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Additive Effects of *Vernonia amygdalina* and *Ocimum gratissimum* on the performance and Egg Quality Parameters of laying Hens

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A R T I C L E I NFO

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egg qualities

and performance.

ABSTRACT

Sixteen (16) weeks feeding trial was conducted to determine the additive effects of dietary inclusion of Vernonia amygdalina (bitter leaf) and Ocimum gratissimum (scent leaf) leaf meals, performance and egg quality parameters on 150 ISA Brown layers. The bitter leaf (BL) and scent leaf (SL) were analyzed to contain 91.87% and 90.03%; 13.52% and 15.52%; 12.22% and 10.39%; 8.55% and 10.55%; 8.18% and 6.73%;1.34% and 1.18%; 6.00% and 3.60%; 2.08% and 1.96%; 0.148mg/dl and 0.096 mg/dl; and ++ and +++ for dry matter, crude protein, crude fibre, ether extract, ash, alkaloid, tannin, flavonoid, steroid and saponins respectively. Five isonitrogenous diets were formulated to contain BL and SL, 0%:0% which is the control (T1); 0%:0% with antibiotics (T2); 75%:25% (T3), 50%:50% (T4) and 25%:75% (T5) respectively. Each of the dietary treatments were replicated thrice, with ten birds per replicate, in a completely randomized design. The pullets (8 weeks) were first maintained on the control diet, up to 12 weeks of age, and were randomly distributed for the administration of the experimental pullets' diets containing approximately 2500Kcal/Kg ME and 14% crude protein. At 14 weeks of age, the birds were administered the experimental diets for a period of 4 months. Data were collected on some performance characteristics and egg internal and external qualities parameters. The results obtained indicated that at the combination level of 25%BL and 75% SL, reduced feed intake, feed efficiency, percent hen day and final body weight. Similarly, at 25% BL and 75%SL, a corresponding increase in the eggshell thickness, yolk colour, yolk weight, haugh unit and egg shape were recorded. Conclusively, laying birds can be fed 25% BL and 75% SL inclusion level to obtain good performance and acceptable egg quality.

1.0 Introduction

The decrease in acceptability to solely rely upon conventional medication (drugs), for a therapeutic approach to animal health, is intensifying. As the global human population continues its upward mobility, we are inevitably faced with the challenges to ensure our access to safe, nutritious, and healthy food. Globalization and the incident of rapid travel, have made transboundary diseases a great concern for food safety and food security (Garcia, et al, 2020). It cannot be overemphasized that transboundary diseases are highly transmittable animal diseases that result in high morbidity and mortality in animals. In an attempt to solve the concomitant animal and human infections due to pathogens, the awareness that antibiotic resistance is a global health challenge leaves us with a strong need to escape this ominous threat to life. Larsson and Flach (2022), observed that evolutionary events that lead to the emergence of new resistance factors in pathogens are rare and difficult to predict, but may be associated with vast ramifications. Ziggers, (2012), reported that more people in hospitals are becoming infected with resistant bacteria that doctors cannot treat with antibiotics, whereas in Europe around 25,000 people die yearly because of this. He further stressed that because of the long and intensive use of antibiotics in animal husbandry, these bacteria have become resistant to most antibiotics used in hospitals. Consumers are always thinking or concerned about the residual effect of antibiotics in poultry products, such as eggs. Muaz, et al (2018) reported that antibiotics may cause the beneficial gut microbiota not to function effectively, and as well increase drug resistance among harmful microorganisms in poultry. Consequently, nutrition is being more widely used as a practical solution for maintaining animal health. To develop a beneficial nutrition-based health strategy, it is necessary to consider the basic interactions between health and nutrition, challenge the current concepts of nutrition and challenge the current concepts of nutritional requirements based on avoidance of deficiency symptoms. Feed components and nutrients influence health in many different ways. By manipulating or selecting them, feed quality may be maintained and the rise of mycotoxin contamination may be reduced. Nutritional components have a positive effect on maintaining the gastrointestinal tract and alleviating the threat of enteric diseases. They influence many non-infectious diseases through the control of oxidative stress. It is now a common reflection in raising animals for food, that nutrition is all there is for a holistic production. Therefore, a nutrition-based health strategy must play a major role in the conventional development of animal production.

