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EFFECT OF MASHED LIME FRUIT JUICE AS AN ALTERNATIVE TO ANTIBIOTICS ON GROWTH PERFORMANCE, VILLI MORPHOMETRY AND CAECAL MICROFLORA OF GROWING RABBITS

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ABSTRACT

The experiment was conducted with thirty clinically healthy mixed breed growing male rabbits with average age of 11 weeks old. The rabbits were assigned to six dietary treatments (0, 500, 1000, 1500 2000 ml mashed lime fruit juice (MLFJ) per 100kg feed and Oxytetracycline; Oxyt) in completely randomized design. A basal diet formulated was designated as 0; to which 500, 1000, 1500 and 2000 ml of mashed lime fruit juice per 100kg feed was added to form treatments 500 - 2000 respectively while for Oxyt treatment, 55 g/100kg Oxytetracycline was added to the basal diet as recommended by the manufacturer. Treatments 0 MLFJ and Oxyt served as negative and positive controls respectively. Five growing rabbits were assigned to each dietary treatment and each rabbit served as a replicate. Parameters measured were acidity level, growth performance, villi morphometry and caecal bacterial cell numbers. The trial lasted for 56 days. The results showed lime fruit juice contained 0.6% ascorbic and 8.02% citric acids. Growth performance results showed improvement in final body weight being significantly ($P<0.05$) higher on 1000 ml MLFJ than other treatments except 500 ml MLFJ and Oxyt treatments. Feed intake and feed conversion ratio of MLFJ groups was comparable to the controls. Feed cost per kg gain was better on the Oxyt and 1000 ml MLFJ groups and worst on 1500 and 2000 ml MLFJ groups. There was significant ($P<0.05$) differences in all villi morphometric parameters measured except villi width. Villi surface area was significantly ($P<0.05$) lower on 1000 ml but similar to 500 and 1500 ml MLFJ than the other treatments Villi perimeter and Villi height were significantly ($P<0.05$) higher on 1500 and 2000 ml than 500 and 1000 ml MLFJ groups but similar to 0 ml MLFJ and Oxyt groups. Crypt depth was significantly ($P<0.05$) higher on 1500 ml and Oxyt than all others treatment groups. Villi height/crypt depth ratio was significantly ($P<0.05$) higher on 2000 ml than 1000, 1500 ml MLFJ and Oxyt groups. The population of *lactobacilli and Bacilli spp* increased as MLFJ increased with the highest value (10.67 and 13.97 $x10^6$ CFU/ml) for rabbits fed 1000 ml dietary MLFJ while those of *E. coli, Clostridium and Staphylococci spp* decreased as MLFJ increased. Results showed that rabbits could tolerate dietary mashed lime fruits juice up to 2000 ml/100kg without negatively affecting feed intake, weight gain, villi morphometric and caecal microflora. However, at 1500 and 2000 ml MLFJ, FC/kg G was increased which had a diminishing effect on the gross margin. It is therefore recommended that mashed lime fruit juice could be included in the diets of growing rabbits at 1000 ml/100kg feed.

Key words: Antibiotics, Organic acids, Lime, Villi morphometry, caecal microflora.

INTRODUCTION

High level of production, least cost feed and efficient feed conversion are the needs of modern rabbit production. This to a certain extent could be achieved by the use of specific feed additives. Antibiotic growth promoters (AGPs) such as Zinc bacitracin among others was the most used in rabbit feed (Falcão-e-Cunha *et al.,* 2007). They stabilized the intestinal microflora and prevented some specific intestinal pathogens leading to better gain and feed conversion (Waldroup *et al.,* 2005). However, despite these good qualities, it had been observed that antibiotics have their negative impact because of their residual effect in both animals and humans, leading to public health concern (Dibner, 2004; Paul *et al.,* 2007). This has necessitated the search for alternatives to AGPs.

As alternative to AGPs, organic acids among others have been reported to be efficacious by modifying intestinal pH, improving digestion and absorption of nutrients (Adams, 1999). However, organic acids currently in use are synthetic types which are very expensive and most farmers do not have access to them, hence the need to explore and exploit natural sources which can compare favourably with AGPs (Ndelekwute and Enyenihi, 2017). Fruits are major sources of organic acids especially *citrus spp* (lime, lemon, and grapefruit) which contain citric and ascorbic acids. Citrus have been reported to have some health benefits due to the presence of bioactive compounds such as ferrulic acid, hydrocinnamic acid, cyanidin glucoside, hisperidine, carotinoid and naringin (Xu *et al.*, 2008).

Citrus fruit extracts have antibacterial, antioxidant, anti-inflammatory, anti-tumour, anti-fungal and blood clot inhibition activities (Abeysinghe *et al.,* 2007; Ndelekwute *et al.,* 2019). Lime fruit juice contains between 1.20-3.89% and 1.60-4.23% of ascorbic and citric acids (Penniston *et al.,* 2008; Ndelekwute *et al.,* 2015 Tareen *et al.,* 2015; Jamil *et al.,* 2016; Ndelekwute and Enyenihi, 2017; Ndelekwute *et al.,* 2019), and a pH of 3.5-4.50 (Ndelekwute *et al.,* 2015; Ndelekwute and Enyenihi, 2017; Ndelekwute *et al.,* 2019), and could serve these purposes. However, there is dearth of information on their use as dietary source of organic acids and other bioactive compounds in rabbits feeding. This study therefore evaluated the effect of mashed lime fruit juice as an alternative to antibiotics on growth performance, villi morphometry and caecal microflora of growing rabbits.

