# Effect of Nitrogen Source and Phenological Stage on the *in vitro* Digestibility of Oba Super II Maize Fodder

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#### **ABSTRACT**

This experiment was conducted at the laboratory of Department of Pasture and Range Management to evaluate the effect of nitrogen source (swine manure, N.P.K. 15:15:15 as well as the control) and phonological stages on the in vitro digestibility and post incubation parameters of Oba Super II maize fodder. The experiment was in a split plot design. The nitrogen source was applied at the rate of 120 kgN/ha two weeks after planting. Maize was harvested at silking, blister, and dough stage, oven dried and milled. Samples were taken to laboratory for in vitro analysis. Data collected was subjected to analysis of variance (ANOVA) using General Linear Model (GLM).

The result of this study showed that nitrogen source and age at harvest significantly influenced (p>0.05) the volume of gas produced throughout the period of incubation. Oba Super II maize fertilized with swine manure harvested at the dough stage had the highest dry matter digestibility value to be 84.80%. It was concluded that Oba Super II maize fertilized with swine manure and harvested at dough stage had the highest gas volume and can be recommended as a potential source of nutrient for ruminant animals.

(Keywords: in vitro, fodder, nitrogen source, phenological, livestock feed).

#### INTRODUCTION

Forage maize is required to meet the energy requirements for rapid animal growth, animal productivity and maintenance. Quality forage maize can be achieved when the soil fertility is

high but most tropical soils are low in fertility and this can be corrected by adding fertilizer to the soil. This fertilizer can be organic or inorganic.

Maturity is an important pre-harvest factor that affects nutrient content and digestibility of forages (Mussadiq et al., 2012). Forage yield in maize increases and forage quality decreases rapidly as plant matures (Jung and Barker, 1973 and Kunelius et al., 1974), so harvesting at early heading stage is generally considered to be a good time to produce high forage yield with high quality. In any production system, it is usually recommended that cutting should be taken early rather than to be late at the head maturity stage in order to obtain the best combination of yield and quality (Fales et al., 1990). This study was carried out to evaluate the effect of nitrogen source and age at harvest on the in vitro digestibility of Oba Super II maize fodder.

### **MATERIALS AND METHODS**

The field experiment was carried out at Pasture Section of the Directorate of University Farms (DUFARMS) and the *in vitro* digestibility was carried out at the laboratory of Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Nigeria. After land preparation, representative of soils was collected from plots at the depth of 0-15 cm using soil auger and was used to determine the preplanting nutrient status of the soil.

The study was a split plot design with three nitrogen sources (NPK, swine manure, as well as the control) and three phonological stages (silking, blister, and dough stage) as the main

plot and the sub-plot, respectively. All nitrogen sources were applied at the rate of 120 kgN/ha.

Oba super II maize fodder was planted at 50cm x 50cm. At every harvest stage, samples were collected and dried to a constant weight for dry matter content determination. Oven dried samples were milled to pass through a 1mm sieve and stored for chemical analysis. The *in vitro* gas production was determined according to the modified procedure of Menke and Steingass (1988). Data collected were subjected to analysis of variance (ANOVA) using General Linear Model. Level of significance will be taken at 5% probability level using SAS 1999 package.

#### **RESULTS AND DISCUSSION**

The unfertilized maize fodder produced the lowest value of crude protein (13.94) compared to (15.98) and (15.97) produced by the swine and NPK fertilized fodder, respectively. The Dry Matter content (DM), *in vitro* organic matter digestibility (IVOMD), and Acid Detergent Fiber (ADF) were not significantly affected by nitrogen source. NPK fertilized maize had the lowest value for NDF (59.07) which is significantly different from the swine fertilized (60.40) and unfertilized maize (60.97).

At dough stage, the CP content was highest (16.45) compared to (14.31) and (15.12) gotten at silking and blister stages, respectively.

The DM content at the dough stage was highest while at the silking and blister stage, it was significantly similar. IVOMD was not significantly affected (p>0.05) at the three phonological stages. At silking stage, NDF was highest (61.12) and was significantly similar to the value obtained at blister stage (60.35) while at dough stage, NDF was lowest (38.98). ADF was highest at the silking stage which significantly differ from (37.18) and (36.69) obtained from blister and dough stage respectively (Table 1).

