*IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*
*e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 3 Ver. II (March 2018), PP 71-76*
*www.iosrjournals.org*

# *In Vitro* Gas Production Parameters and Fermentation Characteristics Of Maize Stovers Ensiled With Or Without Additives

[1]Onaleye, K. J., [2]Ayoade, J. A., [2]Okwori, A. I. [2]Oloche, J.
*1. Department of Animal Production and Health, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria*
*2. Department of Animal Production, University of Agriculture Makurdi, Benue State*

***Abstract:*** *The in vitro gas production parameters and gas production characteristics of six silage types (T1 – T6) from chopped green maize stover (CGMS) and Chopped dry maize stover (CDMS) ensiled with or without ground dried cassava peels GCP) or dried poultry litter DPL) were assessed using the gas production technique. The chemical composition of the silages was also analyzed. Crude protein (CP) was highest (11.94%) in T3 (CGMS + 36% w/w DPL) and lowest (5.06%) in T5 (5.06%). Nitrogen free extract (NFE) ranged from 52.02% in T3 (CGMS + 36% w/w DPL) to 63.78% in T5 (CDMS + 5% w/w GCP). In vitro gas production volume (IVGP), organic matter digestibility (OMD %), metabolizable energy (ME), methane ($CH_4$) and short chain fatty acid (SCFA) were not significantly ($P > 0.05$) different among the silages but dry matter degradability (DMD %) significantly differ ($P < 0.05$), T1 being the highest (60.53%) and T4 the lowest (29.27%). Gas production characteristics were significantly different among the silages. It was concluded that ensiling maize stover with ground dried cassava peels or dried poultry litter improved the gas production characteristics and the nutritive value of the silage.*

***Keywords:*** *in vitro, gas production characteristics*

---

Date of Submission: 07-03-2018 Date of acceptance: 23-03-2018

---

## I. Introduction

Several assessments have indicated that increases in demand for livestock products, consequent to increases in human population growth, income growth and urbanization, will continue for at least the next three decades [1]. To meet the protein requirement of the ever increasing human populace, our huge livestock population needs special attention of nutritionists for supplying sufficient nutrients not only to fulfill their hunger but also to maintain optimum productivity potentials [2].

Since increased human population and urbanization is accompanied by reduced hope of increasing cultivated area for green forage or regenerating degraded pastures, the feasible alternative is efficient utilization of crop residues for ruminant feeding. Crop residues output has steadily increased during the last four decades in developing countries and have consistently gained recognition as ruminant feedstuffs as they constitute a major portion of roughages. The high percentage of structural carbohydrates and low nitrogen contents of crop residues, however, result in low palatability and poor nutrient utilization in ruminants [3]. Ensiling feedstuffs with water soluble carbohydrates or poultry litter have been found beneficial [4,5]

Feed evaluation is essential for feed producers to know the energy, protein and major mineral levels of the feeds so as to develop balanced, least-cost diets for livestock [6]. The *in vitro* gas production technique has, for many years, enjoyed so much of advocacy in its use for studying nutritive value of ruminant feedstuffs, having the advantages of being less expensive, less time consuming and allowing the screening of small quantities of feed samples [7, 8].

## II. Materials And Method

***Preparation of samples***

Samples were taken from each of the ensiled stovers and oven-dried at 105$^oC$ to constant weight. The dried samples were milled through 1mm sieve hammer mill and 200mg milled sample of each silage type was weighed into incubation bag before placement in the cylinders of 100ml plastic syringes in triplicates. Each treatment had triplicates of syringes without substrates called blanks. The pistons were gently fitted and silicon rubber tubes properly clipped. Samples were also collected for proximate composition

***Collection and preparation of rumen fluid***

Rumen fluid was collected from three (3) goats under the same feeding regime using suction tube as described by Babayemi and Bankole [9], prior to morning feeding. The fluid was collected into thermos flask and taken to the laboratory where it was strained through a four-layered cheese cloth into warm flask, constantly flushed with $CO_2$ gas.