MATERIALS AND METHODS

Study Location

The study was conducted at the Rabbit Research Unit of Swine and Rabbit Research Programme, National Animal Production Research Institute, Shika-Zaria, Nigeria, located in the Northern Guinea Savannah ecological zone. The area lies between latitude 10°11' N and longitude 7°8' E, 650m above sea level (Ovimaps, 2019). The dry season begins in the middle of October, with dry cold weather that ends in February. This is followed by relatively hot dry weather from March to May, when the rain begins. The mean minimum and maximum daily temperature is from 14 to 24^0C during the cool season and from about 19 to 38.3^0C during the hot season. The relative humidity varies between 19 and 35% in the dry season and between 63 and 85% in the wet season. The wet periods are between May and October with annual rainfall ranges from 1110 to 1580 mm, (IARMS, 2019).

Processing of Lime Juice

Green lime fruits were harvested from a farm in Gboko Local Government Area of Benue State and some were purchased from Samaru market, Sabon Gari Local Government Area, Kaduna State. The lime fruits were mixed, washed and cut into pieces with a sharp knife and mashed (ground) using a grinding machine. Cheesecloth was used to extract the juice. The juice was stored in a refrigerator at temperature of 4 degree Celsius to reduce oxidation.

Determination of Ascorbic and Citric Acids Content and pH of Lime Fruit Juice

The determination of the ascorbic and citric acids content and pH was carried out at the Central Laboratory, National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria. The juice was analyzed for citric and ascorbic acids content by titration method according to Novella (2014) and Helmenstine (2014). The juice was tested for pH using a digital pH Meter (Model PHS-25). The pH meter was connected to the electricity source, switched to mini volt and allowed to warm for 30 minutes to attain electronic temperature. The temperature was set to room temperature and the electrode was dipped into distilled water. The pH meter was calibrated and standardized with buffer solutions, first with buffer 7 and then checked with other buffers (4 and 9) to ensure that the calibration and standardization were perfectly done. The electrode was inserted into a beaker (100 ml) containing 50 ml of the lime fruit juice and the pH value was recorded according to the method of Holden *et al*. (2005). The pH was carried out in triplicate.

Experimental Design and Management of Animals

The experiment was conducted with thirty clinically healthy mixed breed growing male rabbits with an average age of 11 weeks old. The rabbits were assigned to six dietary treatments (0, 500, 1000, 1500 2000 MLFJ and Oxyt) in a completely randomized design. A basal diet formulated as shown in Table 1 was designated as 0; then 500, 1000, 1500 and 2000 ml of the mashed lime fruit juice per 100kg feed was added to the basal diet to form treatments 500 - 2000 respectively while Oxyt was formed by the addition of 55 g/100kg Oxytetracycline (Oxyt) to the basal diet as recommended by the manufacturer. The treatments 0 MLFJ and Oxyt served as negative and positive controls respectively. Ten kilogramme of the feed was compounded for each treatment every 10 days. Five growing rabbits were assigned to each dietary treatment and each rabbit served as a replicate. The rabbits were housed individually in galvanized wire cages of 60 x 60 x 40 cm in a well-ventilated building. The rabbits were treated for internal and external parasites by injecting Ivermectin Super® at 0.2 ml/kg live weight and oral administration of coccidiostat (Amprolium® at 1 g to 1 L of drinking water) before the commencement of the trial.

Growth Study

Initial and final weights of the rabbits were taken at the start and at the end of the feeding trial. The growth study lasted for 8 weeks. The rabbits were fed 200 g of their dietary treatments daily in flat bottom earthen feeders at 09:00 hours. Feed leftover and/or wastage was weighed and recorded daily before feeding. The rabbits were weighed weekly and their body weights recorded using 20kg Camry scale before feed was offered. Routine management practices were carried out daily. The trial lasted for 56 days. Parameters determined were feed intake, weekly body weight gain, feed conversion ratio, feed cost per kg and mortality.