Poultry scientists are becoming interested in how poultry production can run without using antibiotics and other drugs because of the criticism and argument on their uses. Pandey et al (2019), stated that animals having an optimum growth performance, need to operate on high health status, of which the use of proper additives is a popular argument in such cases. They further opined that with the upgraded standards in the industry, and consumer awareness, as well as demand for healthy animal-based food products, there is increased pressure on the industry for more phyto-based and non-residual alternatives, than the conventional feed additives which are losing popularity. Some herbs, spices and extracts have been found to stimulate feed intake and endogenous secretions or possess antimicrobial coccidiostats or anthelminthic activities (Hossain, 2009); Karimi *et al* (2010) suggested that these herbal products may serve as environmentally friendly alternatives to the antibiotic growth promoters commonly used in animal and poultry feed. The expected mode of action of herbal products is attributed to their antimicrobial characteristics, oxidative-resistant activity, immune system boost, and concomitant improvement in overall poultry performance. Recent studies that involved the use of such herbs as bitter leaf and scent leaf in poultry feed have given profound results in production performance. Thus, the objective of this study was to determine the additive effects of bitter leaf and scent leaf on the productive performance and egg quality parameters of laying hens.

2.0 Materials and Methods

This experiment was conducted at the poultry research unit of Delta State University, Asaba Campus. The bitter leaf and scent leaf were the test ingredients used for this study and were procured from Asaba and the immediate neighbourhood. They were air-dried at room temperature and later milled for subsequent inclusion at specified levels in the diets of growing pullets and laying pullets.

One hundred and fifty (8 weeks old) pullets, managed on deep litter, were used for this trial. There were five dietary treatments with three replicates per treatment and ten birds per replicate. A completely randomized design was used and the experimental rations were administered at twelve (12) weeks of age. Ration 1, which had no bitter leaf and scent leaf, served as the control. Ration 2 had no bitter leaf and scent leaf meals but had antibiotic inclusion. Rations 3 to 5 had bitter leaf and scent leaf meals inclusion at these respective levels, 75% BL + 25% SL, 50% BL+ 50% SL, and 25% BL+ 75% SL (Tables 2 and 3). Proximate analysis and phytochemical constituents were carried out on the bitter leaf and scent leaf meals respectively as shown in Table 1.

Table 1: Proximate Composition and Phytochemical Constituents of Bitter Leaf (BL) and Scent Leaf (SL)

Parameters	BL	SL
Dry matter (%)	91.87	90.03
Crude Protein (%)	13.52	15.52
Crude Fibre (%)	12.22	10.39
Ether Extract (%)	8.85	10.55
Ash (%)	8.18	6.73
Nitrogen free extract (%)	49.10	46.84
Phytochemicals		
Alkaloid (%)	1.34	1.18
Tannin (%)	6.00	3.60
Flavonoid (%)	2.08	1.96
Steroid (mg/dl)	0.148	0.096
Saponins (+)	++	+++

Table 2: Composition of Experimental Diets (g/Kg) for growing pullets

Inclusion Levels (%) Treatments Ingredients	0BL 0SL 1 (Control)	*0BL *0SL *2	75BL 25SL 3	50BL 50SL 4	25BL 75SL 5
Maize	374.00	374.00	350.00	350.00	350.00
Full fat soybean	30.00	30.00	34.00	34.00	34.00
Groundnut cake	18.0	18.00	18.00	18.00	18.00
Fish meal	15.00	15.00	15.00	55.00	15.00
Wheat Offal	505.00	505.00	505.00	505.00	505.00
Bitter Leaf (BL)	0.00	0.00	15.00	10.00	5.00
Scent Leaf	0.00	0.00	5.00	10.00	15.00
Antibiotic +	-	+	-	-	-
Palm oil	√	√	√	√	√
Bone Meal	20.00	20.00	20.00	20.00	20.00
Oyster Shell	30.00	30.00	30.00	30.00	30.00
Salt	2.00	2.00	2.00	2.00	2.00
Premix**	3.00	3.00	3.00	3.00	3.00
Methionine	2.00	2.00	2.00	2.00	2.00
Lysine	1.00	1.00	1.00	1.00	1.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Calculated Analysis					
Crude Protein (%)	14.09	14.09	14.08	14.10	14.09
Metabolizable Energy (Kcal/Kg)	2,495.62	2,495.62	2,490.62	2,490.62	2,490.62

