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Performance, Nutrient Utilization and Intestinal Environment of Weaned Rabbits Fed Diets Supplemented with Organic Acids in the Humid Tropics

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Target Audience: Rabbit farmers, Feed millers, Animal Scientists, Veterinarians.

Abstract

The performance, nutrient utilization and intestinal environment of weaned rabbits fed diets supplemented with organic acids (acetic acid, citric acid and formic acid) were investigated with 24 (6-week old) rabbits in a completely randomized design. The control diet was not supplemented while others were supplemented with 0.5% of each of the organic acids. Measurements were live weight, live weight gain, feed intake, carcass and organ characteristics, feed cost, nutrient digestibility, gut pH and intestinal microbial population. Organic acids had no significant ($P>0.05$) effect on the performance of the rabbits except mortality. Formic acid supplemented diet produced the highest values for shank (2.18%), back-cut (13.47%) and intestine (5.60%). Mortality was significantly ($P<0.05$) reduced with use of acetic acid diet (16.67%) against 50% for the control diet. Organic acid supplemented diets did not significantly ($P>0.05$) affect nutrient digestibility coefficients and feed cost indices. Rather, acetic and formic acids significantly ($P<0.05$) reduced the pH of the small and large intestine digesta compared to the control diet while organic acids had no significant ($P>0.05$) effect on the pH of caecum digesta. There were no significant ($P>0.05$) differences in the microbial population in the small intestine, large intestine and caecum. It was concluded that supplementation of rabbit diets with acetic or formic acid have some beneficial effect on rabbit production, significantly reduced mortality and intestinal pH but had no significant effect on feed cost, apparent nutrient digestibility, carcass and organ characteristics, and bacteria (Salmonella spp. and Escherichia coli) population in the intestine.

Key words: Intestinal environment, nutrient utilization, organic acids, performance, weaned rabbits.

Description of Problem

Rabbits have tremendous capacity for sustainable production of animal protein for human consumption (1) and could be promoted as a healthful natural meat (2). Rabbit meat is high in protein and low in fat, cholesterol, and sodium compared to the meat of other animals (3).

Rabbits are sensitive to multi-factor digestive disorder, which could cause high mortality and morbidity rate (4). During the early growing period, changes in feeding behavior and housing conditions, together with an incomplete maturation of the digestive immune system could promote the development of potentially pathogenic microflora that could cause digestive problems and lower performance of rabbits (5). Antibiotics were often added to feed or water from weaning in order to prevent enteric diseases and reduce high economic losses (6). Criticisms on the use of antibiotics in animal feeding (antibiotic resistance, human health and animal welfare) have stimulated interest in non-antibiotic substances that might have similar effect on the intestinal health of rabbits (6). Among the substances proposed include the use of organic acids, although reports of their effect on microflora population, mucosal immunity and growth performance are few and often contradictory in rabbits (6).

When the gut environment of rabbit is alkaline (high in pH), digestive enzymes are unable to function properly; therefore, nutrient digestion and absorption diminishes (7). There is therefore the need to source for alternative feed additives that would serve as growth promoter, digestibility enhancer and microbial inhibitor without detrimental effects on the health of rabbits and would also pose no danger to humans who would consume their meat. The addition of organic acids to rabbit diet could also be of importance because the weaned rabbit lacks sufficient capacity to acidify its own stomach content (hydrochloric acid in the stomach may wait for at least two hours after feeding to be optimal), leading to sub-optimal protein digestion and insufficient killing of pathogenic microbes by low pH value (8) during this period.

The application of organic acids reduces the buffering capacity of the feed, increases the speed at which stomach content reaches the optimal pH value of about 3, at which the proteolytic enzyme (pepsin) has its optimum efficiency (9; 10). According to (11), organic acids have been reported to improve growth performance, feed efficiency, mineral absorption and phytate-P utilization when supplemented in non-ruminant diets. Also a consistent improvement of nutrient digestibility has been reported by (12).

Un-dissociated part of organic acids could easily penetrate the microbial cell wall (especially gram-negative bacteria), dissociate inside the cell thereby reducing its pH (13). An attempt by the bacteria cell to get rid of the protons (H^+) released by the acids would exhaust the bacteria metabolism while the remaining anion could also destroy the cytoplasm or the cell nucleus and as a consequence, the bacteria cell will die (13). Some organic acids show more antimicrobial strength than others (14) with medium chain fatty acids having a higher antimicrobial strength (15).

Studies on the feeding of organic acids to rabbits are still few and their results inconsistent (16). Also no pathogenic bacteria were detected in rabbits fed diet of 0.4% formaxol-encapsulated formic acid, citric acid and essential oils in a micro-granule, a result that was attributed to low caecum pH (17). The objective of this study was therefore to determine the performance, carcass and organ characteristics, feed cost, apparent nutrient digestibility, pH of the intestinal digesta, and microbial population in the caecum of weaned rabbits fed diets treated with either of the three organic acids (acetic, citric and formic acids) in the humid tropics.