Table 1: Ingredient composition and calculated nutrients of the experimental diet fed to growing rabbits

Ingredients	Quantity (%)
Maize	34.85
Groundnut cake	20.00
Maize offal	22.00
Rice offal	20.00
Bone ash meal	2.00
Common salt	0.30
Vitamin/mineral premix*	0.25
Lysine	0.30
Methionine	0.30
Total	**100.00**
Calculated Nutrient Analysis	
Metabolisable energy, (kcal/kg)	2515.93
Crude protein (%)	16.00
Crude fibre (%)	12.74
Ether extract (%)	4.43
Calcium (%)	0.83
Available phosphorus (%)	0.41
Lysine (%)	0.71
Methionine + cystine (%)	0.53
Feed cost (₦/kg)	78.59

*Biomix broiler starter premix supplies the following per kg diet: vit.A1000 IU; Vit. D3, 2000 IU; Vit. E, 5.0mg; Vit. K, 2mg; Vit. B1, 1.8mg; Vit B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vit.B6, 0.30mg; Vit. B12, 0.015mg; Folic acid, 7.5mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Villi Morphometric Study

Intestinal segment samples (approximately 2 cm in length of duodenum, jejunum and ileum) were taken from slaughtered rabbits using a sharp knife into labelled universal sterile sample bottles for the evaluation of villi morphometric indices. The morphometric indices that were evaluated include villus perimeter, villus height, from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, villi surface area, and the villus height to crypt depth ratio as described by Zhang *et al.* (2009). This was carried out at the Histology Preparation Laboratory, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria.

Caecal Microbial Study

At the end of the trial, caecal digesta samples were collected from the caecum of the slaughtered rabbits using swab sticks into labelled universal sterile sample bottles for the determination of bacterial cell numbers in the caecum. *Escherichia coli, Lactobacilli spp, Clostridium spp, Salmonella spp* and *Bacilli spp* were determined by using different selective media (Eosin Methyelene Blue Agar, Mannitol Salt Agar and *Salmonella* Shigella Agar) for isolation of bacteria groups and characterization, based on sugar fermentation using Microbact 12E kit and conventional biochemical methods as described by O.M.P (2015 a, b and c). This was carried out at the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Statistical Analysis

Data generated were subjected to analysis of variance using General Linear Model procedure

of the Statistical Analysis System (SAS, 2002) software package. Orthogonal pair wise difference was used to separate significant means. Below is the model for the analysis:

$Y_{ij} = \mu + T_i + e_{ij}$

Where,

Y_{ij} = performance of j^{th} rabbit fed i^{th} diet

μ = the overall mean of observations of interest

T_i = the fixed effect of the i^{th} treatment

e_{ij} = random error

RESULTS AND DISCUSSION

Organic Acids content and pH of Lime Fruit Juice

The results indicated that lime fruit juice contained 0.6% ascorbic acid and 8.02% citric acid with a pH of 4.35. This finding is a clear confirmation that the lime fruit juice was acidic agreeing with the report of Novella (2014) and Ndelekwute *et al.* (2015). However, the ascorbic acid content did not agree with the report of Shrestha *et al.* (2012), Ndelekwute *et al.* (2015) and Ndelekwute and Enyenihi (2017) who reported values of 0.08 and 1.20% of ascorbic acid. Physicochemical composition of the fruits especially ascorbic acid, total soluble solids and titreable acids depend on their maturity stage and exposure to sun light (Izumi *et al.* 1988). Shrestha *et al.* (2012) reported that green colour orange has less ascorbic acid than the orange (ripened) coloured ones when harvested at the same time. In sweet orange fruits, the exterior (sunlight) part contains more ascorbic acid and sugar with significant correlation between sucrose and ascorbic acid content (Izumi *et al.* 1988). The citric acid content obtained in this study is higher than was reported by Penniston *et al.* (2008) and Ndelekwute *et al.* (2015) who reported values of 4.6 and 1.60% respectively. The high value of citric acid recorded however, is similar with the values of 8.3-9.1 and 7.9% respectively reported by Fang and Roose (1997) and Shrestha, *et al.* (2012). These differences may be as a result of difference in agro-ecological zones, stage of maturity of the fruits and seasonal variations at harvest of fruits. The pH value obtained is similar with pH range of 3.5-4.50 reported by Ndelekwute *et al.* (2015), Ndelekwute and Enyenihi, (2017) and Ndelekwute *et al.* (2019).

Growth performance of growing rabbits fed diets containing graded levels of Mashed Lime Fruit Juice

Table 2 shows the growth performance of growing rabbits fed dietary levels of mashed lime fruit juice. There were no significant ($P>0.05$) differences in all parameters considered, except final body weight and feed cost per kg gain (FC/kg G). Final body weight was significantly ($P<0.05$) higher on 1000 ml MLFJ than other treatments except 500 ml MLFJ and Oxyt treatments. Rabbits fed 500, 1500 and 2000 ml mashed lime fruit juice diets also had similar final body weight with the Oxyt group. The lowest final body weight was obtained for rabbits on 0 ml mashed lime fruit juice though it was similar to 500, 1500 and 2000 ml MLFJ groups. FC/kg G was significantly ($P<0.05$) higher for rabbits on 2000 ml MLFJ than on Oxyt treatment which was similar to 0, 500, 1000 and 1500 ml mashed lime fruit juice. No mortality was recorded during the period of the study.