***Preparation of Buffer Solution***

The buffer used consisted of 9.8g $NaHCO_3$ + 2.77g $Na_2HPO_4$ + 0.57g KCl + 0.47g NaCl + 0.12g $MgSO_4.7H_2O$ + 0.16g $CaCl_2.2H_2O$ per litre and was stored in dark bottle. $CaCl_2.2H_2O$ was the last solute to be added after the others have been in the solution. Before use, a volume of buffer was warmed at 30$^o$C under continuous flushing with $CO_2$. During this process, Urea was added to the buffer at the rate of 1.0g/litre.

***In vitro* gas production**

The *in vitro* gas production was done according to the method described by Menke and Steingass [10]. Inoculum for incubation of samples was prepared by mixing strained rumen liquor with buffer at the ratio 1:4 (v/v). The syringes containing the samples were pre-warmed for an hour in the incubator at 39$^o$C before the start of incubation. The steel clip on the silicon rubber tube of each syringe was loosened and 30ml of inoculum dispensed into the syringes through the rubber tubing. The same volume of inoculum was introduced into the blanks (syringes without substrates). The level of pistons of the syringes and the time of inoculation were recorded.

The set-up was incubated at 39$^o$C in the incubator for 24 hours while the volume of gas produced was recorded at 3-hour intervals as 6, 9, 12, 15, 18, 21, 24 hours. The volume of gas was read by measuring the head space formed between the top of the piston and the mixture in the syringe.

***Measurement of methane gas produced***

After 24 hours of incubation, 4ml of 10M NaOH was introduced into the syringes through the silicon tube and properly shaken for proper mixing and absorption of $CO_2$ gas by the NaOH solution introduced, according to the method described by Fievez, *et al.* [11]. After all the $CO_2$ gas might have been absorbed, the volume of methane left in the syringes was recorded in ml. The average volume of gas produced from the blanks was deducted from the volume of gas per sample to estimate the net gas produced for each sample.

***Calculation***

The gas production characteristics were estimated using the Fit curve macro NEWAY Excel developed by Chen, [12] for estimating rumen degradability and gas production characteristics, adapting the non-linear equation of McDonald [13]: $Y = a + b\,(1-e^{-c\,(t-tl)})$ for feedstuffs with lag time tl;

Where Y= volume of gas produced at time t,

a = volume of gas produced from soluble fraction

b = volume of gas produced from insoluble but degradable fraction,

(a+b) = total volume of gas produced,

c = rate of gas production

t = incubation time,

tl = lag time

The post incubation parameters such as metabolizable energy (ME, MJ/kg DM), Organic Matter Degradability (OMD, %) and Short Chain Fatty Acids (SCFA) were estimated at the 24 hours post gas production exercise according to [10]

ME = 2.20 + 0.136 Gv + 0.057 CP + 0.0029 CF

OMD (%) = 14.88 + 0.889 Gv + 0.45 CP + 0.0651 XA

SCFA= 0.0239 Gv – 0.0601

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

CP= Crude protein

CF= Crude fiber,

XA= Total ash of the incubated sample

Data collected were subjected to analysis of variance (ANOVA) using the SAS [14] software package in a completely randomized design. Where significant differences existed in means, they were separated using Duncan [15] Multiple range multiple F-test

## III. Result

The results of the proximate composition of the silages are presented in Table 1. The CP ranged from 5.06% in T5 to 11.94% in T3. The CF ranged from 20.10% in T3 to 23.37% in T6. Silage DM ranged from 28.10 in T4 to 38.30 in T6. Ash and EE were lowest (3.25 and 0.95% respectively) in T1 and highest (10.35 and 2.4% respectively) in T6 while NFE content ranged from 57.02% in T3 to 63.78% in T5.

Neutral detergent fibre (NDF) was highest (59.43%) in T1 (CGMS only) and with highest (30.12%) amount of hemicellulose. Lignin was highest (17.02%) in T4 (CDMS only) and lowest (8.11%) in T5 (CDMS + GCP).

