fish in that treatment, hence the inferior growth rate observed in those fish. Jan (1995) explained that there are differences between metabolic energy for production (MEp) and metabolic energy for body maintenance (ME<sub>m</sub>). The ratio between these levels of energy varies within body weight and water temperature due to the interactive effect of feeding level and temperature on the fish body weight. Similarly, Ali (2006) reported that temperature affects the growth rate of fish by affecting a variety of metabolic processes including respiration, feed intake and digestion. That, any divergence from the normal ranges of the metabolic processes could alter the optimal range for fish health and growth. Although, the feed consumption may be high but greater proportion could have been used for body maintenance.

There were no significant ( $P>0.0$ ) differences among treatments in dissolved oxygen and pH values. While all the treatments had the same pH value (7.1) the dissolved oxygen values were 7.1, 6.8, 7.3 and 7.5 for T1, T2, T3 and T4, respectively. The physico-chemical properties obtained in the experiment were within the tolerable values for catfish production. Davies et al. (2006) Cruz et al. (2000), Boyd (1982), Michael (1999) and Michael et al. (2005) showed that the dissolved oxygen and pH in pond should not be below 2.5mg/l and pH of 5.0 - 8.0 for catfish production. It has been shown that the most dictating factor in fish production is the water quality of the pond as governed by water temperature, pH and dissolved oxygen. Considering the fact that the time of day has a good bearing on the water temperature, pH and dissolved oxygen in addition to feeding management, it does seem that feeding time has a great impact on performance of African cat fish, especially on feed intake and growth rate.

Table 5 shows the economic aspect of the various treatments used in the experiment. The cost implication of feeding the fish at the various treatments levels showed a significant difference ( $P<0.05$ ) for the total cost of fish



at the prevailing market price with the highest value of ₦6,969.00 for T4, ₦6,360.84 for T3, ₦6,243.60 for T1 and ₦5,346.40 being the lowest for T2. There was also a significant difference ( $P < 0.05$ ) among treatments in total cost of feed consumed by the fish in the course of the experiment. The value of ₦4, 280.79 was recorded for T4, ₦4, 053.66 for T1, ₦4, 011.81 for T3 and the lowest value of ₦3, 389.25 for T2. The observed significant differences ( $P < 0.05$ ) in the total cost of feed consumed were as a result of the different body weight gained of the fish in accordance to their different feed of the fish. However, the profit margin percentage recorded ranged from 54.10% to 62.90% showing no significant differences ( $P > 0.05$ ). This observed marginal profit percentages agreed with the 40-60% range of profitability recorded by Adebayo and Adesoji (2008) and Davies et al. (2006). This means that though there were significant differences ( $P < 0.05$ ) in the cost of the fish and feed used, the profit recorded were still high and that the feeding methods used were also economical. There were also no significant differences ( $P > 0.05$ ) in the feed cost per kilogram weight gain of the fish as their values ranged from N390.00, N380.00, N379.00 and N368.00 for T1, T2, T3 and T4 respectively. This was due to the profit margin recorded which lie also within economic level.

**Table 5 - Cost implication of feeding the fish at different time interval**

Parameters/Treatments	T1	T2	T3	T4	SEM
Total cost of fish at ₦600.00/Kg	6,243.60	5,346.54 <sup>c</sup>	6,360.84 <sup>b</sup>	6,969.00 <sup>a</sup>	0.01
Total cost of feed at ₦300.00/Kg	4,053.66 <sup>a</sup>	3,389.25 <sup>c</sup>	4,011.81 <sup>b</sup>	4,280.79 <sup>a</sup>	0.33
Profit margin (₦)	2,189.94	1,957.29	2,349.03	2,688.21	0.01
Profit margin %	54.10	58.06	58.73	62.9	1.98
Feed cost per Kg weight gain (₦)	390.00	380.00	379.00	368.00	0.72

<sup>abc</sup> = Means with different superscripts on the same row are significantly ( $P < 0.05$ ) different, SEM = Standard error of mean

## CONCLUSION

It is therefore evident from the results obtained in the present study that catfish should be fed twice daily; morning and evening time of the day. However, it is pertinent to consider when the prevailing physico-chemical characteristics (water temperature, pH, dissolved oxygen, and so on) that affect fish feeding behaviour are at optimal levels in the pond. Other factors such as stocking density, stocking integration and aggression, feed composition, feeds size, fish type and feed preparation should also be considered in determining best time of the day to feed catfish.