The superior final body weight obtained from the dietary mashed lime fruit juice groups with the best performance by rabbits fed 1000 ml/100kg diet, compared to the controls in this study, is an indication that the juice had impacted positively on the growth performance of the rabbits. This is similar to the report of Ndelekwute and Enyenihi (2017), who recorded non -significant differences in all parameters measured except final body weight of broiler chicken at finisher phase. Although there were no statistical differences in weight gain and average daily weight gain (ADWG), numerically, the dietary mashed lime fruit juice treated groups were higher than the negative

control with 1000 ml being higher than the positive control. This was in agreement with the report of Scapinello *et al*. (2001) and Michelan *et al*. (2002), who found that the inclusion of 1.5% fumaric acid in the feeds of growing rabbits tended to improve both the daily gain and the feed efficiency, but the differences were not statistically significant. Abd El-Latif *et al.* (2019) and Elwan *et al*. (2019) also had a similar result.

Table 2: Growth performance of growing rabbits fed diets containing graded levels of Mashed Lime Fruit Juice

	Levels of Lime Mashed Fruit Juice (ml)						
Parameters	**0**	**500**	**1000**	**1500**	**2000**	**Oxyt**	**SEM**
Initial weight (g)	1530.00	1500.00	1500.00	1490.00	1490.00	1480.00	199.21
Final weight (g)	2490.00^{c}	2590.00^{abc}	2800.00^{a}	2550.00^{bc}	2580.00^{bc}	2760.50^{ab}	109.90
Weight gain (g)	960.00	1090.00	1300.00	1060.00	1090.00	1280.50	185.63
ADWG (g)	17.14	19.47	23.21	18.93	19.46	22.87	3.24
Feed intake (g)	6866.00	7205.00	6741.00	6665.00	7184.00	6716.00	386.72
ADFI	122.61	128.66	124.23	119.02	128.29	119.93	6.34
FCR	7.15	6.61	5.35	6.29	6.59	5.24	1.09
FC/kg G ₦	561.00^{ab}	559.14^{ab}	484.67^{b}	607.55^{ab}	676.07^{a}	430.15^{b}	97.36
Mortality (%)	0	0	0	0	0	0	0.00

ab Means with different superscript along rows are significantly different at (P<0.05); Oxyt = Oxytetracycline; SEM = Standard error means; ADWG = Average daily weight gain; ADFI = Average daily feed intake; FCR = Feed conversion ratio; FC/kg G = Feed cost per kg gain.

Feed intake of mashed lime fruit juice treated groups was comparable to the controls. Average daily feed intake (ADFI) followed the same trend as the feed intake. It could be observed that the best feed conversion ratio (FCR) numerically was recorded for the Oxyt group which was similar to 1000 ml and the worst on the negative control (0 ml) group. The lower FCR suggest that the rabbits utilized the diet more efficiently when mashed lime fruit juice was added to the feed. In summary the dietary mashed lime fruit juice had better FCR compared to the negative control. The non-significant difference in FCR is an indication of comparable utilization of the experimental diets. This is similar to the report of Oluremi *et al.* (2018) who reported no significant differences in FCR when they studied the growth performance and blood profile of rabbits fed biodegraded-sweet orange (*Citrus sinensis)* peel based diets. Abd El-Latif *et al.* (2019) and Elwan *et al*. (2019) also recorded no significant (P>0.05) differences in feed conversion among rabbits fed dietary supplementation of 0.5 tumeric, 0.5 ginger and 1.0% dried lemon during their experiment. Ndelekwute and Enyenihi (2017) also recorded similar result.

Feed cost per kg gain (FC/kg G) was better on the Oxyt group and worst on 2000 ml while all the other treatments were statistically similar but numerically better than the negative control. Savings per kg gain obtained varied from N1.86-76.33 between the 500 and 1000 ml mashed lime treatments and the negative control. The lower FC/kg G according to Ndelekwute *et al.* (2018) was advantageous in that it took lower feed cost to produce a unit gain in weight when lime juice was added to the

feed. Feed cost/kg gain was higher by N46.55-115.70 between the 1500 and 2000 ml mashed lime juice and the negative control indicating that addition of mashed lime juice at these levels was counterproductive to production. The fact that no mortality was recorded in this study is an indication that the mashed lime fruit juice was safe for rabbits. This corroborated the report of Ndelekwute *et al.* (2018) who recorded a similar result with broiler chickens.

Villi Morphometric of growing rabbits fed diets containing graded levels of Mashed Lime Fruit Juice

Table 3 shows the effect of diets containing graded levels of mashed lime fruit juice on villi morphometry of growing rabbits. The result showed significant (P<0.05) differences in all parameters measured except villi width. Villi surface area (VSA) was significantly (P<0.05) lower on 1000 ml MLFJ but similar to 500 and 1500 ml MLFJ than the other treatments.