**Table 1:** Chemical Composition (%) of Maize Stovers ensiled with or without ground dried cassava peels or dried poultry litter

| **Nutrients** (%) | CGMS Only (T1) | CGMS + 5% GCP (T2) | CGMS + 36% DPL (T3) | CDMS only (T4) | CDMS + 5% GCP (T5) | CDMS +36%DPL (T6) |
|---|---|---|---|---|---|---|
| Dry matter | 28.90 | 32.90 | 36.80 | 28.10 | 32.00 | 38.30 |
| Crude protein | 8.75 | 7.00 | 11.94 | 6.23 | 5.06 | 6.19 |
| Crude fibre | 23.36 | 21.30 | 20.10 | 23.50 | 22.59 | 23.37 |
| Ash | 3.25 | 6.66 | 9.02 | 5.97 | 6.67 | 10.35 |
| Ether extract | 0.95 | 1.71 | 1.92 | 1.68 | 1.90 | 2.40 |
| NFE | 63.69 | 63.33 | 57.02 | 62.62 | 63.78 | 57.69 |
| **Fibre fraction (%)** | | | | | | |
| NDF | 59.43 | 40.72 | 43.93 | 52.38 | 48.67 | 47.55 |
| ADF | 29.31 | 29.50 | 28.72 | 30.60 | 39.14 | 37.48 |
| ADL | 11.26 | 10.41 | 9.35 | 17.02 | 8.11 | 8.79 |
| Hemicellulose | 30.12 | 11.22 | 15.21 | 21.78 | 9.53 | 10.07 |
| Cellulose | 18.05 | 19.09 | 19.37 | 13.58 | 29.61 | 28.69 |

CGMS = Chopped Green Maize Stover only (CGMS only)
CDMS = Chopped Dry Maize Stover only (CDMS only)
GCP = Ground Dried Cassava Peels
DPL = Dried Poultry Litter
+ = ensiled with
**N**FE = Nitrogen Free Extract
NDF = Neutral detergent fibre
ADF = Acid detergent fibre
ADL = Acid detergent lignin

The result of the *in vitro* gas production parameters is presented in Table 2. There were no significant differences (P > 0.05) in total gas volume (TGV), *in vitro* organic matter digestibility IVOMD, metabolizable energy (ME), short chain fatty acid (SCFA) and methane ($CH_4$) production. Absolute values however indicated that T4 (CDMS only) had higher values than the other silages in all the parameter stated above. *In vitro* dry matter degradability was significantly (P < 0.05) higher (60.53%) in T1 than T4 (29.27%) but similar to T2, T3, T5 and T6.

**Table 2:** *In vitro* fermentation parameters of ensiled maize stover and concentrate supplements

| Parameter | *Experimental treatments T1 | T2 | T3 | T4 | T5 | T6 | SEM |
|---|---|---|---|---|---|---|---|
| Total GasVolume (ml/200 mg DM) | 64.67 | 65.80 | 63.81 | 76.78 | 61.00 | 55.90 | 11.08$^{ns}$ |
| Methane (ml/200 mg DM) | 16.67 | 26.00 | 20.00 | 23.33 | 21.33 | 19.33 | 3.28$^{ns}$ |
| IV Organic Matter Digestibility (%) | 76.34 | 76.96 | 77.57 | 86.33 | 71.83 | 68.05 | 9.85$^{ns}$ |
| IV Dry Matter Digestibility (%) | 60.53$^{a}$ | 43.57$^{ab}$ | 48.10$^{ab}$ | 29.27$^{b}$ | 35.57$^{ab}$ | 45.23$^{ab}$ | 9.65* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Metabolizable Energy (MJ/Kg DM) | 11.54 | 11.61 | 11.62 | 13.06 | 10.85 | 10.23 | $1.51^{ns}$ |
| Short Chain Fatty Acids (mmol/200mgDM) | 1.54 | 1.57 | 1.52 | 1.83 | 1.45 | 1.33 | $0.26^{ns}$ |

*Means with different superscripts are significantly different (P < 0.05)*
*T1- T6 as in table 1
IV = *In vitro*
SEM = Standard error of mean

The *in vitro* gas production characteristics are presented in Table 3. The gas produced from soluble fraction ‘a’, from insoluble but degradable fraction ‘b’ and from the potentially degradable fraction ‘a + b’ were all significantly (P < 0.05) different among the treatments with T5 and T6 having similar but higher values than other silage types. All the silages had negative ‘a’ values. Potential extent of gas production from insoluble but degradable fraction ‘b’ ranged from 55.21(ml/200mg DM) in T2 to 162.26 (ml/200mg DM) in T6. Gas production from the potentially degradable portion ‘a + b’ was highest (159.01ml/200mg DM) in T6 and lowest (33.54ml/200mg DM) in T1.