NPK fertilized maize at the dough stage had the highest CP while the unfertilized maize at silking stage had the lowest CP. The CP increased steadily at the three phenological stages for all nitrogen sources. NPK fertilized maize at dough stage had the highest value of DM while the unfertilized at dough stage had the lowest value. There was no significant difference in the values of the IVDMD. The unfertilized maize at silking and dough stage had significantly similar values of NDF with the swine fertilized maize at blister and dough stage. At silking stage, NPK fertilized maize had the highest value for ADF (Table 2).

**Table 1:** Main Effect of Nitrogen Source and Phonological Stage on the Chemical Composition of Oba Super II Maize Fodder.

Nitrogen	DM	СР	NDF	ADF					
Swine	93.04	15.98a	60.40a	37.20					
NPK	93.30	15.97a	59.07b	37.38					
Control	92.74	13.94b	60.97a	38.27					
SEM	0.24	0.35	0.44	0.52					
Phenological Stage									
Silking	92.66b	14.31b	61.12a	38.98a					
Blister	92.64b	15.12b	60.35a	37.18b					
Dough	93.78a	16.45a	58.98b	36.69b					
SEM	0.19	0.39	0.45	0.49					

a, b: Means with different alphabet in each column are significantly( p<0.05) different; SEM = Standard Error of Mean

**Table 2:** Interaction Effect of Nitrogen Source and Phonological Stage on the Chemical Composition of Oba Super II Maize Fodder.

Nitrogen	Phenological Stage	DM	СР	NDF	ADF	
Swine	Silking	92.75bc	14.80cd	61.41a	38.94ab	
	Blister	92.59bc	15.94abc	60.25ab	36.36bc	
	Dough	93.79ab	17.18ab	59.57ab	36.31bc	
NPK	Silking	92.96abc	15.02cd	61.69a	40.15a	
	Blister	92.52bc	15.34bc	59.24b	37.19abc	
	Dough	94.42a	17.54a	56.28c	34.80c	
Control	Silking	92.28c	13.10d	60.26ab	37.84abc	
	Blister	92.82bc	14.08cd	61.55a	37.99ab	
	Dough	73.13abc	14.62cd	60.26ab	38.97ab	
SEM		0.14	0.25	0.30	0.32	

a, b, c, d: Means with different alphabet in each column are significantly(p<0.05) different; SEM = Standard Error of Mean

**Table 3:** Main Effect of Nitrogen Source and Phonological Stage on the *in vitro* Gas Production (ml/200mgDM), *in vitro* Dry Matter Digestibility, and Post Incubation Kinetics (b, c, and lag) of Oba Super II Maize Fodder.

Factors		Incubation Time (Hours)					DMD	В	С	LAG
	3	6	12	24	36	48				
	Nitrogen Source									
Swine	2.33a	4.00a	6.67	11.67	15.67a	19.00a	71.11	41.66	0.02	2.28
NPK	1.67ab	3.67ab	7.00	11.33	14.67ab	18.67a	67.33	33.47	0.04	1.89
Control	1.00b	2.67b	6.67	9.67	13.33b	15.67b	63.91	34.66	0.03	1.97
SEM	0. 34	0.35	0.78	0.87	0.84	0.86	4.95	7.02	0.01	0.42
	Phenological Stage									
Silking	1.33b	2.67b	5.00b	10.00b	14.00b	18.33	56.64b	50.27a	0.01 b	2.39ab
Blister	1.33b	3.33ab	6.00b	9.00b	12.67b	16.33	72.30a	40.21a	0.01b	2.55a
Dough	2.33a	4.33a	9.33a	13.67a	17.00a	18.67	73.40a	19.31b	0.07a	1.19b
SEM	0.34	1.05	0.51	0.65	0.67	0.87	4.25	4.82	0.01	0.34

a, b : Means with different alphabet in each column are significantly(p<0.05) different SEM = Standard Error of Mean, b: insoluble fraction, c: constant gas production rate, lag: lag time

At the 48th hour of incubation the unfertilized maize fodder produced the lowest volume of gas (15.57 ml/200mgDM) compared to (19.00 (18.67 mI/200maDM)ml/200maDM) and produced by the swine and NPK fertilized fodder respectively. Phenological stage also influenced the volume of gas produced. At the 24th hour of incubation, maize fodder harvested at dough stage produced the highest gas volume (13.67 ml/200mgDM) which is significantly different (p<0.05) than those harvested at silking and blister stage while the volume of gas produced did not differ significantly (p>0.05) when the phonological stages were compared at the 48th hour of incubation.