Villi perimeter (VP) was significantly (P<0.05) higher on 1500 and 2000 ml than 500 and 1000 ml MLFJ groups but was similar to 0 ml MLFJ and Oxyt while the lowest value was at 1000 ml MLFJ. Villi height (VH) was also significantly (P<0.05) higher on 1500 and 2000 ml than 500 and 1000 ml MLFJ groups but similar to 0 ml MLFJ and Oxyt groups. Rabbits fed 500 ml dietary mashed lime fruit juice also had higher villi height compared to those fed 1000 ml but similar to 0 ml and Oxyt groups. Crypt depth (CD) was significantly (P<0.05) higher on 1500 ml MLFJ and Oxyt than all other treatment groups. Villi height/crypt depth (VH/CD) ratio of rabbits fed 2000 ml dietary mashed lime fruit juice was significantly (P<0.05) higher than 1000, 1500 ml MLFJ and Oxyt groups but similar to 0 and 500 ml MLFJ treatments. Villi height, crypt depth and VH/CD are considered as criteria that reflect the small intestine morphology and absorption capacity (Montagne *et al.,* 2003). Therefore, an increase in villi height, VH/CD or decrease in the crypt depth is correlated with an improvement in the digestion and absorption of nutrients (Hou *et al.,* 2010; Yao *et al.,* 2012). The significant differences obtained here is not surprising as it was evident in the significant differences recorded in the final body weight of the rabbits.

Table 3: Villi Morphometric of growing rabbits fed diets containing graded levels of Mashed Lime Fruit Juice

	Levels of Lime Mashed Fruit Juice (ml)						
Parameters	**0**	**500**	**1000**	**1500**	**2000**	**Oxyt**	**SEM**
VSA (µm)^2	11765^{a}	10130^{ab}	8833^{b}	10921^{ab}	12701^{a}	11570^{a}	1287.53
VP (µm)	672.65^{ab}	588.99^{bc}	502.50^{c}	695.17^{a}	721.74^{a}	674.51^{ab}	49.14
VH (µm)	308.66^{ab}	288.87^{b}	249.11^{c}	331.65^{a}	337.31^{a}	312.05^{ab}	19.51
VW (µm)	69.50	75.90	59.20	72.60	72.00	70.27	86.60
CD(µm)	93.01^{b}	89.31^{b}	86.07^{b}	124.30^{a}	94.26^{b}	123.38^{a}	6.74
VH/CD	3.47^{ab}	3.25^{ab}	2.93^{b}	2.74^{bc}	3.64^{a}	2.52^{bc}	0.31

^{abc} Means with different superscript along rows are significantly different at (P<0.05); Oxyt = Oxytetracycline; SEM = Standard error of means; VSA = Villi surface area; VP = Villi perimeter; VH = Villi height; VW = Villi width; CD = Crypt depth; VH/CD = Villus height/crypt depth.

According to several authors (Xu *et al*., 2003; Markovic *et al.,* 2009), it is expected that the larger the villi height and length, the more the surface area available for absorption in the intestine which will in turn result in a better growth and development of the host. Rabbits fed dietary mashed lime fruit juices were different from the 0 ml MLFJ. This result is contrary to that of Adil *et al.* (2010) and Agboola *et al.* (2019) who observed no significant differences in these parameters when

they fed diets supplemented with butyric acid to broiler chickens.

Caecal Bacteria Count of growing rabbits fed diets containing graded levels of Mashed Lime Fruit Juice

Table 4 shows the effect of diets containing graded levels of mashed lime fruit juice on caecal bacteria count of growing rabbits. There were significant (P<0.05) differences in all parameters considered, except *clostridium spp*. while *Salmonella spp* was not detected. *Escherichia coli* (E. coli) numbers decreased though not significantly as the level of mashed lime fruit juice increased in the diet being significantly (P>0.05) higher at 0 ml MLFJ than on Oxyt which had the lowest numbers. *Lactobacilli spp* was significantly (P<0.05) higher at 1000 ml but was similar to 500 ml MLFJ. The lowest value was recorded at 0 ml but was similar to 1000 and 2000 ml while 2000 ml MLFJ and Oxyt were also similar. Significant (P>0.05) differences were observed in *Bacilli spp* across treatments with the highest numbers obtained at 1000 ml MLFJ, which was similar to Oxyt treatment, though the lowest value was obtained at 0 ml MLFJ, it was similar to 1500 and 2000 ml MLFJ treatments. *Lactobacilli spp* and *Bacilli spp* had similar increase in number from 0 ml to 1000 ml mashed lime fruit juice with subsequent drop in numbers at 1500 and 2000 ml levels. While Oxyt treatment had lower numbers of *Lactobacilli spp*, they had higher number of *Bacilli spp* compared with the mashed lime juice treatments except 500 and 1000 ml MLFJ treatments. *Staphylococci spp* decreased as level of mashed lime fruit juice increased in the diet. It was significantly (P<0.05) higher at 0 ml than all the treatment groups while 500 ml mashed lime fruit juice diet was significantly (P>0.05) higher than 1000, 1500, 2000 ml mashed lime fruit juice and Oxyt treatments.

