EFFECTS OF TURMERIC (*CURCUMA LONGA*), GARLIC (*ALLIUM SATIVUM*), GINGER (*Zingiber officinate*) POWDER AND THEIR COMBINATIONS AS FEED ADDITIVES ON MICROBIAL GROWTH OF RABBIT MEAT DURING REFRIGERATED STORAGE.

Ademolue, R. O.[1], Sanwo, K. A.[1], Shobayo, R.A.[1], Ayo-Ajasa, O.Y[1], Okanlawon, E. O.[1]
[1]Department of Animal Production and Health, Federal University of Agriculture Abeokuta
Corresponding Author: Ademolue, R.O.; ade_toy@hotmail.com; +2348067150302

ABSTRACT
The study evaluated the microbial growth of meat harvested from rabbits fed turmeric, garlic, ginger powder and their combinations. Forty-eight weaner rabbits (8 weeks old) were randomly allotted into eight dietary groups designated T1 to T8 in a completely randomized design. T1 contained 0% phytobiotic, T2, T3 and T4 contained 0.6% turmeric (TR), garlic (GA) and ginger (GG) powder respectively, T5, T6 and T7 contained combination of 0.3% each of TR+GA, TR+GG and GA+GG while T8 contained 0.2% each of the three phytobiotics. Meat were harvested aseptically, stored (0^0C) for 14days and analyzed for microbial load at days 0, 7 and 14. Total Bacterial Count (TBC) of psychrophilic bacteria and species colony (logCFU.g-1) were evaluated while the microbiological analyses were performed using plate dilution method. Result showed that lowest ($p>0.05$) TBC of 1.01 was recorded in T2. Significantly ($p<0.01$) lower *Staphylococcus aureus* (SA) (0.20) and *Pseudomonas aeruginosa* (PA) (0.03) were recorded in T2 while colony of other isolated bacterial were not significantly ($p>0.05$) different from the control. Similarly, storage duration has no significant effect on the TBC of the meat samples, SA count was reduced ($p<0.01$) from the initial 0.55 at day 0 to 0.06 at day 14 while PA count receded from 0.18 to 0.09 as storage progresses to day 14 ($p<0.01$). However, it has been concluded that dietary turmeric powder at 0.6% inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth while 14days of refrigeration reduced the plate counts of the two bacterial spp. than the control.
Keywords: Turmeric; Garlic; Ginger; Rabbit meat; Microbial growth

INTRODUCTION
Meat is an excellent source of bioactive compounds, high biological value protein as well as many valuable nutrients (Heinz and Hautzinger, 2007) which has been proven cannot be substituted for by plant sources. Rabbit meat offers an inexpensive lean functional proteins with relatively balanced amino-acids, highly digestible with relatively low calorie, thus, it is safe and suitable for special diets (El-Adawy *et al*., 2020). Meat undergoes progressive deterioration due to the unique biological and chemical nature majorly as a result of lipid oxidation and microbial activities which ultimately leads to safety and quality deterioration if the meat is not opportunely handled and preserved. The most prevailing and prominent spoilage organisms in meat is bacteria and due to their ubiquity, they are mostly incorporated in meat from the environment which consequently affect its functional, sensory and nutritional quality.

The emerging problem regarding the negative impact exerted by some synthetic preservatives on the health of consumers versus the benefits imparted by natural antimicrobials and their specificity to foodborne pathogens necessitates the requirement for more systemic research to evaluate the mechanism of action of natural preservatives (Lee and Paik, 2016). Phytobiotics are plant materials containing secondary metabolites and used in nutritionally insignificant quantities as a preservative to kill or prevent the growth of harmful bacteria (Adamson, 2004). Therefore, the practice of complementary and alternative medicine (Phytomedicine) is on the increase in developing countries in response to WHO directives culminating in several studies and providing the scientific basis for the efficacy of many plant materials used in folk medicine (Mancini, *et al*. 2017) and with lesser side effects. Turmeric, garlic and ginger have been studied for their antioxidant and antimicrobial properties (Whitemore and Naidu, 2000; Olatidoye *et al*., 2015) and hence could play an important role in rabbit feeding strategy since this specie of livestock is capable of benefitting from a wide variety of ingredients in its diet due to its digestive physiology which makes it possible to incorporate diverse ingredients in their diet in to modify the characteristics of its meat.