The gas production rate constants ‘c’ were also significantly (P < 0.05) different. T1 had the fastest (0.16 fraction/h) rate and T5 and T6 had similar and lowest (0.01 fraction/h) rate. All the silages exhibited lag phase (l) during the incubation which were also significantly (P < 0.05) different. Lag phase for T5 was significantly (P < 0.05) higher (6.7 h) than T2 (5.67 h) and T3 (5.5h).

**Table no 3 :** *In vitro* fermentation characteristics of ensiled maize stover and concentrate supplements

| | *Experimental treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | T5 | T6 | SEM |
| a | $-34.60^{c}$ | $-11.86^{ab}$ | $-0.79^{a}$ | $-17.27^{a}$ | $-0.48^{a}$ | $-3.25^{a}$ | $4.14^{*}$ |
| b | $68.14^{b}$ | $55.21^{b}$ | $69.68^{b}$ | $77.69^{b}$ | $139.92^{a}$ | $162.21^{a}$ | $13.32^{*}$ |
| a+b | $33.54^{b}$ | $43.35^{b}$ | $68.90^{b}$ | $60.42^{b}$ | $139.44^{a}$ | $159.01^{a}$ | $15.12^{*}$ |
| c | $0.16^{a}$ | $0.07^{b}$ | $0.04^{bc}$ | $0.08^{b}$ | $0.01^{c}$ | $0.01^{c}$ | $0.02^{*}$ |
| l | $5.80^{bc}$ | $5.67^{c}$ | $5.50^{c}$ | $6.10^{bc}$ | $6.70^{a}$ | $6.30^{ab}$ | $0.18^{*}$ |

*Means with different superscripts are significantly different (P < 0.05)*
*T1- T6 as in table 1
a = Gas from soluble fraction
b = Potential extent of gas production
a + b = Gas production from potentially fermentable fraction
c = Rate of fermentation
l = Lag time

## IV. Discussion

***Chemical composition***

The crude protein (CP) content of 5.06, 6.19, 6.23 and 7.00% for T5, T6, T4 and T2 respectively were lower than the 1.35% N (8.44% CP) minimum requirement for optimum activities of the rumen microbes [16]. Silages T1 (8.75% CP) and T3 (11.94% CP), however meet the minimum CP requirement prescribed by Norton [16]. T3 also meet the 11.3% minimum CP requirement for growth in ruminants [17]. The lower CP contents of T2 and T5 compared to the CP of their corresponding control silages (T1 and T4 respectively) may be attributed to the dilution effect of starch from the ground cassava peels (GCP) used as ensiling additive and corroborates the report of Deaville and Givens [18].

The NDF was highest (59.43%) in T1 and lowest (40.72%) in T2 although a larger portion of the NDF in T1 is hemicellulose which is more digestible by rumen microbes. T4 had a lignin content (17.00%) similar to the 16.80% reported by Amuda *et al.* [19] for maize stover silage. All other silages had lower lignin content than the 14.00 – 16.8% reported by [19].

***Gas production parameters***

Although there were no significant differences ($P < 0.05$) among the silages in TGV, $CH_4$, OMD, ME and SCFA, absolute values indicated that T4 produced the highest (77.78ml/200mg DM) volume of gas, a substantial amount (23.33ml) of which is methane, a nutritionally wasteful product [20].

Similarly, although T1 and T4 (both control) had similar values of NFE and ME, T1 had significantly ($P < 0.05$) higher (60.53%) IVDMD than T4 (29.27%). T2 and T5 (ensiled with 5% GCP) had similar NFE and ME, T2 had higher (43.57%) absolute value of IVDMD than T5 (35.57%). Similarly, although T3 and T6 were similar in NFE and ME values, T3 had higher (48.10%) absolute value of IVDMD than T6 (45.23%).