***Antibiotic (Tyl- dox extra wsp: Tylosin Tartrate 20g, Doxycycline Hyclate 15g)**
+ Antibiotic added only to treatment 2
***** Vitamin-mineral premix provided the following vitamins and minerals per kg of diet. Vit A, 15,000 I.U; Vit D3, 3000 I.U; Vit E, 30 I.U; Vit K, 2.5mg; Vit B12, 0.2mg; Niacin, 40mg Pantothenic acid; Folic acid, 1.0mg; Biotin, 0.08mg; Choline, 500mg; Mn, 6mg; Fe, 24mg; Cu, 6mg; I, 1.4mg; Se, 0.25mg; Co, 0.4mg; Antioxidant, 125mg.***

Table 3: Composition of Experimental Diets (g/Kg) for layers

Inclusion Levels (%) Treatment Ingredients	0BL 0SL 1 (Control)	*0BL *0SL *2	75BL 25SL 3	50BL 50SL 4	25BL 75SL 5
Maize	452.00	452.00	427.00	427.00	427.00
Full fat soybean	160.00	160.00	165.00	165.00	165.00
Groundnut cake	40.00	40.00	40.00	40.00	40.00
Fish meal	30.00	30.00	30.00	30.00	30.00
Wheat Offal	205.00	205.00	205.00	205.00	205.00
Bitter Leaf (BL)	0.00	0.00	15.00	10.00	5.00
Scent Leaf	0.00	0.00	5.00	10.00	15.00
Antibiotic +	-	+	-	-	-
Bone Meal	30.00	30.00	30.00	30.00	30.00
Oyster Shell	75.00	75.00	75.00	75.00	75.00
Salt	2.00	2.00	2.00	2.00	2.00
Premix**	3.00	3.00	3.00	3.00	3.00
Methionine	2.00	2.00	2.00	2.00	2.00
Lysine	1.00	1.00	1.00	1.00	1.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Calculated Analysis					
Crude Protein (%)	17.03	17.03	17.01	17.01	17.00
Metabolizable Energy (Kcal/Kg)	2,524.40	2,524.40	2,504.40	2,504.40	2,504.40

***Antibiotic (Tylo-dox extra wsp: Tylosin Tartrate 20g, Doxycycline Hyclate 15g)**
+ Antibiotic added only to treatment 2
***** Vitamin-mineral premix provided the following vitamins and minerals per kg of diet. Vit A, 15,000 I.U; Vit D3, 3000 I.U; Vit E, 30 I.U; Vit K, 2.5mg; Vit B12, 0.2mg; Niacin, 40mg Pantothenic acid; Folic acid, 1.0mg; Biotin, 0.08mg; Choline, 500mg; Mn, 6mg; Fe, 24mg; Cu, 6mg; I, 1.4mg; Se, 0.25mg; Co, 0.4mg; Antioxidant, 125mg.***

The diets and water were given *ad libitum* to the birds for the four months the experiment lasted. The initial and final weights of the birds per replicate were taken at the beginning and at the end of the experiment, respectively. Feed intake was measured weekly while egg production records were taken daily.

All eggs collected from each replicate group were used in estimating the average egg weight of the eggs using the Labtech^R 1400 electronic platform scale. After weighing, two eggs from each replicate were broken out into a flat, white plate and with the aid of vernier callipers, the albumin heights were measured for the calculation of Haugh unit values according to the formula of Oluyemi and Roberts (1979) as follows:

$$HU = 100 \log (H + 7.57 – 1.7 W^{0.37})$$

Where: HU = Haugh unit,

H = observed albumen height in millilitres

W= weight of the eggs in grams

The yolk height and weight were measured for the calculation of the yolk index. Yolk colour was rated by matching it with pH coloured paper strips. The shells were dried for three days and weighed per replicate. The shell thickness was measured using the vernier callipers.

From the primary data collected other data such as feed conversion efficiency, feed cost per kg egg and per cent hen-day production were generated. All data were subjected to the analysis of variance (steel and Torrie, 1980) and the significance between treatment means was separated using Duncan Multiple Range Test (Duncan, 1955 as contained in the SPSS (2010)).