MATERIALS AND METHODS
The meat analysis was carried out at the meat processing laboratory of the Department of Animal Production and Health, Federal University of Agriculture, Abeokuta. Forty-eight 8 weeks old weaner rabbits were used for

the study and were randomly allotted into eight (8) dietary groups designated T1 to T8 in a completely randomized design (CRD) with each treatment having 3 replicates of two (2) rabbits each. T1 (control) is with 0% phytobiotic, treatment 2, 3 and 4 contained 0.6% each of turmeric (TR), garlic (GA) and ginger (GG) powder respectively while T5, T6 and T7 contained combination of 0.3% each of TR+GA, TR+GG and GA+GG while T8 contained a blend of 0.2% each of the three test ingredients (TR,GA and GG). Phytobiotics powder were added at different graded levels as treated diets while the feeding trial lasted 12 weeks. Replicates of the harvested rabbit meat samples were collected aseptically, frozen (0^0C) for 14days and analyzed for microbial load at days 0, 7 and 14 and this was used to determine the shelf-life of the meat. Microbiological testing including total viable bacterial counts (TVBC), Psychrophilic bacteria, *Enterobacter* spp., and lactic acid bacteria were investigated to identify potentially pathogenic organisms that may be present on the stored meat and was expressed as colony-forming units per gram (CFU.ml-1) while the colonies were counted following 24 and 48 h of incubation at 35± 1ºC (AOAC Official Method 991.04). Data obtained were subjected to one-way ANOVA in 8 by 3 factorial arrangements in a CRD and analyzed using SPSS statistics package version 23 (IBM Corporation, 2015).

RESULTS AND DISCUSSION

Table 1 shows the effect of diets supplementation on the microbial load of refrigerated rabbit meat and it shows that inclusion of phytogenic blends was able to significantly ($P<0.01$) curtailed the colony forming unit per ml. (CFU.ml-1) count of *S. aureus* in samples harvested from rabbit group fed T2 (0.20 CFU.ml-1). Conversantly, a significantly ($p<0.05$) tapered *Pseudomonas spp.* of 0.03 was recorded in meat samples from rabbits fed T2 while there were no significant ($p>0.05$) dietary effects in the colony count of other bacteria spps. isolated on the meat samples. The average TBC in the stored meat was 1.35 and the highest count was recorded in T3. This result is lower than the mean TBC recorded by Rodriguez-Calleja *et al*. (2004) as well as the microbiological specification standard set for frozen chickens, hence, within the maximum acceptable microbiological limits, and thus fit for human consumption. Lowest ($p>0.05$) TBC count was recorded in samples from rabbit fed 6% turmeric. Many foodborne diseases are associated with consumption of meat hence, the micro flora of raw meats is of interest as an index of processing practices, storage conditions, shelf life, and potential risk to public health (Mor-Mur and Yuste, 2010). Nevertheless, as with other meats, rabbit has the potential to carry food-poisoning organisms from different sources, however, *Salmonella* spp., *L. monocytogenes*, and *Y. enterocolitica* were the most frequent meat contaminants but these were not isolated in this study. Significantly lower *S. aureus* ($p<0.01$) and *P. aeruginosa* ($p<0.05$) counts were recorded in meat from rabbits fed T2 and this could be attributed to the antimicrobial properties of curcumin present in turmeric and at 0.6% inclusion level. According to Shelef *et al*. (1980), the concentration of turmeric required to inhibit bacterial growth is between 0.5 and 5%, which may explain the lack of effect in T5, T6 and T8 where lower turmeric concentrations were fed. This result is in agreement with the report of Franco *et al*. (2007) who evaluated the possible antimicrobial effect of *Curcuma longa* L. essential oil on *S. aureus*, *E. coli* and *Salmonella* spp., and verified that only *S. aureus* growth was inhibited. This results however negates the findings of Lourenço *et al*. (2013) and suggested that turmeric may specifically act against Gram-positive bacteria.