Table 4: Caecal Bacteria Count of growing rabbits fed diets containing graded levels of Mashed Lime Fruit Juice

	Levels of Lime Mashed Fruit Juice (ml)						
Parameters ($x10^6$CFU/ml)	**0**	**500**	**1000**	**1500**	**2000**	**Oxyt**	**SEM**
Escherichia coli	9.00^{a}	8.00^{ab}	6.93^{ab}	4.67^{ab}	4.20^{ab}	3.27^{b}	2.83
Lactobacilli *spp*	5.60^{cd}	9.57^{ab}	10.67^{a}	6.77^{c}	8.33^{bc}	8.77^{b}	0.91
Clostridium *spp*	11.67	9.70	10.93	7.63	5.20	12.00	3.69
Salmonella *spp*	ND	ND	ND	ND	ND	ND	
Bacilli *spp*	6.60^{d}	9.70^{bc}	13.97^{a}	7.40^{cd}	8.03^{cd}	12.07^{ab}	1.51
Staph *spp*	17.07^{a}	10.63^{b}	3.50^{c}	2.93^{c}	2.07^{c}	2.73^{c}	2.23

[ab] Means with different superscript along rows are significantly different at (P<0.05); Oxyt = Oxytetracycline; Staph = *Staphylococci spp*; SEM = Standard error of means. ND = not detected

The addition of mashed lime fruit juice significantly affected the caecal microbiota of the growing rabbits except *clostridium spp.* However, *clostridium spp.* was numerically affected. The number of pathogenic bacteria *spp* decreased as dietary mashed lime fruit juice increased while the beneficial ones increased as the mashed lime fruit juice increased. *Bacilli spp is* one of the beneficial and harmless microbes in the microbiota (Salihu *et al.,* 2020). *Lactobacilli spp are* commensal bacteria that have long been known for their ability to activate the intestinal immune system and increase resistance to disease. These bacteria

have short chain fatty acids which are bacteriostatic for a subset of bacteria spp (Salihu *et al.,* 2020). The most abundant essential oils in *C. aurantifolia* peels are limonene, α-terpineol and γ-terpinen (Jafari *et al.,* 2011). It has been reported (Wenk, 2000; Lan *et al.,* 2003) that many essential oils stimulate the growth of beneficial microbes and limit a number of pathogenic bacteria in poultry. *C. aurantifolia* was reported to have high zone of inhibition against *Shigella spp*., *E. coli* strains and *Salmonella spp* (Reddy, *et al.,* 2012). Mizusawa *et al.* (2014) reported that most strains belonging to *Lactobacillus plantarum* could proliferate well in citrus juice with a pH of 3.5. Uddin *et al.* (2014) reported that by creating acidic conditions, citric acid made the nutrients more available and prevented the growth of harmful microorganisms, which monitored better performance. The result of this study is in line with the report of Salihu *et al.* (2020) who reported significant improvement in beneficial intestinal microbiota and decrease in pathogenic intestinal microbes when they evaluated the effect of *Ocimum gratissimum* and *Ocimum canum* as natural growth promoters on growth performance, intestinal microbiota and villi morphometry of broiler chickens. *Salmonella spp* was not detected in this study. This could be that it was either not present or present in undetectable levels. This finding is consistent with previous report (Hagen *et al*., 1976) that naturally occurring Salmonella infections were uncommon in domestic rabbits. *Salmonella enterica* according to Percy *et al*. (1993) and Kylie (2016) was rarely identified in faecal samples from clinically healthy rabbits.

CONCLUSION

The result had confirmed the acidity of lime fruit juice. Results showed that rabbits could tolerate dietary mashed lime fruits juice up to 2000 ml/100kg without negatively affecting feed intake, weight gain, villi morphometry and caecal microflora. However, at 1500 and 2000 ml MLFJ FC/kg G was increased which had a diminishing effect on the gross margin. It is therefore recommended that mashed lime fruit juice could be included in the diets of growing rabbits at 1000 ml/100kg feed.

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REFERENCES

Abd El-Latif, S. A., Tosson, A. M., Elwan, H.A.M. and Helpawy, E.S. (2019). Effect of dietary growth promoters on performance and carcass traits of growing rabbits. *Acta Scientific Nutritional Health,* 3(7): DOI: 10.31080/ASMS.2019.03.0442

Abeysinghe, D. C., Li, C., Sun, D., Zhang, W. S., Zhou, C. H. and Chen, C. S. (2007). Bioactive compounds and antioxidant capacities different edible tissues of citrus fruits of four species. *Food Chemistry,* 104: 1338-1344.

Adams, C. (1999). Poultry and dietary acids. *Feed International*, 20: 7072.

Adil, S., Bandy, M. T., Ahmad, G., Masood, B., Mir, S. and Rehman, M. (2010). Effect of dietary supplementation of organic acids on performance, intestinal histomorphology and serum biochemistry of broiler chickens. *Veterinary Medicine Internatioonal Volume*, Article ID 479485, 7Pp.

Agboola, A. F., Omidiwura, B. R. O., Ahmed, R. O. and Ayoola, O. D. (2019). Influence of butyric acid supplemented diets on growth response, precaecal nutrient

digestibility, gut morphology and histopathological measurements in broiler chickens. *Nigerian Journal of Animal Production,* 46(4): 117-129.

Dibner, J. (2004). Organic acids: can they replace antibiotic growth promoters. *Feed International,* 25(12): 14-16.