3.0 Results and Discussion

The results of the proximate composition and phytochemical analysis of the BL and SL, the test ingredients and nutrient composition of the diets fed, the performance of the pullet layers and egg quality are presented in Tables 1, 2, 3, 4 (a and b) and 5 respectively. The result of the proximate composition and phytochemical constituents showed that BL and SL respectively, have a fairly high level of crude protein (13.52% and 15.52%) crude fibre (12.22% and 1039%) and ether extract (8.85% and 10.55%) Alkaloid (1.34% and 1.18%),

Table 4a Performance Characteristics of Pullets (14–30 weeks) Fed Experimental Diets.

Inclusion levels (%)	OBL OSL	*OBL *OSL	75BL 25SL	50BL 50SL	25BL 75SL	
	Treatments					
Ingredients	1 (Control)	*2	3	4	5	SEM
Initial weight (Kg)	0.65	0.64	0.71	0.69	0.65	0.23
Final weight (kg)	1.86	1.86	1.89	1.93	1.88	0.01
Weight gain (kg)	1.22	1.22	1.19	1.23	1.23	0.02
Feed intake (Kg)	5.12^{a}	5.08^{ab}	4.98^{bc}	4.98^{bc}	4.96^{c}	0.02
Feed conversion ratio	2.20	2.32	2.20	2.25	2.23	0.03

Antibiotic: (Tylo-dox extra wsp :Tylosin Tartrate 20g, Doxycycline Hyclate 15g)
BL : Bitter leaf SL ; Scent leaf

Table 4b Performance Characteristics of Pullet Layers Fed Graded levels of Bitter Leaf and Scent Leaf Meals

Inclusion levels (%)	0BL 0SL	*0BL *0SL	75BL 25SL	50BL 50SL	25BL 75SL	
	Treatments					
Ingredients	**1 (control)**	***2**	**3**	**4**	**5**	**SEM**
Feed intake (g/bird/day)	91.33^{a}	90.67^{ab}	89.00^{bc}	89.00^{bc}	88.67^{c}	0.33
Feed efficiency (Kg/egg/kg/feed)	2.20	2.32	2.30	2.25	2.23	0.03
Hen-day Production (%)	80.16^{a}	73.81^{a}	76.67^{a}	76.19^{a}	73.81^{a}	0.94
Total egg Production	42.67^{a}	39.60^{a}	42.93^{a}	40.80^{a}	41.60^{a}	0.57
Total egg production/week	5.33^{a}	5.17^{a}	5.17^{a}	5.33^{a}	5.17^{a}	0.59
Total egg weight (Kg)	2.33^{a}	2.19^{a}	2.30^{a}	2.21^{a}	2.24^{a}	0.03

a, b, c, within each row, Means with the same superscripts are not significantly different (P > 0.05).
****Antibiotic: (Tylyo-dox extra wsp: Tylosin Tartrate 20g, Doxycycline Hyclate 15g)***
SEM = Standard error of the mean **BL: Bitter leaf** **SL: Scent leaf**

Table 5: Egg Quality Characteristics of Pullet Layers Fed the Experimental Diets

Inclusion levels (%)	0BL 0SL	*0BL *0SL	75BL 25SL	50BL 50SL	25BL 75SL	
	Treatments					
Ingredients	**1 (Control)**	***2**	**3**	**4**	**5**	**SEM**
Egg weight (g)	55.78	53.68	53.74	53.84	55.42	0.60
Egg shell thickness (mm)	0.45^{ab}	0.42^{ab}	0.41^{b}	0.46^{ab}	0.47^{a}	0.01
Egg shell weight (g)	6.00	5.03	5.26	5.61	5.95	0.16
Egg shell weight (% egg wt)	10.79	9.35	9.67	10.43	10.72	0.27
Albumen weight (g)	32.30	32.60	30.82	30.63	30.85	0.53
Yolk weight (g)	14.98^{b}	15.40^{b}	16.43^{ab}	16.70^{ab}	18.14^{a}	0.39
Albumen weight (% egg wt)	57.88	60.60	57.30	56.25	55.74	0.74
Yolk weight (% egg wt)	26.87^{c}	28.72^{bc}	30.62^{ab}	31.02^{ab}	32.68^{a}	0.68
Haugh unit	76.43^{b}	79.03^{ab}	86.13^{a}	86.16^{a}	86.85^{a}	1.52
Yolk index	0.26	0.29	0.26	0.28	0.28	0.01
Shell Surface Area (SSA)	68.25	66.54	66.58	66.67	68.12	0.51
Egg Shape Index	0.78	0.76	0.77	0.76	0.78	0.01
Yolk colour score	5.23	4.87	5.20	5.03	5.00	0.06