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# APPLICATION OF MOLECULAR MARKERS IN FARM ANIMAL IMPROVEMENT: PROSPECTS AND CHALLENGES

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**ABSTRACT:** *The discovery of genetic polymorphism at the DNA sequence level has been exploited as markers to explain the observed phenotypic variability in animals. Molecular markers have proven to be more reliable than other forms of genetic markers. The overview of the applications of molecular markers in the areas of genetic diversity conservation, identification of disease carriers, parentage determination, marker-assisted selection, transgenesis, sex-determination; and the enumeration of some challenges to the application of these markers in the developing countries, especially Nigeria, form the crux of this paper. Some of the challenges include economic factors, mechanical and logistics factors, lack of funding/grants for research, IPR issues and lack of adequately trained personnel in areas of molecular genetics.*

**Key words:** *Molecular Markers, DNA Sequence, Polymorphism, Challenges*

## INTRODUCTION

Until recently, most genetic progress for quantitative traits in livestock has been made by selection on phenotype or on Estimated Breeding Value derived from phenotype, without knowledge of the number of genes that affect the trait or the effects of each gene (Naqvi, 2007). Recent developments in DNA technologies have made it possible to uncover a large number of genetic polymorphism at the DNA sequence level, and to use them as markers for evaluation of the genetic basis for the observed phenotypic variability.

Molecular markers reveal variations even at the DNA level. They are not normal genes, as they usually do not have any biological effect, but are rather constant landmarks in the genome. They are identifiable DNA sequences found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next (FAO, 2003).

Molecular markers rely on a DNA assay and have proven to be more reliable than other forms of genetic markers. Morphological (e.g. pigmentation) and chromosomal (e.g. structural or numerical variations) markers usually show low degree of polymorphism and hence are not very useful for genetic markers. Biochemical markers which have been tried out extensively have not been found very encouraging as they are often sex-limited, age-dependent and are significantly influenced by the environment. The molecular markers, however, have overcome most of these limitations. They are numerous and ubiquitously distributed throughout the genome, they are not affected by the environment and generally do not have any pleiotropic effects on the Quantitative Trait Loci (QTL). According to Gholizadeh et al. (2008), the ultimate use of molecular markers would be to identify QTL in order to practice genotype selection. The development of molecular techniques has created new possibilities for the selection and genetic improvement of livestock. It entails the identification and mapping of genes and genetic polymorphisms. These polymorphisms and genetic maps are then evaluated to differentiate between markers in the expression of particular traits in a family that might indicate a direct effect of these differences in terms of genetic determination on the trait. More probably, they can prove some degree of linkage of the QTL affecting the trait and the marker. The use of molecular markers in genetic analysis offers several advantages. For example, the DNA samples can be conveniently isolated from the blood of live individuals, from tissues like sperm, hair follicle and even from archival preparations (Mitra et al., 1999). Some common types of molecular markers include, Restriction Fragment Length Polymorphism (RFLP), Polymerase Chain Reaction (PCR), Minisatellites, Microsatellites, Randomly Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphisms (AFLP).

Notwithstanding the many benefits accruable to the application of molecular markers in animal improvement, the technology is plagued by many challenges, especially in developing countries as Nigeria. This paper sets out to showcase some of these benefits and enumerate the challenges being faced in molecular markers application, with emphasis on Nigeria.

