



Effect of *Alchornea Cordifolia* and *Costus Afer* based diet on Gut Microbes of Male Rabbits.

Elijah, I. Ohimain¹, Medubari B. Nodu², Okes, J. Imoni¹

¹Agricultural and Veterinary Microbiology Research Unit, Microbiology Department, Niger Delta University, Wilberforce Island, Bayelsa State

²Animal Science Department, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

Abstract

The gut microbiome of animals affects the health and performance of the animal. This study was carried out to evaluate the effect of *Alchornea cordifolia* and *Costus afer* leaf meal on gut microbiome of young male rabbits. Twenty-four (24) 5-6 months old rabbit bucks with an average weight of 5.3kg were used for this study. The completely randomized design was used and the rabbit bucks were distributed into four (4) feeding /treatment groups (T) designated as T1 (the control fed with regular rabbit feed only), T2 (fed with *Alchornea cordifolia*), T3 (fed with *Costus afer*) and T4 (fed with a mixture of *Alchornea cordifolia* and *Costus afer*). The rabbits were fed the experimental diet for eight weeks and samples for microbiological analysis were collected from the different sections of the rabbit gut (stomach, ileum, caecum and colon) and droppings and analyzed for total heterotrophic bacteria (THB) and total coliform counts (TCC). The population density of THB was highest in T4 at the stomach ($5.03 \pm 0.87 \times 10^5$ cfu/g) and ileum ($5.32 \pm 1.19 \times 10^5$ cfu/g), but in T3 at the caecum ($4.83 \pm 0.95 \times 10^5$ cfu/g), colon ($4.15 \pm 0.60 \times 10^5$ cfu/g) and droppings ($3.35 \pm 0.65 \times 10^5$ cfu/g). The population of TCC was highest in T4 in the stomach (i.e. $2.72 \pm 0.40 \times 10^5$ cfu/g) and ileum $2.67 \pm 0.53 \times 10^5$ cfu/g), in T3 at the caecum ($3.53 \pm 0.69 \times 10^5$ cfu/g) and droppings ($2.02 \pm 0.37 \times 10^5$ cfu/g), but T1 in the colon ($2.77 \pm 0.60 \times 10^5$ cfu/g). The feed intake was highest in T4, followed by T3 and T1, but least in T2 ($P < 0.05$), whereas there was no significant difference ($P > 0.05$) in the weight gain of the rabbits (1.75 kg). We found that while *Alchornea* seemed to be reducing the population, *Costus afer* was enhancing their population and the overall effect on weight gain was insignificant.

Keywords: Coliforms, Heterotrophic bacteria, Medicinal plants, Gut microbes, Phytobiotics, Phytogenics, Rabbit microbiome

Introduction

Due to poverty, protein malnutrition is common in many developing countries including Nigeria, where the source of animal protein is mostly beef, chicken and wildlife. Rabbit is a source of protein that is underutilized. Rabbit farming is not common in the country. Two of the major challenges in animal farming is the availability of good quality feed (Ohimain and Ofongo, 2014) and problem of microbial infections (Ofongo and Ohimain, 2019; Ofongo and Ohimain, 2015), which prompted the use of antibiotic growth promoters (AGP) in animal farming (Ohimain and Ofongo, 2012, Ofongo and Ohimain, 2019). But due to the problems of antibiotic resistance in humans and animals, the use of synthetic antibiotics is either restricted or banned in several countries. Without antibiotics, microbial infections would threaten animal farming. Common microbial infections in animal farming are caused by bacteria (*Salmonella*, *Clostridium perfringes*, *Escherichia coli*) and parasites particularly *Eimeria* leading to diseases, poor growth and even mortality (Ohimain and Ofongo, 2012). This has prompted the scientific community to research into alternatives to AGP. Several alternatives to AGP have emerged, which could be classified as photogenics and phytobiotics, probiotics, prebiotics and enzymes (Ofongo-Abule et al., 2016, Abule and Ohimain 2016).

Meanwhile, two herbs common in traditional African medicine are *Alchornea cordifolia* and *Costus afer*. The phytochemical analysis of the leaves of these herbs revealed the presence of several bioactive substances of medical importance including Alkaloids, Saponins, Tannins, Flavonoids, phenols, glycosides and terpenoids (Momoh et al, 2011; Ukpabi et al, 2012 and Anaga et al 2004). These plants have been used in diverse forms. Due to its high anti-inflammatory properties (Osadabe and Okoye, 2003), *Alchornea cordifolia* have been used for the treatment of chancre, yaws, wounds and ulcers, gum inflammation and conjunctivitis (Neuwinger 2000). *Costus afer* have been reported to have hypoglycaemic property (Momoh et al. 2011),

and has ability to alleviate hepatic oxidative stress and toxicity (Ukpabi et al. 2012).