Elwan, H.A.M., Dawood, D.H., El-Safei, S.M.A., Abd El-Rahaman, A.A., Abdel-Latif, S.A., Mohany, M...Al-Rejaie, S.S. (2019). The potential role of *citrus lmon* powder as a natural feed supplement to boost the productive performance, antioxidant status, and blood biochemistry of growing rabbits. *Animals,* 9(426): doi:10.3390/ani9070426

Falcao-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J., Mour, J.L. (2007). Alternatives to antidote growth promoters in rabbit feeding: A review. *World Rabbit Science,* 15: 127-140.

Fang, D. Q. and Roose, M. L. (1997). Identification of Citrus Cultivars with Inter-Simple Sequence Repeat Markers, *Theoretical and Applied Genetics*, 95: 408-417.

Hagen, K. W., Gorham, J. R., Wash, P. and Flatt, R. E. (1976). Domestic Rabbits: Diseases and Parasites. Agriculture Handbook No. 490. Agricultural Research Service United States Department of Agriculture. Pp 10.

Helmenstine, A. M. (2014). Vitamin C determination by iodine titration. Hewitt, C. D., Innes, D. J., Savory, J. and Wills, M. R. (eds) Normal biochemical and hematological values in New Zealand White Rabbits. *Clinical Chemistry*, 35 (8): 1777.

Holden, J. M., Bhagwat, S. A., Haytowitz, D. B., Gebhardt, S. E., Dwyer, J. T., Peterson, J. and Balentine, D. (2005). Development of a database of critically evaluated flavonoids data: Application of USDA's data quality evaluation system. *Journal of Food Composition and Analysis,* 18:829–44. I

Hou, Y., Wang, L. and Ding, B. (2010). Dietary alpha-ketoglutarate supplementation ameliorates intestinal injury in lipopolysaccharide-challenged piglets. *Amino Acids,* 39(2): 555-564.

IARMS, (2019). Institute for Agricultural Research Metrological Station, weather report, Ahmadu Bello University, Zaria.

Izumi, H., Ito, T. and Yoshida, Y. (1988). Relationship between ascorbic acid and sugar content in citrus fruit peel during growth and development. *Journal of the Japanese Society for Horticultural Science,* 57: 304-311.

Jafari, S., Esfahani, S., Fazeli, M. R., Jamalifar, H., Samadi, M., Samadi, N., Khanavi, M. (2011). Antimicrobial activity of lime essential oils against food-borne pathogens isolated from cream-filled cakes and pastries. *International Journal of Biological Chemistry,* DOI: 103923/ijbe.2011

Jamil, N., Jabeen, R., Khan, M., Riaz, M., Naeem, T., Khan, A., Fahmid, S. (2016). Quantitative assessment of juice content, citric acid and sugar content in oranges, sweet lime, lemon and grapes available in fresh fruit market of Quetta City. *International Journal of Basic and Applied Science,* 15(1): 22-28.

Kylie, J. (2016). An investigation into the faecal microbiota of domestic rabbits (*oryctolagus cuniculus*) and factors influencing its composition. A PhD Thesis, University of Guelph. Guelph, Ontario, Canada. Pp 63-72.

Lan, P. T. N., Binh, L. T. and Benno, Y. (2003). Impact of two probiotics *Lactobacillus* strains feeding on faecal *Lactobacilli* and weight gains in chickens. *Journal of Genetics and Applied Microbiology,* 49: 29-36.

Markovic, R., Sefer, D., Krstic, M. and Petrujkic, B. (2009). Effect of different growth promoters on broiler performance and gut morphology. *Archive Medical Veterinary*, 41: 163-169.

Michelan, A.C., Scapinello, C., Natali, M.R.M., Furlan, A.C., Sakaguti, E.S., Faria, H.G., Hernandes, A.B. (2002). Utilização de probiotico, ácido orgânico e antibiótico em dietas para coelhos em crescimento: ensaio de digestibilidade, avaliação da morfometria intestinal e desempenho. *Revista Brasileira de Zootecnia*, 31: 2227-2237.

Mizusawa, N., Iino, T., Masuoka, N., Nagaoka, N., Shibata, J. Gomi, A., ... Ishikawa, F. (2014). Beneficial effects of citrus juice fermented with lactobacillus plantarum yit 0132 on japanese cedar pollinosis. *Bioscience of Microbiota, Food and Health*, 33(4): 147–155.

Ndelekwute, E. K., Assam, E.D., Unah, U.I., Assam, E.M. and Okonkwo, A.C. (2019). Antibacterial action and dietary effect of lemon juice on nutrient digestibility and growth performance of broiler chickens. *Nigerian Journal of Animal Production*, 46(2): 102-110.

Ndelekwute, E. K. and Enyenihi, G.E. (2017). Lime juice as a source of organic acids for growth and apparent nutrient digestibility of broiler chickens. *Journal of Veterinary Medicine and Surgery*, 1(1): 1-5.

Ndelekwute, E.K., Udoh, U.H. and Enyenihi, G.E. (2018). Effect of Mashed Lime Fruit Juice on Carcass Yield, Internal Organs, Economics and Nutritive Value of Meat of Broiler Chickens. *Journal of Animal Husbandry and Dairy Science*, 2(2): 27-33

Ndelekwute, E. K., Okereke, C. O., Essien, E. B. and Udorok, U. E. (2015). Effect of lime (*Citrus aurantifolia*) juice on meat yield and profitability of broilers. *Nigerian Journal of Agriculture, Food and Environment*, 11(2):53-58.