REVIEW ARTICLE



## APPLICATIONS OF MOLECULAR MARKERS

### Genetic Diversity Conservation:

Consequent upon the rampant crossbreeding of exotic animals with local breeds in order to exploit heterosis, there has been an irreversible loss of genetic diversity among our local animal breeds. The conservation of genetic diversity is important in the sense that it encourages high level of heterozygosity in the population. Gholizadeh et al. (2008) posit that genetic variation is a prerequisite for populations to be able to face future environmental changes. Frankham et al. (2003) added that genetic diversity is necessary to ensure long-term response to selection, either natural or artificial, for traits of economic or cultural interest.

Potentially unique genes in populations should be conserved with studies using DNA markers, as their contribution to biodiversity would be greater. The primary aim of studying genetic diversity is to understand the extent of differentiation of populations within species. Population-specific genetic markers (alleles) can be generated using a range of methods available for detection of polymorphic loci (Gwakisa, 2002). The genetic characterization of populations, breeds and species allows evaluation of genetic variability. Molecular markers have been exploited to access this variability as they contribute information on every region of the genome (Pandey et al., 2006). Gwakisa (2002) reported that the most widely used molecular techniques for the study of genetic variations at the DNA level include RFLP, RAPD, AFLP, microsatellites and minisatellites.

### Identification of Disease Carrier:

Infectious diseases are responsible for great losses in economic returns to the livestock farmer. Most of the serious incurable diseases result not from infectious disease-causing organisms but by defective genomes of the individual animals. Certain allelic variations in the host genome lead to susceptibility or resistance to a particular disease (Mitra et al., 1999). Kingsbury (1990) reported that a particular RFLP in the Prion protein gene was responsible for the variation in host's response to the causative agent, and the incubation time of bovine spongiform encephalopathy (BSE).

DNA polymorphism occurring within a gene helps to understand the molecular mechanism and genetic control of several genetic and metabolic disorders and allows the identification of heterozygous carrier – animals which are otherwise phenotypically indistinguishable from normal individuals. The PCR-RFLP assay has been used to identify carrier animals possessing the defective recessive allele in bovine leucocyte adhesion deficiency in cattle (Shuster et al., 1992), hyperkalemic periodic analysis in horses and malignant hyperthermia in pigs (Fujii et al., 1991). Georges et al. (1993) identified carrier animals of weaver disease in cattle using microsatellite (TGLA 116) marker.

### Determination of Parentage:

The identification of parentage in segregating populations generally takes place by means of the exclusion principle. That is, presence at some genetic locus in the offspring of an allele not found in either of the putative parents effectively excludes the particular parental pair from biological parenthood. Highly polymorphic DNA fingerprinting markers have been reported to be very useful in parentage testing (Mitra et al., 1999). Molecular markers can be employed for sire identification in Artificial Insemination programmes.

### Marker-Assisted Selection:

This is a genetic engineering technique which involves the incorporation of DNA markers for selection, to increase the efficiency of the traditional methods of breeding based on phenotypic information. Molecular marker analysis allows the identification of genome segments, QTL contributing to the genetic variance of a trait and thus to select superior genotype by environment interaction (Gholizadeh et al., 2008). Therefore selection for favourable QTL effects based on molecular marker studies has great benefits to offer for the improvement of such economic traits.

### Transgenesis:

This is a procedure in which a gene or part of a gene from one individual is incorporated into the genome of another one. According to Mitra et al. (1999) findings, the starting point of this technology is the identification of the genes of interest. In this context, molecular markers can serve as points of reference for mapping the relevant genes that would be the first step towards their manipulation. Molecular markers could as well be used to identify animals carrying the transgenes for the purpose of multiplication.

### Sex Determination of Offspring:

Molecular markers can be applied in the determination of sex of pre-implantation embryos. This can be achieved by using as probes, Y-chromosome-specific (male-specific) DNA sequence. Peura et al. (1991) reported that using the PCR-based method of sex determination has the advantage of being carried out in less than five hours with almost 100% accuracy. It is less invasive, unlike other cytogenetical methods, and can be done at an early stage of the embryo (Machaty et al., 1993).