Each of the pairs compared comprised of silage from fresh and dry maize stover with fresh maize stover silage having higher IVDMD than the dry maize stover silage. The observed differences in IVDMD between comparative silages agree with the report of Cone, *et al.* [21], who noted that the stage at harvest affects the digestibility of feed resources and that two maize silages with the same energy value may differ in animal performance (DMD inclusive) because of different energy composition ( starch vs cell wall).

The extremely low (29.27%) IVDMD recorded for T4 as compared to other silage treatments could be attributed to its high (17.0%) lignin content. Depeters & Heguy [22] earlier noted that high ADL depresses digestibility of feed by rumen microbes. Considering the minimum required degradability range of 40 – 50% [23], silages T1, T2, T3 and T6 could serve as dry season maintenance ration for ruminants with little or no concentrate supplementation while T4 and T5 must necessarily be supplemented to contribute to dry season ruminant feed [23].

***Gas production characteristics***

The potential extent of gas production from the insoluble but degradable fraction 'b' and gas production from the potentially degradable fraction 'a + b' were significantly different ($P < 0.05$) among the silages and both follow the same trend. T5 and T6 were similar but significantly ($P < 0.05$) higher than the other silages in 'b' and 'a + b' values. Among the silages from dry maize stover (T4 – T6), T6 had the highest (159.01ml/200mg DM) while T4 had the lowest (60.42ml/200mg DM). Also among the silages from fresh maize stover (T1 – T3), T3 had the highest (68.90ml/200mg DM) absolute value of 'a + b' while T1 had the least (33.54ml/200mg DM) value.

Although T5 and T6 or T2 and T3 were statistically similar, absolute value of T6 was higher than T5 and that of T3 higher than T2. This suggests that the use of poultry litter as ensilage additive improves gas production characteristic better than the use of ground cassava peels [4]. The observed differences might also be due to the interplay of lignin and CP content of the different samples as high ADL had been noted to depress digestibility [22].

All the silages exhibited lag phase (L) which was the time taken for the microbes to effectively colonize the substrate. The earliest to start gas production was T3 (5.50 h) which also had the highest (11.94%) CP. This observation agrees with the earlier finding of Norton [16]. The highest (0.16 mg/h) rate of gas production 'c' was however observed in T1 and the lowest (0.01mg/h) rate in T5 and T6. This perhaps may be due to the presence of more fermentable carbohydrates (hemicelluloses) in T1 as compared to other silage types [20].

## V. Conclusion

It was concluded from this study that dried poultry litter or ground cassava peels as silage additives improved the gas production characteristic of maize stover silage and could be used to ensile maize stover as dry season feed for ruminants.

## Acknowledgement

The authors acknowledge the Tertiary Education Training Fund (TETFund) and the management of Federal University Wukari Nigeria for the financial support. The Management of the Department of Animal Production Makurdi is also acknowledged.

## References

[1]. Thornton, P.K.. (2010). Livestock Production: Recent Trends, Future Prospects, In: Charles, H., Godfray, J., John, R.B., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.P., Robinson, S. and Toulmin, C. (Eds), (2010) "Food Security: Feeding the World in 2050" *Philosophical Transactions of the Royal Society of London, B series, Biological Sciences*. 365 (1554): 2853–2867 *doi: 10.1098/rstb.2010.0134*

[2]. Waje, S.H., Singh, S.K. and Vishal M. (2010) Effect of using forest grass based complete rations on growth and nutrient utilization in growing crossbred calves. *Animal Nutrition and Feed Technology*, 10: 229-234

[3]. Dhuria, R.K, Sharma, T. and Purohit, G.R. (2011) Effect of densification of gram straw based complete feed on rumen and haemato-biochemical parameters in Magra lambs. *Animal Nutrition and Feed Technology* 11: 133-141

[4]. Jordaan, J.D. (2004). The Influence of Bedding Material and Collection Period on the Feeding Value of Broiler and Layer Litter, M.Sc. Thesis, University of Free State, South Africa. 84pp.