Olawosulu and Ibrahim (2006) screened five medicinal plants and reported that they can be explored as alternative feed stuffs of protein for the formulation of animal diets in order to reduce the competition and cost of protein in animal feeds. Several other researchers have used different plant species to supplement for protein and as alternative to antibiotics in the diet of animals especially the monogastrics (Karsin et al, 2008, Edeoga and Okoli (2000) Egena et al, 2007; Obun and Ayanwale, 2008). *Alchornea cordifolia* have been used as protein supplements in feed to boost the performance of many reared animals including rabbits (Alikwe et al., 2014a), rats (Alikwe et al., 2014b), chicken (Alikwe et al., 2014c) and Snail (Alikwe et al., 2014d). However, the use of *Alchornea* in rabbit diet have led to various outcomes. Timibitei et al (2014a) showed that *A cordifolia* in the diet of rabbits resulted in the decrease in the weight of some organs including liver, kidney and heart of rabbits, while Timibitei et al., (2013) claimed that there was no difference in organ weight including the gonads when feed with *Alchornea cordifolia* leaf meal up to 5-15g/kg feed. *Alchonea* seemed to affect the reproductive health of rabbits. Timibitei et al., (2014b) reported that *Alchornea* in feed caused a progressive decrease in estrogen and progesterone levels of rabbits, while it was recently demonstrated that it can boost the reproductive health of male rabbit (Nodu et al., 2020). But studies on the effects of *Alchornea* meal on gut microbes of rabbit are not common, Kigigha and Atuzie (2012) tested the antibiotic activities of extracts of *Alchornea cordifolia* in-vitro and found it to be effective against *Staphylococcus aureus* and *Escherichia coli*, which appears to justify their use in traditional medicine. Hence, this study was aimed at investigating the effect of feeding *Alchornea cordifolia*, *Costus afer* and their mixture on the gut microbial densities of young male rabbits.

Materials and Methods

The study was conducted in the rabbitry unit of the Teaching and Research Farm, Faculty of Agriculture, Niger Delta University. Twenty-four (24) domestic mixed breed adult (*Oryctolagus cuniculus*) rabbits with a mean weight of 5.3kg were assigned randomly to four treatments (T=4) with six rabbits (n=6) per treatment and were further subdivided into three replicates (r=3) per treatment in a completely randomized experimental design (CRD). *Alchornea cordifolia* and *C. afer* leaves were harvested fresh from the Teaching and Research Farm, the leaves were plucked,

washed and dried, ground and fed along with growers' mash according to the different treatments diets (T). Treatment 1 (T1) i.e. the control group was fed with concentrate feed as shown in Table 1 and other forages such as *Puereria*, *Ipomea batata* leaf and *Aspillia africana*, Treatment 2 (T2) was fed with 50% concentrate feed and 50% *Alchornea cordifolia*, Treatment 3 (T3) with 50% concentrates and 50% *Costus afer*, and treatment 4 (T4) with concentrates (50%) and a mixture of *Alchornea cordifolia* and *Costus afer* at 25:25 ratio. The rabbits were fed ad libitum for a period of 8 weeks.

Table 1: Percentage composition of experimental diet

Ingredients	% composition
Maize	40.00
Soybean meal	26.00
Wheat offal	15.00
Palm kernel cake	6.00
Crayfish dust	8.00
Bone meal	4.30
Mineral/vitamin premix	0.50
Salt	0.20
Total	100.00

Nodu *et al* (2020)

Performance

Feed Intake and weight gain were measured and used to assess performance. The quantity of feed consumed by the rabbits in the different experimental groups was measured. The feed intake was measured by subtracting the leftovers from the total feed administered. The weight of the rabbits was weighed at the beginning and ending of the experiment and the difference is the weight gain.

Microbiological Analysis

At the end of the experiment, the rabbits were slaughtered and the contents were collected aseptically at different sections of the gut (ileum, caecum, stomach and colon) and droppings and analyzed for total heterotrophic bacteria counts (THB) and total coliform counts (TCC). The samples were serially sterilized diluted up to 10^7 using distilled water and plated on nutrient agar and MacConkey

agar using pour plate method respectively for THB and TCC. MacConkey Agar was used as selective and differential medium for cultivation and presumptive identification of coliform organisms. The plates were incubated at 35°C for 24-48 hours. Colonies of coliforms appears red or pink on MacConkey Agar. The colonies were counted using a conolony counter. Only colonies within the range of 30 – 300 were counted as valid and was recorded as colony forming units per gram (cfu/g).