Novella, T. (2014). What fruits have citric acid? Available on: http:// www.livestrong.com/article/132609-what-fruits-have-citric-acid/

Oluremi, O.I.A., Gabriel, O.S., Ipirakwagh, E.N., Ekwue, C.O. and Afolabi, E.T. (2018). Performance and blood profile of biodegraded-sweet orange (*Citrus* sinensis) peels based diets. *Nigerian Journal of Animal Science*, 20(3): 287-294.

OMP (2015a). Oxoid Microbiological Products. Eosin Methylene Blue Agar. www.oxoid.com/UK//blue/prod-detail/prod-detail.asp?pr=CM0069&org=66

OMP (2015b). Oxoid Microbiological Products. *Salmonella Shigella* Agar. http://www.oxoid.comUK//blue/prod-detail/prod-detail.asp?pr=CM0099&org124&c=UK&lang=EN

OMP (2015c). Oxoid Microbiological Products. Manithol Salt Agar. www.oxoid.com/uk//blue/prod-detail/prod-detail.asp?pr=CM0085&org

Ovimaps, (2019). Ovilocation map; ovi earth imaginary. Dated March, 2019.

Paul, S. K., Samanta, G., Halder, G. and Biswas, P. (2007). Effect of combination organic acid salts as antibiotic replacers on the performance and gut health of broiler chickens. *Livestock Research and Rural Development*, 19: 115-120.

Penniston, K. L., Nakada, S.Y., Holmes, R.P. and Assimos, D.G. (2008). Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. *Journal of Endourology*, 22(3): 567–570.

Percy, D. H., Muckle, C. A., Hampson, R. J., and Brash, M. L. (1993). The enteritis complex in domestic rabbits: A field study.

Canadian Veterinary Journal, 34(2): 95-102.

Reddy, L. J., Jallil, R. D., Jose, B. and Gopu, S. (2012). Evaluation of antibacterial & antioxidant activities of the leaf essential oil & leaf extracts of *Citrus aurantifolia*. *Asian Journal of Biochemical and Pharmaceutical Research*, 2(2): 346-354.

Salihu, E. A., Onimisi, P. A., Omage, J.J., Afolayan, M., Moses, O. and Reuben, R.S. (2020). Evaluation of *Ocimum gratissimum* and *Ocimum canum* as natural growth promoters on the performance, intestinal microbiota and villi morphometry of broiler chickens. *Nigerian Journal of Animal Production*, 47(1): 186-196.

SAS, (2002). Statistical Analysis System Institute Inc. *Users' Guide Statistic Version* 9. Carry, North Carolina, USA.

Scapinello, C., Garcia de Faria, H., Furlan, A. C. and Michelan, A.C. (2001). Efeito da utilização de oligossacarídeo manose e acidificantes sobre o desempenho de coelhos em crescimento. *Revista Brasileira de Zootecnia*, 30: 1272-1277.

Shrestha, R. L., Dhakal, D. D., Gautum, D.M., Paudyal, K. P. and Shrestha, S. (2012). Variation of physiochemical components of acid lime (citrus aurantifolia swingle) fruits at different sides of the tree in Nepal. *American Journal of Plant Sciences*, 3: 1688-1692.

Tareen, H., Mengal, F., Masood,Z., Mengal,R., Ahmed,S., Bibi,S... Nawaz, Z. (2015). Determination of Vitamin C content in Citrus Fruits and in Non-Citrus Fruits by Titrimetric method, with special reference to their nutritional importance in Human diet. *Biological Forum – An International Journal*, 7(2): 367-369.

Uddin, M. J., Islam, K. M. S., Reza, A. and Chowdhury, R. (2014). Citric acid as feed additive in diet of rabbit- effect on growth performance. *Journal of Bangladesh Agricultural University*, 12(1): 87–90.

Waldroup, A., Kanlawato, S., and Mauromuos-Takos, A. (2005). Performance characteristics and microbiological aspects of broilers fed diets supplemented with organic acids. *Journal of Food Protection*, 58: 68-72.

Wenk, C. (2000). Recent advances in animal feed additives such as metabolicmodifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. *Asian Australian Journal of Animal Science, 13: 86-95.*

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y. and Shi, J. (2008). Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chemistry.* 106: 545-551.

Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A. and Wang, M.Q. (2003). Effect of dietary fructo-oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science*, 82: 1030-1036.

Yao, K., Yin, Y. and Li, X. (2012). Alpha-ketoglutarate inhibits glutamine degradation and enhances protein synthesis in intestinal porcine epithelial cells. *Amino Acids*, 42(6): 2491-2500.

Zhang, K. Y., Yan, F., Keen, C. A. and Waldroup, P.W. (2009). Evaluation of microencapsulated essential oils and organic acids in diets for broiler chickens. *International of Poultry Science*, 4: 612-619.