Material and Methods

The study was conducted at the Rabbit Unit, Teaching and Research Farm of College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike. It bears a coordinate of $5^{0}28^{1}$ North and $7^{0}33^{1}$ East and lies at an altitude of 122 meters above sea level.

The area is characterized by annual rainfall of about 2177 mm, relative humidity of about 75% and temperature range of 22^{0}C to 30^{0}C.

Management of Experimental Animals

Twenty four weaned hybrid (New Zealand White x Chinchilla) rabbits with average live weight of 645.83 g and average age of 42 days (40-44 days) were randomly allotted into four treatment groups of six rabbits per treatment. The rabbits were housed in a standard three – tier hutch (120 cm x 150 cm). The hutches which were housed in a well–ventilated house had trays underneath for faecal collection. Feed and water were given to the animals *ad libitum* and the study lasted 86 days with precautions taken to avoid feed wastage.

Experimental Diets

The experimental concentrate diet was composed of maize 35%, soybean meal 11.60%, wheat offal 25%, palm kernel meal 25%, bone meal 2.5%, salt 0.25%, lysine 0.20%, methionine 0.20% and vitamin premix 0.25%. The calculated CP and ME values were 17.32% and 10.57 MJkg^{-1}, respectively. The concentrate diet supplemented with no organic acid (OA) served as control (diet 1). Diets 2, 3, and 4 were supplemented with 0.5% acetic acid, 0.5% citric acid or 0.5% formic acid, respectively. The rabbits received supplementary *Centrosema pubescence* forage (25 g per rabbit/d) since rabbits require forage for normal living.

Experimental Design

The experimental design was completely randomized design (CRD) with model:

$Y_{ij} = \mu + T_i + e_{ij;}$ Where:

Y_{ij} = The j^{th} observation on the i^{th} treatment

μ = The overall mean

T_i = Effect of the i^{th} treatment

e_{ij} = Experimental error

There were four treatments each replicated 3 times with two rabbits (male and female) per replicate.

Data Collection

The rabbits were weighed individually at the commencement of the experiment and subsequently on a weekly basis with a top loading sensitive balance. Each morning, concentrate feed and forage not consumed were weighed before cleaning the individual hutches. The amount of feed consumed was calculated as the difference between the quantity of feed offered and quantity not consumed. The average daily weight gain and feed intake were calculated and recorded at the end of each week.

The digestibility study was conducted using male rabbits placed in metabolism cages. They were acclimatized for seven days when they were fed the experimental concentrate and forage diet *ad libitum* to establish feed intake. Thereafter, they were fed 90% of their *ad libitum* intake to avoid feed wastage and ensure total consumption of the feed. Faecal collection was for five days using an aluminum tray placed under the cages. The collected faecal droppings were dried with a GallenKamp^R oven at 60^{o}C for 12 hours and stored for proximate determination.

Carcass Evaluation

At the end of the experimental period, one female rabbit per replicate was selected for carcass analysis. Before slaughter, the animals were fasted over-night to clear the guts. They were weighed, stunned, bled by cutting the jugular vein. The tail was cut very close to the base followed by the removal of the pelt, head, feet and evisceration (removal of all internal organs).

The cut parts, internal organs, and gastro-intestinal components (stomach, small intestine, large intestine and caecum) were weighed using sensitive electronic balance (Ohaus^R, 0.01 g) and expressed as a percentage of the slaughter weight. The contents of the small intestine, large intestine and caecum were collected for pH determination using membrane pH meter (Hanna Instruments; H1314). Samples were also collected from the same caecum, small intestine and large intestine for bacteriological determination. The bacterial counts were conducted using a Microbial Colony Counter.

Chemical and Data Analyses

Proximate analysis of the concentrate diets was carried out according to methods of (18). Data collected were subjected to analysis of variance (ANOVA) for a completely randomized design as outlined by (19) and where significant differences existed, means were separated using the Duncan Multiple Range Test (20).

Results and discussion

The proximate composition of the concentrate diet fed to the weaned rabbits (Table 1) showed that the crude protein content (17.71%) met the requirement of the growing rabbits. The organic acids had no significant effect (P>0.05) on the performance of the rabbits except mortality (Table 2). This lack of influence on the daily weight gain, feed intake and live weight of rabbits could be due to higher level (0.5%) of organic acids used in this study. According to (21), levels of organic acids higher than 0.25% caused growth depression and poor feed conversion while (21) and (22) reported that lower level of organic acids yielded significantly higher body weight gains. But numerically, rabbits fed diet treated with acetic acid had the highest value for final live weight, daily weight gain, protein efficiency ratio (PER) and better FCR (Table 2). However, (23) had reported that broiler chickens fed diets supplemented with organic acids had significantly ($P<0.05$) improved body weight gains and FCR, and that organic acid supplementation, irrespective of type and level of acid used, had a beneficial effect on the performance of broilers.