a, b, c, within each row, Means with the same superscripts are not significantly different (P > 0.05).
****Antibiotic: (Tylo-dox extra wsp: Tylosin Tartrate 20g, Doxycycline Hyclate 15g)***
SEM = Standard error of the means **BL: Bitter leaf** **SL: Scent leaf**

Tannin (6.00% and 3.60%), Flavonoid (2.08% and 1.96%), Steroid (0.148% and 0.096%) and Saponin (++ and +++). Feed intake was found lowest in birds fed 25%BL + 75%SL level of inclusion (treatment 5). This can be explained as being due to the astringent taste of the feed, having a higher percentage of SL with high saponin level, which would have affected the aroma and palatability of the feed, and palatability plays an important factor in feed intake (Gazwi et al, 2022). However, weight gain was found high in birds fed treatment 5. This is an indication of good utilization of the feed at that level of inclusion of the test ingredients. Bawa *et al* (2003) had argued that it is not the absolute value of protein and energy in the diets of laying hens that is important but the quality and bioavailability of the protein and energy that is of paramount importance.

Per cent hen-day production though not significantly (P>0.05) difference was found least in treatments 2 (0% BL + 0%SL, with antibiotics) and 5 (25%BL + 75% SL), and the latter inclusion level (treatments 5), the birds were able to utilize the feed efficiently. Birds fed treatments 1 and 5 came into lay within six (6) days interval, of which the former (control) had the first lay. However, the birds in all the other treatments groups came into lay within twenty-eight (28) days interval from that of the control. This notable variation at the age of the first egg between the control group and the other treatments groups 2, 3 and 4 may suggest that treatment 5 had a better inclusion level and combination of the test ingredients.

The relatively small size of the first laid eggs recorded for the treatment groups 1 and 5 may be connected with their relatively earlier age at lay compared with other treatment groups. The average egg weight indicated a positive response in laying birds fed the test ingredients which compared well with the control. The weight gains recorded for the birds fed all the dietary treatments is an indication of a positive nutrient balance. The similarity in the egg weights, total egg produced and feed efficiency from all treatment groups, further attest to the nutritional adequacy of the diets, thereby signifying the test ingredients as possible additives in layer diet.

Egg shell quality is of particular importance in commercial table eggs production to reduce wastage from egg breakage in the cages. Data on egg qualities presented in Table 5 showed shell thickness to be most superior when the test ingredients were used at 25% BL and 75% SL inclusion, which varied significantly (P<0.05) to that of 75% BL and 25% SL inclusion level. Perhaps some intrinsic factors associated with the inclusion levels may be responsible. Belyavin (1988) stated that shell thickness and weight, shell surface area (SSA) and egg shape index (ESI) are important indices for measuring shell strength. These parameters with the exception of shell thickness, were similar among the treatments. Furthermore, no shelless egg was laid. All these are positive indications that adequate minerals required for egg shell formation were available from the diets supplemented with bitter leaf and scent leaf meals. These findings agree with the report of Odunsi *et al* (2002) when they fed layers with *gliricidia sepium* leaf meal.

Haugh limit, an index of protein utilization, was high in eggs laid in all the treatment groups, though that of control was significantly (P<0.05) lower than that of treatments 3, 4, 5. This indicates that dietary proteins provided by the different dietary treatments irrespective of the level of inclusion of test ingredients were effectively utilized by laying chickens. The Haugh unit values obtained in this study agreed with the standard values of each other workers including Oluyemi and Roberts (2000).