Statistical Analysis

Data obtained from the laboratory analysis were subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT) using SPSS version 17 (SPSS Inc, Illinois, Chicago).

RESULTS

The population density of THB in the stomach was highest ($5.03 \pm 0.87 \times 10^5$ cfu/g) in Treatment 4 i.e. when both *Alchornea* and *Costus* were incorporated in the diet of the male rabbits, though, not significantly different ($P > 0.05$) with the other treatments including the control (Table 2). At the ileum, the highest THB population of $5.32 \pm 1.19 \times 10^5$ cfu/g was also reported in Treatment 4, which was significantly ($P < 0.05$) different from the other treatments, followed by Treatment 3 and least in Treatment 2. At the caecum, the highest

THB density of $4.83 \pm 0.95 \times 10^5$ cfu/g was recorded in Treatment 3, which was significantly different ($P < 0.05$) from the other treatments. At the colon, the highest THB population of $4.15 \pm 0.60 \times 10^5$ cfu/g was recorded for Treatment 3, which was not significantly different ($P > 0.05$) from the others. The population of THB in the droppings was also highest in Treatment 3 being $3.35 \pm 0.65 \times 10^5$ cfu/g, but was also not significantly different ($P > 0.05$) from the others.

Trt #	Treatment Diet	Stomach	Ileum	Caecum	Colon	Droppings
1	Control. Concentrate & forage	$3.65 \pm 1.06a$	$2.31 \pm 0.42ab$	$2.05 \pm 0.28a$	$1.97 \pm 0.77a$	$1.93 \pm 0.22a$
2	Alchornea cordifolia supplemented with concentrate	$3.30 \pm 0.91a$	$1.44 \pm 0.25a$	$1.48 \pm 0.42a$	$2.45 \pm 1.01a$	$1.94 \pm 0.80a$
3	Costus afer with concentrates	$3.24 \pm 0.82a$	$4.07 \pm 0.83bc$	$4.83 \pm 0.95b$	$4.15 \pm 0.60a$	$3.35 \pm 0.65a$
4	Costus + Alchornea + concentrates	$5.03 \pm 0.87a$	$5.32 \pm 1.19c$	$2.82 \pm 0.65a$	$3.33 \pm 1.21a$	$2.32 \pm 0.50a$

Along the column, mean \pm standard error (n=6) with the same alphabets are not significantly different ($P > 0.05$) according to Duncan Statistics

The population of TCC in the stomach of the rabbits was highest in Treatment 4, i.e. $2.72 \pm 0.40 \times 10^5$ cfu/g, which was not significantly different ($P > 0.05$) from the other treatments (Table 3). At the ileum, the highest population of $2.67 \pm 0.53 \times 10^5$ cfu/g was reported in Treatment 4, which was significantly different ($P < 0.05$) from the values recorded in Treatments 1 and 2. At the caecum, the highest TCC of $3.53 \pm 0.69 \times 10^5$ cfu/g was recorded in Treatment 3, which was

significantly different ($P < 0.05$) from the other treatments. In the colon, the highest population of coliforms of $2.77 \pm 0.60 \times 10^5$ cfu/g was recorded, which was significantly different ($P < 0.05$) from the control, but not from the other treatments ($P > 0.05$). In the droppings, the highest coliform density of $2.02 \pm 0.37 \times 10^5$ cfu/g was recorded in Treatment 3, which was not significantly different ($P > 0.05$) from the other treatments.

Table 3: Changes in coliform in the gut of male rabbits fed with *Alchornea cordifolia* and *Costos afer* based diets, x 10⁵ cfu/g

Trt #	Treatment Diet	Stomach	Ileum	Caecum	Colon	Droppings
1	Control. Concentrate & forage	1.76±0.67a	1.03±0.32.08a	0.93±0.26a	0.95±0.24a	0.97±0.07a
2	Alchornea cordifolia supplemented with concentrate	2.01±0.44a	0.78±0.12a	0.79±0.35a	1.34±0.45ab	1.12±0.43a
3	Costus afer with concentrates	1.40±0.35a	1.83±0.42ab	3.53±0.69b	2.77±0.60b	2.02±0.37a
4	Costus + Alchornea + concentrates	2.72±0.40a	2.67±0.53b	1.78±0.40a	1.86±0.59ab	1.31±0.35a

Along the column, mean ± standard error (n=6) with the same alphabets are not significantly different (P>0.05) according to Duncan Statistics

Feed intake and weight gain was used to assess performance (Table 4). The feed intake was highest in T4, followed by T3 and T1, but least in T2 (P <0.05). Notwithstanding the differences in feed intake, there was no

significant difference (P >0.05). in the weight gain of the rabbits. The weight gain of 1.75 kg was the same in T1, T3 and T4 but slightly lower in T2.