Table 2: Performance of weaned rabbits fed diets supplemented with organic acids.

Parameters	Control	Acetic	Citric	Formic	SEM
Initial live weight (g)	650.00	645.83	645.83	641.67	40.01
Final live weight (g)	1783.33	1791.67	1744.16	1695.83	91.73
Daily weight gain (g)	13.49	13.64	13.07	12.54	0.72
Daily feed intake (g)	58.54	55.46	56.03	53.57	4.86
FCR	4.35	4.04	4.27	4.35	0.22
Protein intake (g)	10.37	9.82	9.91	9.48	0.81
PER	1.32	1.39	1.37	1.15	0.25
Mortality (%)	50^{a}	16.67^{c}	33.3^{b}	33.3^{b}	0.68

a,b, c: Means with different superscripts and within the same row are significantly ($P<0.05$) different. SEM = Standard error of means. PER = Protein efficiency ratio.

The organic acid supplemented diets significantly ($P<0.05$) influenced the mortality of the rabbits. Rabbits fed the control diet had the highest (50%) and those fed acetic acid diet, the lowest (16.67%) mortality. Generally, mortality was significantly reduced by 22.2% and this could be as a result of acidification of diets by various organic acids thereby reducing the production of toxic components by colonizing pathogens on the intestinal wall, and consequently preventing the damage of epithelial cells that could lead to death (24).

The carcass and organ characteristics of rabbits fed diets supplemented with organic acids were as presented in Table 3. Organic acid diets significantly ($P<0.05$) improved the proportion of back, shoulder and shank over the control diet. At the same time, heart, spleen and intestine (small+large intestine) were significantly affected without regular pattern. It could be observed that the dressed weight and thigh were not affected by the diets supplemented with organic acids probably as a result of the non-significant effect on final live weight of the rabbits.

Table 3. Carcass Characteristics of weaned Rabbits fed diets supplemented with Organic acids.

Parameter	Control	Acetic	Citric	Formic	SEM
Slaughter weight (g)	1741.67	1783.33	1766.67	1650.00	36.34
Dressed weight (%)	47.74	50.38	48.87	49.82	0.62
Thigh (%)	10.48	8.75	9.12	8.78	0.47
Back (%)	9.18^{c}	11.84^{ab}	11.55^{b}	13.47^{a}	0.51
Shoulder (%)	7.10^{b}	8.02^{a}	7.94^{a}	7.83^{a}	0.13
Loin (%)	4.48	5.17	4.83	4.85	0.12
Shank (%)	1.72^{b}	2.03^{a}	2.07^{a}	2.18^{a}	0.06
Skin (%)	9.57	10.00	10.64	11.34	0.31
Heart (%)	0.36^{c}	0.45^{ab}	0.46^{a}	0.40^{bc}	0.01
Liver (%)	8.39	3.08	3.01	3.21	0.07
Spleen (%)	0.28^{b}	0.39^{a}	0.40^{a}	0.20^{b}	0.002
Stomach (%)	1.35	1.39	1.39	1.49	0.03
Small+Large intestine (%)	4.70^{b}	4.70^{b}	4.99^{ab}	5.60^{a}	0.15

a,b, c: Means in the same row with different superscripts and are significantly ($P<0.05$) different. SEM = Standard error of mean.

The carcass and organ characteristics obtained in this study were in line with the report of (25) that carcass weight was not influenced by organic acid supplements, although they did not indicate the effect on carcass components. However, our results suggest that supplementation of rabbit diets with acetic or formic acids could be desirable for farmers considering the slight increase observed in the dressing percentage of rabbits.

The organic acids did not significantly ($P>0.05$) reduce the daily feed cost, cost of feed consumed and cost per kg live weight gain (Table 4). This could have been due to the high level of organic acids used in supplementing the diets. However, acetic, citric and formic acids reduced the daily feed cost by 5.19, 4.55 and 8.44%, respectively and correspondingly reduced the cost of feed consumed by 4.40, 3.44 and 7.67%, respectively. This suggests that the supplementation of rabbit diets with organic acid(s) could reduce feed cost as (23) had earlier reported that organic acids contributed to profitability in poultry production.

Table 4. Cost of Diets supplemented with Organic Acids fed to Weaned Rabbits

Cost	Control	Acetic	Citric	Formic	SEM
Daily feed cost (₦)	3.08	2.92	2.94	2.82	0.24
Cost of feed consumed (₦)	36.62	35.01	35.36	33.81	2.81
Total feed intake (g)	702.50	665.57	672.36	642.87	4.57
Cost per kg weight gain (₦)	228.08	212.85	224.77	223.11	11.45

SEM = Standard error of mean.