Table 1: Effect of Dietary phytobiotics on Microbial Load of Refrigerated Rabbit Meat (CFU.ml-1)

	Experimental Diets									
Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM	F-Value
Total Bacterial Count (TBC)	1.34	1.01	1.75	1.14	1.42	1.42	1.09	1.59	0.19	0.69
Staphylococcus aureus	0.45^{ab}	0.20^{d}	0.59^{a}	0.47^{ab}	0.24^{c}	0.58^{a}	0.43^{abc}	0.32^{bc}	0.08	5.63**
Escherichia coli	0.34^{ab}	0.42^{ab}	0.46^{ab}	0.16^{b}	0.33^{ab}	0.29^{ab}	0.30^{ab}	0.61^{a}	0.10	1.46
Proteus vulgaris.	0.13^{ab}	0.19^{a}	0.16^{ab}	0.13^{ab}	0.16^{ab}	0.16^{ab}	0.07^{b}	0.14^{ab}	0.09	1.45
Pseudomonas aeruginosa	0.07^{b}	0.03^{c}	0.17^{ab}	0.07^{b}	0.18^{a}	0.16^{ab}	0.10^{ab}	0.12^{ab}	0.04	2.58*
Klebsiella pneumonia	0.15^{ab}	0.17^{ab}	0.07^{ab}	0.11^{ab}	0.21^{a}	0.13^{ab}	0.02^{b}	0.20^{a}	0.05	1.84
Enterobacter spp.	0.20^{ab}	0.00^{b}	0.30^{a}	0.20^{ab}	0.30^{a}	0.10^{ab}	0.17^{ab}	0.20^{ab}	0.03	1.54

^{a,b,c,d,} Means in the same row with different superscripts differ significantly (0<0.05). ** Significant difference at 1% (P<0.01) * Significant difference at 5% (P<0.05); KEYS: T1- Control, T2- Basal diet + 0.6% of turmeric powder, T3- Basal diet + 0.6% garlic powder, T4- Basal diet + 0.6% ginger powder, T5- Basal diet + 0.3% each of turmeric and garlic powder, T6- Basal diet + 0.3% of turmeric and ginger powder, T7- Basal diet + 0.3% each of ginger and garlic powder, T8- Basal diet + 0.2% each of phytobiotic

Table 2 represents the effect of storage duration on microbial count of rabbit meat samples. There were no significant ($p>0.05$) reduction in colony forming units per ml (CFU.ml-1) of total bacterial count (TBC), *E. coli*, *Proteus spp*. and *Klebsiella spp*. in days 0, 7 and 14 of storage. However, progressive storage days significantly ($p<0.01$) diminished average CFU.ml-1 of *S. aureus* from 0.55 at day 0 to 0.41 and later to 0.28 at day 14 day. Similarly, significantly heightened ($p<0.01$) *Pseudomonas spp*. was recorded at day 0 (0.18) and day 7 (0.13) but receded ($p<0.01$) to 0.09 as the storage days increased to day 14. TBC, *E. coli*, *Proteus spp* and *Klebsiella spp* counts followed similar pattern of reduction ($p>0.05$). This growth reduction could be attributed to storage duration and temperature and it is in agreement with the findings of several authors. Akinduro *et al*. (2020) investigated the antibacterial effects of some selected nine spices used in Nigerian pepper soup and reported that the spices significantly ($p<0.05$) reduced bacterial load on the two separate days of storage while attributing the effect to the presence of phytochemicals in them. Various phytochemicals obtained in spices offer a promising alternative to the chemical additives for ensuring the meat safety while their Inhibitory activity on microbial growth has been previously reported.

Table 2: Effect of Storage Duration on Microbial Load of Rabbit Meat (log_{10}CFU\ml)

	Days of Storage				
Parameters	0	7	14	SEM	F-Value
Total Bacterial Count	1.64	1.11	1.09	0.13	1.51
Staphylococcus aureus	0.55^{a}	0.41^{b}	0.28^{c}	0.06	12.03**
Escherichia coli	0.47	0.34	0.41	0.07	1.72
Proteus vulgaris.	0.15	0.11	0.14	0.03	0.13
Pseudomonas aeruginosa	0.18^{a}	0.13^{a}	0.09^{b}	0.02	17.80**
Klebsiella pneumonia	0.18	0.12	0.17	0.03	1.14
Enterobacter	0.11	0.00	0.00	0.01	0.10

^{a,b,c}, Means in the same row with different superscript differ significantly ($p<0.05$). ** Significant difference at 1% ($P<0.01$)

CONCLUSION

Due to rabbit meat moisture status, it is an ideal growth medium for spoiling and pathogenic microorganisms and owing to health problems associated with the use of synthetic preservatives, consumers appeal to the use of natural antimicrobial preservatives. This results affirmed that dietary supplementation of turmeric powder at 0.6% exhibited the highest bacteriostatic efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in stored rabbit meat.