[5]. Moran, J. (2005). Making Quality Silage, In: Tropical Dairy Farming: Feeding management for smallholder dairy farmers in the humid tropics. Landlinks Press, pp 83-97

[6]. Lardy, G. and Anderson, V., (2009) Alternative Feeds for Ruminants. North Dakota State University Extension Service, AS-1182 (Revised) https://www.ag.ndsu.edu/wardcountyextension/livestock/AS1182.pdf. Accessed 02 2018

[7]. Makkar, H.P.S. (2004). Recent Advances in *in vitro* gas method for evaluation of nutritional quality of feed resources. Animal Production and Health section, Joint FAO/IAEA Division, *International Atomic Energy Agency Publications*, pp. 1-10

[8]. Njidda, A.A., Ikhinioya, I and Mohammed I.R. (2010)Feed potential of *Zizipus Mauritian* as bowse to goats in Sudano-sahelian region of Nigeria, *Proceedings of the* 35th *Conference of the Nigerian Society of Animal Production* held on the 14th -17th March, 2010. Univ. of Ibadan, Ibadan, Nigeria. pp 473-476.

[9]. Babayemi, O.J., Bankole, M.A. (2006). Nutritive value of *Tephrosia candida* seed in West African Dwarf Goats. *J. Cent. Eur. Agric.* 7(4): 731-738

[10]. Menke, K.H. and Steingass, H. (1988). Estimation of Energetic Feed Value from Chemical Analysis and *in vitro* Gas Production using Rumen Fluid. *Anim. Res. Dev*. 28: 7-55

[11]. Fievez, V.O., Babayemi, J.O. and Demeyer, D. (2005). Estimation of Direct and Indirect Gas Production in Syringes; A tool to Estimate Short Chain Fatty Acid Production Requiring Minimal Laboratory Facilities. *Anim. Feed Sci. Tech.* 123 (1): 197-210

[12]. Chen, X.B. (1995). Fit Curve Macros for Microsoft Excel (NEWAY EXCEL). Unpublished note pad

[13]. McDonald, I. (1981). A revised model for the estimation of protein degradability in the rumen. *Journal of Agricultural Science* (Cambridge) 96: 251-252

[14]. SAS. (2002). Statistical Analysis Systems, User'Guide, SAS Institute Inc. Cary, North Carolina, USA.

[15]. Duncan, D.B. (1955). Multiple range and Multiple F-Tests. Biometrics, 11: 1 – 42.

[16]. Norton, B. W. (1994). "The Nutritive value of tree legumes". In: Getteridge, R.C. & Shelton, P. (Eds.). Forage Tree Legumes in Tropical Agriculture. CAB International.pp178-191. *http://www.fao.org/ag/agP/agpc/doc/Publicat/Gutt-shelx5556e0j.htm*

[17]. A.R.C (1984). Agricultural Research Council. The Nutrient Requirements of Livestock. Commonwealth Agricultural Bureaux, Slough, England

[18]. Deaville, E.R. and Givens, D.1. (2001). Use of the automated gas production technique to determine the fermentation kinetics of carbohydrate fractions in maize silage. *Animal Feed Science and Technology* 93: 205-215.

[19]. Amuda, A.J., Falola, O.O. and Babayemi, O.J. (2017). Chemical composition and quality characteristics of ensiled maize stover. *FUW Trends in Science and Technology Journal* 2 (1A): 195 – 198 e-ISSN: 24085162; p-ISSN: 24085170

[20]. Oloche, J., Ayoade, J.A. and Oluremi, O.I.A. (2013). *In Vitro* Gas Production Parameters and Characteristics of Four Types of Sweet Orange (C*itrus Sinensis*) Peels Meal *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372*. 5( 3): 5-8

[21]. Cone, J.W., Van Gelder, A.H., Van Schooten, H.A. and Groten, J.A.M. (2008). Effects of forage maize type and maturity stage on *in vitro* rumen fermentation characteristics NJAS- Wagenigen *Journal of Life Sciences* 55 (2) 139 - 154

[22]. Depeters, E. D and Heguy, J. (2013). The ABC's of forage analysis – fiber & digestibility

[23]. http://www.milkproduction.com/Library/Scientific-articles/Nutrition/The-ABCs-of-forage-analysis--fiber--digestibility/

[24]. FAO (1986). Food and Agriculture Organization, FAO Production Year Book, 40: 95 and 247


1Onaleye, K. J "In Vitro Gas Production Parameters and Fermentation Characteristics Of Maize Stovers Ensiled With Or Without Additives." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.3 (2018): 71-76.