Apparent Nutrient digestibility

There were no significant (P>0.05) differences in the apparent nutrient digestibility coefficient of Dry Matter (DM), Crude Protein (CP), Ether Extract, Crude Fibre (CF), Crude Ash and Nitrogen Free Extract (NFE) of rabbits fed diets supplemented with organic acids (acetic, citric and formic acids) and the control diet (Table 5). Improvements in diet digestibility of rabbits are variable, often not significant (6) although, (26) had reported an increase in diet digestibility by adding sodium butyrate. Total tract apparent digestibility of DM or organic matter of growing rabbits was not affected by 0.5% fumaric acid supplementation (27), although digestibility of crude protein and crude fibre significantly increased. It has also been stated (28) that there was no positive effect of the inclusion of organic acids in the diet on apparent digestibility of DM, CP, CF, ether extract, Ash and NFE of rabbits. It was expected that the supplementation of the diets with organic acids would improve apparent digestibility coefficient of nutrients as (7) had reported that organic acid in diets reduced small intestinal pH, enhanced enzymatic activities and absorption capabilities of the small intestine.

Table 5: Apparent digestibility coefficients of rabbits fed diets supplemented with organic acids

Parameter	Control	Acetic	Citric	Formic	SEM
Dry matter (%)	73.96	71.57	72.57	73.57	2.33
Crude protein (%)	83.64	82.40	81.40	81.58	1.31
Crude fibre (%)	79.13	76.63	83.14	84.37	3.94
Ether extract (%)	84.78	82.78	82.32	83.41	1.15
Crude ash (%)	63.35	61.55	56.48	59.10	3.06
Nitrogen free extract (%)	62.90	58.00	60.32	60.08	3.10

SEM = Standard error of mean.

GIT characteristics

The pH of the sections of the rabbit intestine as influenced by diets supplemented with organic acids is presented in Table 6. The organic acids significantly (P<0.05) reduced the pH of digesta of the small intestine. Acetic and formic acids significantly ($P<0.05$) reduced the pH of the large intestine compared to the control diet while the organic acids had no significant ($P>0.05$) effect on the pH of caecum digesta. This result is in line with the report of (7) and may have been due to the intrinsic acid activity and buffering capacity of diets (6; 29). The non-significant effect of the organic acids on caecal pH could also be due to the fact that either a major absorption of the organic acids occurred before reaching the caecum or it may be due to a significant buffering capacity of caecal content (28).

Table 6: Intestinal pH of rabbits fed diets supplemented with organic acids.

GIT	Control	Acetic	Citric	Formic	SEM
Small intestine	7.28^{a}	7.19^{b}	7.19^{b}	7.15^{b}	0.16
Large intestine	6.62^{a}	6.51^{b}	6.56^{ab}	6.49^{b}	0.13
Caecum	6.25	6.05	6.16	6.07	0.14

a,b, c: Means in the same row with different superscripts and are significantly (P<0.0 different. SEM = Standard error of mean.

Bacterial population

There were no significant ($P>0.05$) differences in the *Salmonella spp*. count in the small intestine and *Escharichia coli* count in the large intestine and the caecum (Table 7). *Salmonella spp*. could not be found in the large intestine and caeca. There were also no *Escharichia coli* in the small intestine of rabbits fed control and organic acid supplemented diets. Formic acid however numerically reduced the population of *Salmonella* in the small intestine more than acetic or citric acids while acetic acid reduced the population of *Escherichia coli* in the large intestine more than other acids considered. The result of this study is in agreement with the report of (30) that organic acids added to the diet of rabbits did not prevent *salmonella spp* and *E. Coli* growth but reduced their population in relation to non-acid supplemented diet

Table 7: Bacterial population in the Intestine of rabbits fed diets supplemented with organic acids

Parameter	Control	Acetic	Citric	Formic	SEM
Salmonella spp. in S. I. (x10^{3}cfu/g)	2.03	2.00	2.11	1.51	0.14
Escherichia coli in L. I. (x10^{3}cfu/g)	1.98	1.50	1.63	1.97	0.24
Escherichia coli in caecum (x10^{3}cfu/g)	0.93	1.25	1.63	1.75	0.21

SEM = Standard error of mean. S.I. = Small Intestine, L. I. = Large intestine, CFU = Colony forming Unit.

Conclusion and Application

1. Supplementation of rabbit diets with acetic or formic acid has some beneficial effect on rabbit production.
2. It significantly reduced mortality and intestinal pH.
3. Organic acid supplementation had no significant effect on feed cost, apparent nutrient digestibility, carcass and organ characteristics and bacteria (*Salmonella spp*. and *Escherichia coli*) population in the intestine.
4. An investigation of the appropriate inclusion level(s) of the organic acids could be necessary for better rabbit performance.

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