Table 4: Performance of male rabbit fed with *Alchornea codifolia* and *Costus afer* based diets

Parameter	T1	T2	T3	T4
Feed Intake, g	42.00±2.65 ^b	39.64±2.79 ^c	42.97±2.48 ^b	52.74±2.52 ^a
Weight Gain, kg	1.75±0.14 ^a	1.67±0.17 ^a	1.75±0.15 ^a	1.75±0.17 ^a

(P<0.05) Means on the same row with different superscript are significantly different.

Discussions

Overall, the results tend to show that *Alchornea* extracts results in the reduction in the population of heterotrophic bacteria, while *Costus* tend to enhance it. The reason for these differences is not known, but it could be due to the antibiotic property of *Alchornea cordifolia*, which have been established by Kigigha and Atuzie (2012). It should be noted that heterotrophic bacteria are not pathogens, they mostly consist of the normal flora/ microbiome of the intestinal tract that protects the gut from pathogenic species. They compete with pathogens for food and attachment surface and become beneficial to the animal.

The results do not seem to show that the herbs result in any significant reduction of coliforms in the rabbits. Coliforms, though, a normal flora in the intestinal tract, could become pathogenic especially when the immunity of the animal is weakened, hence, they can easily become opportunistic pathogens.

The results of the performance show that that the presence of *Alchornea cordifolia* in the diet tend to result in decreased feed intake significantly and weight gain insignificantly. The reason for this pattern of growth is not fully understood. But it has been observed that *Costus afer* is sweet, while *Alchornea* is not. Hence, the rabbits'

preference of *Costus* to *Alchornea*. It should be noted that control of pathogenic microbes often results in the enhancement of performance. Other authors have reported significance increases in performance when they incorporated *Alchornea* in the feed of rabbits (Alikwe *et al.*, 2014a).

Conclusion

In an attempt to find alternative AGP for the farming of rabbits, we studied the effect of *Alchornea cordifolia* and *Costus afer* and their combination-based diet on the gut heterotrophic bacteria and total coliform in vivo. We found that while *Alchornea* seemed to be reducing the population, *Costus afer* was enhancing their population of the microbes in the GIT. The feed intake was significantly different among the treatments, with a preference for *Costus*, but the overall weight gain was not significantly different.

Acknowledgement

This work was carried out as postgraduate research project of the third author under the supervision of the first and second authors. The in-vivo study was carried out in the Teaching and Research Farm, Faculty of Agriculture, while the microbiological analysis was carried out in the Microbiology Department, Niger Delta University, Wilberforce Island, Bayelsa State.

Disclosure Statement

We declare no conflict of interest in the research, writing, editing and publication of this paper. All authors significantly contributed to the success of this paper.

References

Abule, R.T.S., Etebu, E. and Ohimain, E.I. (2016): Effect of diet type and enzyme supplementation on gut pH in broilers. *International Journal of Agriculture Innovations and Research* Vol 4 (5) 964 – 967

Abule, R.T.S. and Ohimain, E.I. (2016): Enzyme supplemented wheat offal diet improves performance of broilers. *International Journal of Agriculture Innovations and Research* Vol 4 (5) 968 – 971.

Alikwe, P.C.N., Akinbosola P. J. Ohimain, E. I. (2014b). Performance characteristics of wistar rats gavaged with aqueous extract of *Alchornea cordifolia* leaf meal. *American Journal of Experimental Agriculture*. 4 (8): 971 – 977

Alikwe, P.C.N., Ohimain, E. I., Kester, A. E. (2014a). Performance evaluation of New Zealand white rabbits fed *Alchornea cordifolia* leaf meal as replacement for soya bean meal. *American Journal of Agriculture and Forestry*. 2 (2): 51-54

Alikwe, P.C.N., Tuagha, B., Ohimain, E. I., 2014c. Tropical Christmas bush (*Alchornea cordifolia*) Leaf Meal as Unconventional Protein Supplement in the Diet of Broiler Chicks. *International Journal of Applied Research and Technology* 3 (2): 40-47