There was no significant difference (P>0.05) in the dry matter digestibility (DMD), insoluble fraction (b), constant gas production rate (c) and the lag time with the nitrogen sources imposed. Dry matter digestibility and the value for c increased significantly (p<0.05) with increasing phonological stage while the reverse was the case for b. The lag value obtained at the Dough stage was significantly different (p<0.05) and lower compared to 2.55 ml/200mgDM obtained from plant harvested at dough stage.

Nitrogen source and phonological stage (Table 3) had a significant influence (p>0.05) on the volume of gas produced throughout the period of incubation. The unfertilized maize harvested at

dough stage significantly (p<0.05) produced the highest gas volume from the 6<sup>th</sup> to the 18<sup>th</sup> hour of incubation. At the 24<sup>th</sup> hour of incubation the highest gas volume (14.00 ml/200 mgDM) was obtained from both the unfertilized maize and maize fertilized with swine manure harvested at dough stage.

From the 30<sup>th</sup> to the 48<sup>th</sup> hour of incubation, the unfertilized maize harvested at blister stage had the lowest gas production which ranged from 9.00 ml/200mgDM to 13.00 ml/200mgDM and maize fertilized with swine manure had the highest which ranged from 14.00 to 20.00 ml/200mgDM Dry matter digestibility, values for b and lag were not significantly (p>0.05) affected by the interaction of the nitrogen sources and the phonological stages. Mean values for c significantly ranged from 0.01 ml/200mgDM to 0.07 ml/200mgDM (Table 4).

#### DISCUSSION

The crude protein (CP) content of the maize fodder in this study were higher than the minimum (6.5–8.0 %) prescribed for optimal performance for ruminants in the tropics (Minson, 1981). The CP content of the maize affected by the manures is above the critical limit below which intake of forages by ruminants and rumen microbial activity would be adversely affected (Van Soest, 1994).

The NDF of maize fodder recorded in this study is within the range of 600-650 g/kg suggested as the critical limit above which efficiency of utilization of tropical forages by ruminants would be impaired (Van Soest, 1982; Muia, 2000) and the consistent decrease in the concentration of NDF with advancement in plant age is in line with the submission of Estrada-Flores et al. (2006).

The addition of swine manure raised the volume of gas produced when compared with the unfertilized maize in the present study. This is at variance with the report of Dele (2012) who observed a higher gas volume from the unfertilized grasses relative to the fertilized grasses. The reason for this observation might be because the manure raised the level of nutrient present in the maize plant as such there might have been a higher amount of carbohydrate reserve present in the maize plant available for microbial degradation. Coelho, et al. (1998) reported that gas production is a result of substantial changes in carbohydrate fraction while Wolin (1960) reported that gas production from protein fermentation is relatively small compared to carbohydrate fermentation when contribution of fat to gas production is negligible. This might be the reason for the higher gas volume observed with the swine fertilized maize.

**Table 4:** Interaction Effect of Nitrogen Source and Phonological Stages on the *in vitro* gas Production (ml/200mgDM), *in vitro* Dry Matter Digestibility and Post Incubation Kinetics (b, c, and lag) of Oba Super II Maize.