The sexing of pre-implantation embryos can serve as an important tool for improving a herd for a desired purpose.



## CHALLENGES TO THE APPLICATION OF MOLECULAR MARKERS

### **Economic factors:**

According to Dekkers and Hospital (2002), "economics is the key determinant for the application of molecular genetics in genetic improvement programmes. The use of markers in selection incurs the costs that are inherent to molecular techniques." Developing costs (e.g. identifying molecular markers on the genome, detecting association between markers and the traits of interest) and running costs (e.g. typing individuals appropriate in the selection programme) can be quite expensive. Besides, the cost of importation of the technology from developed countries could be so outrageous that it may out-weigh whatever benefits that could be derived from it.

### **Mechanical and Logistics factors:**

In Africa presently, functional Biotechnological and Genomic Centres are not very common. Apart from the International Livestock Research Institute, Nairobi, Kenya and the University of Agriculture, Abeokuta, Nigeria, many other centres are lacking in equipment for processes such as DNA extraction & electrophoresis, PCR, hybridization, and amplification. Omitogun (2007), noted that even many well-equipped laboratories in some of the Research Institutes, Universities and Polytechnics in Nigeria, have become 'white elephants' because of lack of materials or consumables to fully use the equipment available. Since molecular markers have to be imported from countries like the USA and the UK, researchers have to place orders long in advance when the need to use such markers arise, and the delivery of these markers to their point of use may take several days. This long delays impacts negatively on the potency of the imported markers, which consequently complicate or distort experimental results.

### **Lack of Funds/Grants to Researchers:**

The researches involving molecular technologies are being hampered in Nigeria and other developing nations due to the inability of researchers to access grants and funds. Many times in Nigeria, researchers are denied opportunity to secure research grants because their institutions or their basic affiliations could not provide the basic equipment/facilities required to effectively carry out some researches (Olowofeso, 2011). Sometimes when research grants are provided, the amount is hardly sufficient to procure all the necessary reagents and other consumables. But it is common knowledge that meaningful research especially molecular studies require a lot of funds.

### **Erratic Power Supply:**

In Nigeria and some other African countries, power supply is very erratic and unsteady. At times for days running into months, some areas do not have electric power supply due to one problem or another, and when provided, might last for few hours. Many Universities and Research Institutes are not left out of this malady. Students and researchers alike have been forced to terminate their experiments involving constant power supply as a result of this menace. Olowofeso (2011) argues that this erratic power supply appears to be the most challenging factor impeding human activities in developing countries. Molecular markers need very cool environment at all times and storage materials like refrigerators and deep freezers connected to a regular supply of electricity is necessary, as markers devoid of a cooler environment will not work when employed in PCR technology.

### **Lack of adequately trained Personnel:**

The application of molecular markers to the improvement of animal species in Nigeria is also being hampered by the non-availability of enough number of adequately trained personnel with the requisite practical experience in the Universities. Some who are well trained have been rendered redundant because of non-motivation, while others have opted to move to the developed countries to work. It is therefore advocated that training and re-training of personnel be carried out to forestall the problem of inadequate human resources.

### **Intellectual Property Rights (IPRs) issues:**

IPRs is playing an ever greater role on food and agriculture in developing countries. It is influencing generally in the negative sense, the quality of agricultural research carried out and the nature of research collaborations between the public and private sector and between developing and developed countries. It is obvious that IPRs may also impact on developing countries such as Nigeria. Where patents are not sought, information on innovations is kept secret, and has negative impact by denying the developing countries access to potentially useful information.

## CONCLUSION

It is no doubt that molecular markers have the potentiality of improving the genetic lot of animal species. It is advocated that Government be more pro-active in tackling the challenges enumerated herein. Public and private sectors are enjoined to look into partnering with Universities and Research Institutes to develop our own molecular technologies.

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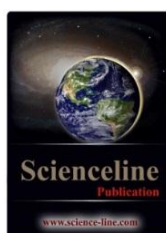
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