Yolk colour is of commercial importance (Belyavin and Marango, 1989). The comparable yolk colour obtained for this study is a positive indication that bitter leaf and scent leaf meals could be used as feed additives in the diet of laying chickens without affecting the consumers' preferred egg yolk colour. This report is in agreement with the egg yolk colour result of Esonu *et al* (2013), who fed a few stages cooked *Canavalia plagion sperma* (Piper) seed meals as a feed ingredient in layers diets. Yolk weight was found highest in the diet containing 25% BL and 75% SL which varied significantly from that of control and treatment 2 (0% BL + 0% SL, with antibiotic). This shows that the test ingredients are good in egg yolk pigment, and as well exhibit synergic nutritive values. The non-significant (P>0.05) difference in the albumin weight was also indicative of the high nutritive rating of bitter leaf and scent leaf in the diets of laying chickens.

4.0 Conclusion

Conclusively, BL and SL can be fed at 25% and 75% level respectively for optimum performance in terms of production and egg quality parameters. Moreover, it is recommended that poultry farmers should endeavour to cultivate both plants in their neighbourhood, to reduce the cost of production (synthetic drugs) and, also to increase their profit margin.

References

A.A.O.C (1990). Official Methods of Analysis Association of Official Analytical Chemists. 15**th** Edition, Washington, D.C.

Aletor, V.A. (1986). Some agro-chemical by-products and waste in livestock feeding. A review of prospects and problems. *World rev of Anim. p*rod. xxii (4): 35-41.

Bawa, G.S; Tegbe, T.S.B. and Ogundipe, S.O. (2003). Effects of feeding graded levels of lablab seeds as a replacement for soyabeans on the performance characteristics of young pigs. Proc. 28th NSAP conf. Ibadan, Nigeria. Vol. 28 pp 230-232.

Belyavin, C. G. and Marangos, A. G. (1989). Natural Products for Egg yolk pigmentation. In Recent Developments in Poultry nutrition. Ed. Cole D. J. A. and Haresign, W. Butterworths. London.

Borutora, R. (2011) Less medication through effective mycotoxin control. World Poultry No. 27 Volume 07 pp 26-27.

Esonu, B. O. Anumni, P.E. Udedibie, A. B. I. Emenalom, O. O., Etuk, B., Odoemelam, V. and Okorie, K. C. (2013). Evaluation of two stage cooked *Canavalia plagiosperma* (Piper) Seed meal as feed ingredient in layer Diets. *Nigerian J. Anim. Sci.* 2013 Vol. 15: 95 – 103.

Gazwi, H. S. S., Mahmoud, M. E. and Toson, E. M. A. (2022). Analysis of the phytochemicals of *Coriandrum sativum* and *Cichorium intybus* aqueous extracts and their biological effects on broiler chickens. Sci Rep 12, 6399. https://doi.org/1o. 1038/s41598-022-10329-2.

Hossain, M.M. (2009) Herbs versus drugs: Aspect World poultry. Engonormix.com/./165/po.htm.

Karimi, A., Yan, F., Coto, C., Park, J.H., Min, Y., Lu, C. Gidden, J.A. Lay, J.O. and Waldroup, P.W. (2010). Effects of level and source of oregano leaf in starter diets for broiler chicks. *Journal of Applied Poultry Research* Vol. 19 No. 2 137-145.

Muaz, K., Riaz, M., Akhtar, S., Park, S. and Ismail, A. (2018). Antibiotic residues in chicken meat: Global Prevalence, Threats, and Decontamination Strategies: A Review. J Food Prot 81(4): 619-627.

Odunsi, A. A., Ogunleke, M. D., Alagbe, O. S. and Ajam, T. O. (2002). Effect of feeding *gliricidia sepium* leaf meal on performance and egg quality of layers. International Journal of Poultry Science. 01/2002. Retrieved September 1st 2014 from www.researchgatenet/publication/45.

Oluyemi J.A. and Roberts, F.A. (2000). Poultry production in Warri West Climates pp 148-156. Spectrum Books Limited, Ibadan. Revised Edition.

Pandey, A. K., Kumar, P. and Saxena, M. J. (2019). Feed Additives in Animal Health DOI: 10. 1007/978-3-030-04624-8-23 In Book: Nutraceuticals in Veterinary Medicine PP. 345-362.

Steel, R. G. and Torrie, J. H. (1980). Principles of procedures of Statistics. McGraw-Hill book Company, New York.

Ziggers, D. (2012) Antibiotics will kill us. All about feed Vol. 20 No 1. Pp 5.