Nitrogen	Phenological	Incubation Time (Hours)						DMD	В	С	LAG
Source	stage	3	6	12	24	36	48				
Swine	Silking	3.00a	4.00ab	6.00bcd	11.00abc	16.00abc	20.00a	50.53	46.16	0.01c	2.87
	Blister	2.00ab	4.00ab	6.00bcd	10.00abc	13.00bcd	17.00ab	78.00	54.28	0.01c	2.88
	Dough	2.00ab	4.00ab	8.00abc	14.00a	18.00a	20.00a	84.80	24.53	0.04bc	1.10
NPK	Silking	1.00ab	3.00abc	5.00cd	11.00abc	14.00abcd	19.00ab	64.04	57.36	0.01c	1.44
	Blister	1.00ab	4.00ab	7.00bcd	10.00abc	14.00abcd	19.00ab	71.95	28.62	0.02bc	3.13
	Dough	3.00a	4.00ab	9.00ab	13.00ab	16.00abc	18.00ab	66.00	14.43	0.10a	1.09
Control	Silking	0.00b	1.00c	4.00d	8.00bc	12.00cd	16.00ab	55.36	47.28	0.01c	2.87
	Blister	1.00ab	2.00bc	5.00cd	7.00c	11.00d	13.00b	66.96	37.72	0.01c	1.65
	Dough	2.00ab	5.00a	11.00a	14.00a	17.00ab	18.00ab	69.39	18.97	0.07ab	1.38
SEM		0.22	0.27	0.47	0.54	0.52	0.56	2.82	3.97	0.07	0.24

a, b, c, d: Means with different alphabet in each column are significantly( p<0.05) different SEM = Standard Error of Mean, b: insoluble fraction, c: constant gas production rate, lag: lag time

The stage at harvest is an important factor affecting nutritive value of forages. However, plant harvested at the dough stage produced the highest gas volume. Normally it is expected that with maturity of the plant, digestibility will reduce, reverse was however the case in the present study. The reason for this might be due to the presence of grain as the full stalk (fodder and grain) were fed to the rumen inoculum hence the higher gas produced might be due to the inclusion of larger quantity of maize cob. This is in conformity with the findings of Mussadig, et al. (2012) that maturity is an important pre-harvest factor that affects nutrient content and digestibility of forages. This is in line with report observed as maturity had positive effect on gas production in the plant.

The fermentation of the insoluble fraction (b) which was measured by the volume of gas produced in time (t) was higher than the value of 3.20-3.70 ml/200mgDM reported (Colkesen, 2005) for treating barley gain with formaldehyde. This might be as a result of the nitrogen sources and stage of harvest of the maize

The value obtained for the constant gas production rate (c) of maize as affected by the nitrogen source and the harvest stage was higher than values of 0.03-0.04mlhr-1 reported by Sodeinde, et al. (2009). It showed that they are highly digestible and this may be attributed to chemical constituent as influenced by the different nitrogen sources.

The rate at which different chemical constituents are fermented is a reflection of microbial growth and accessibility of the feed to microbial enzymes (Getachew et al., 2004). Similarly, Khazaal et al. (1996) suggested that the intake of a feed was mostly explained by the constant gas production rate (c) which affected the rate of passage of the feed through the rumen. Thus the high value obtained for the (c) indicated a better nutrient degradation for rumen microorganism in animals.

The difference in lag time might be due to the difference in the rate of degradation. The in vitro dry matter digestibility of maize fertilized with swine manure was higher and it increased with increasing harvest stage. This is in contrast with reports of Zinash et al. (1995) who reported a depressed IVDMD of grass species harvested at relatively advanced stages.

## CONCLUSION

From the result of this study, it can be concluded that the NPK fertilized maize harvested at the Dough stage had the highest crude protein and swine manure fertilized maize had the highest gas volume and dry matter digestibility. Oba super II maize fodder harvested at the Dough stage after planting had higher gas volume compared to those harvest at the blister and silking stages. Swine manure fertilized maize produced the highest dry matter digestibility and post incubation kinetics.