REFERENCES

Adamson, M.W. (2004). Food in Medieval Times. Greenwood Press, 88 Post Road West, Westport, CT 06881 (An imprint of Greenwood Publishing Group, Inc.).

Akinduro, V.O.; Asaniyan, E.K. and Osho, I.B. (2020). Antibacterial effects of some selected spices used in Nigerian pepper soup on cooked broiler meat on days 3 and 6 of refrigeration storage. *Journal of Agricultural Science and Practice* Volume 5(4), pages 174-183.

El-Adawy, M.M.; Salem, A.Z.; Khodeir, M.H.; Khusro, A.; Elghandour, M.M.; Hernández, S.R. and Al-Shamandy, O.A. (2020). Influence of four tropical medicinal and aromatic plants on growth performance, digestibility, and blood constituents of rabbits. Agrofor. Syst. **2020**, 94, 1279–1289.

Franco, A.P.; Oliveira, T.B.; Ferri, P.H.; Bara, M.T.F. and Paula, J.R. (2007). Avaliação da composição química e atividade antibacteriana dos óleos essenciais de *Aloysia gratissima*, *Ocimum gratissimum* L. and e *Curcuma longa* L. (Açafrão). Revista Eletrônica de Farmácia 2007; 4(2):208-220.

Gideon, P.K.; Sugumar, R. and David, D.C. (2017). An *in vitro* study of antibacterial and antifungal activity of *Cynodon dactylon. National Journal of Physiol Pharm Pharmacol* 2017; 7(4):381-385.

Heinz, G. and Hautzinger, P. (2007). *Meat Processing Technology for Small-to Medium-Scale Producers*, Food and Agriculture Organization of the United Nations, Rome, Italy, 2007.

Lee, N.K. and Paik, H.D. (2016). Status, antimicrobial mechanism, and regulation of natural preservatives in livestock food systems. *Korean Journal for Food Science of Animal Resources*, vol. 36, no. 4, p. 547-557. https://doi.org/10.5851/kosfa.2016.36.4.547.

Lourenço T.C.; Mendonça E.P; Melo R.T; Silva, P. and Rossi, D.A. (2013). Antimicrobial effect of turmeric (*Curcuma longa*) on chicken breast meat contamination. Bras. Cienc. Avic. vol.15 no.2 Campinas.

Mancini, S.; Preziuso, G.; Fratini, F.; Nuvoloni, R.; Dal, B. A. and Paci, G. (2017). Qualitative improvement of rabbit burgers using *Zingiber officinale* Roscoe powder. World Rabbit Sci. **2017**, 25, 367.

Mor-Mur, M. and Yuste, J. (2010). Emerging bacterial pathogens in meat and poultry: An overview. Food and Bioprocess Technology. 3(1):24-35 DOI: 10.1007/s11947-009-0189-8.

Olatidoye, O.P.; Sobowale, S.S.; Oluwafemi, R. A. and Alabi, A. O. (2015). Effects of Adding Ginger extracts (Zingiber officinale) on minced cow Meat during refrigerated storage. *American Journal of Food Science and Nutrition Research*; 2(6): 165-171.

Rodriguez-Calleja, J.M.; Santos, J.A.; Otera, A. and Garcia-Lopez, M.L. (2004). Microbiological quality of rabbit meat. In Journal of Food Protection, vol. 67, 2004, no. 5, p. 966-971.

Shelef, L.A.; Naglik, O.A. and Bogen, D.W. (1980). Sensitivity of some common food-borne bacteria to the spices sage, rosemary and allspice. *Journal of food science*. Vol. 45, issue 4/pp 1042-1044.

Whitemore, B.B. and Naidu, A.S. (2000). Thiosulfinates. In A. S. Naidu (Ed.), Natural food antimicrobial systems. pp. 265–380. Boca Raton, FL: CRC Press.