#### REFERENCES

- Coelho, M., F.G. Hembry, F.E. Barton, and A.M. Saxton. 1998. "A Comparison of Microbial, Enzymatic, Chemical and Near Infrared Reflectance Spectroscopy Method in Forage Evaluation". Animal Feed Science and Technology. 20:219.
- Colkesen, M., A. Kamalak, O. Canbolat, Y. Gurbuuz, and C.O. Ozkan. 2005. "Effect of Cultivar and Formaldehyde Treatment of Barley Grain on Rumen Fermentation Characteristics using In Vitro Gas Production". South African Journal of Animal Science. 35(3): 206-212.
- Dele, P.A. 2012. "Evaluation of Dry Matter Yield and Nutritive Quality of Forage, Hay and Silage Produced from Three Legumes Fertilized with Animal Manures". Ph.D. Thesis. Department of Pasture and Range Management, University of Agriculture: Abeokuta. 263.
- Estrada-Flores, J.G., M. González-Ronquillo, F.L. Mould, C. M. Arriaga-Jordán, and O. A. Castelán-Ortega. 2006. "Chemical Composition and Fermentation Characteristics of Grain and Different Parts of the Stover from Maize Land Races Harvested at Different Growing Periods in two Zones of Central Mexico". Animal Science. 82: 845-852.
- Fales, S.L., R.R. Hill, and R.J. Hoove. 1990. "Chemical Regulation of Growth and Forage Quality of Cool Season Grasses and Sulphur in Tropical Regions". *Journal of Agricultural Science* 120 (3): 295-299.
- Getachew, G., E.J. Depeters, and P.H. Robbinson. 2004. "In vitro Gas Production Provides Effective Method for Assessing Ruminant Feeds". California Agriculture. 58: 1-12.

- Jung, G.A. and R.S. Barker. 1973. "Orchard Grass" In: M.E. Heath et al (ed). Forges 3<sup>rd</sup> edition. 285-296. lowa State University Press. Ames, Iowa.
- Khazaal, K., Z. Parissi, C. Tsiouvaras, A. Nastis, and E.R. Orskov. 1996. "Assessment of Phenolics-Related Anti-Nutritive Levels using the *in vitro* Gas Production Technique: A Comparison between Different Types of Polyvinyl/Polyroliodone or Polyethylene Glycol". *Animal Science*. 71: 405-414.
- Kunelius, H.T., L.B. Macleod, and F.W. Cadler. 1974. "Effect of Cutting Management on Yields Digestibility, Crude Protein, and Persistence of Timothy, Brome Grass, and Orchard Grass". Canadian Journal of Plant Science. 54:55 – 64.
- Menke, K.H. and H. Steingass. 1988. "Estimation of the Energetic Feed Value Obtained from Chemical Analysis and *In vitro* Gas Production using Rumen Fluid". *Animal Research and Development*. 28: 7-55.
- Minson, D.J. 1981. "Nutritional Differences between Tropical and Temperate Pastures". In: Grazing Animals. Disciplinary Approach". World Animal Science. Volume 34, Elsevier, Amsterdam, Netherlands. 143-157.
- Muia, J.M.K. 2000. "Use of Napier Grass to Improve Smallholder Milk Production in Kenya". Ph.D. Thesis. Wageningen Agricultural University. The Netherlands.
- Mussadiq, Z., M. Hetta, C. Swensson, and A. Gustavsson. 2012. "Plant Development, Agronomic Performance and Nutritive Value of Forage Maize Depending on Hybrid and Marginal Site Conditions at High Latitudes". Acta Agriculturae Scandinavica Section B Soil and Plant Science. 62: 420–430.
- Sodeinde, F.G., J.A. Akinlade, O.A. Aderinola, S.R. Amao, J.A. Alalade, and A.T. Adesokan. 2009.
   "The Effect of Poultry Manure on Proximate Composition and in vitro Gas Production of Panicum maximum cv T 58 in the Derived Savanna Zone of Nigeria". Pakistan Journal of Nutrition. 82: 1262-1265.
- Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant: Ruminant Metabolism, Nutritional Strategies, the Cellulolytic Fermentation and the Chemistry of Forages and Plant Fibres. O&B Books: Corvallis, OR. 375 pp
- Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant, 2<sup>nd</sup> ed. Comstock Publishing Associates/Cornell University Press: Ithaca, NY. 476pp

- 17. Wolin, M.J. 1960. "A Theoretical Rumen Fermentation Balance". *Journal of Dairy Science* 43: 1452-1459.
- Zinash, S., G. Lulseged, and T. Tadesse. 1995. "Effect of Harvesting Stage on Yield and Quality of Natural Pasture in the Central Highlands of Ethiopia". In: Proceedings of third National Conference of the Ethiopian Society of Animal Production (ESAP), pp. 316 – 322. April 27-29, Addis Ababa, Ethiopia.

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