Alikwe, P. C. N. Yeigba, J, Akinnusi, B. Oyenike F A, and Ohimain, E.I. 2014d. Performance and Carcass Characteristics of Giant African Land Snails fed *Alchornea cordifolia* Leaf Meal in Replacement for Soybean Meal. *International Journal of Research in Agriculture and Food Science*. 1 (6): 1-4

Anaga A.O, Njoku C.J, Ekejiuba E.S., Esiaka M.N and Asuzu I.U (2004). Investigation of the methanolic leaf extract of *Costus afer* for pharmacological activities invitro and in vivo. *Phytomedicine*. 11:242-248.

Edeoga, H.O., and Okoli, B.E., (2000) Chromosome number of *Costus lucrmusianus* (Costaceae) in Nigeria folia. *Geobotanica* 33: 315-318.

Egena, S.S., Yahaya, A., Shaiwoya, S.K., and Usman, A. (2007). Effect of anaerobic fermentation/lye treatment and roasting of flamboyant seed on nutrient digestibility and carcass characteristics of broilers. *International Journal of Tropical Agricultural and Food System* Pp 373-374.

Karsin, P.D., Oyawiye, E.O., Bawa G.S., Musa, U. and Edache, J.A. (2008) performance of Japanese quails fed graded levels of pigeon pea (*Cajanus cajan*) Meal. *Proc. 33rd Ann. Conf NSAP. Ayetoro, Ogun State*. 18th-20th March. Pp:390-392.

Kigigha LT and Atuzie MN. 2012 Assessment of traditional medicinal application of

- Alchornea cordifolia*. African Journal of Biotechnology. 11(8): 2083-2086.
- Momoh, S., Yusuf O.W., Adamu M.M., Agwu C.O.C and Atanu F.O. (2011) Evaluation of Phytochemical composition and hypoglycaemic activities of methanolic leave extracts of *Costus afer* in albino rats. *British journal of pharmacological resources* 1:237-239.
- Neuwinger, H.D. (2000): Africa Traditional Medicine: A dictionary of plant use and application. *Medpharm Scientific Stuttgart, Germany* pp. 589.
- Nodu M. B., Patani I, Bennett A. B., Ufondu N. G., Ohimain E. I. 2020. Effects of *Alchornea cordifolia* and *Costus afer* leaf meal on semen electrolytes and metabolites of young male rabbits. *Annals of Applied Science*. Accepted
- Obun C.O. and ayanwale B.A (2008), Performance, nutrients digestibility and cost evaluation of raw and roasted *Afrelia africana* Seed Meal to finisher Broiler Chicks. *Nig. J. of Animal Reproduction* 35:9-17.
- Ofongo – Abule, R.T.S., and Ohimain, E.I, 2015: Proximate composition and anti – microbial effect of *Ocimum gratissimum* on broiler gut micro flora. *Journal of Advances in Biological and Basic Research*. (1): 24 – 27.
- Ofongo – Abule, R.T.S., Etebu, E. And Ohimain, E.I. 2016: Performance and molecular identification of bacteria isolated from the gut of broiler birds after antibiotic administration and enzyme supplementation. *Journal of Microbiology, Biotechnology and Food Sciences*. 6 (3): 924 – 929.
- Ofongo, R. T. S. and Ohimain, E. I. (2019). Effect of enzyme supplementation and plant extracts on villus height and microbial counts in broilers. *Nigeria Journal of Animal Production*. 46 (2): 137 – 149.
- Ohimain, E. I. and Ofongo, R. T. S. 2014. Enzyme Supplemented Poultry Diets: Benefits So Far – A Review. *International Journal of Advanced Research in Biotechnology*. 3 (5): 31-39
- Ohimain, E. I. and Ofongo, R.T. S. 2012. The effect of probiotic and prebiotic feed supplementation on chicken health and gut microflora; a review. *International Journal of Animal and Veterinary Advances*. 4(2): 135-143
- Olowosulu A.K., and Ibrahim Y.K.E (2006) Studies on the antimicrobial Screening of Aqueous extracts of five plants used in folk medicine in Nigeria. *West African Journal of Biological Science* 3(5):2-26
- Osadabe, P.O and Okoye F.B (2003) Anti-Inflammatory effect of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. *Journal of Ethnopharmacology* 88:19-24.
- Timibitei, K. O., Alikwe, P. C. N., Ohimain, E. I. and Wekhe, S.N. 2014b. Evaluating the impact of *Alchornea cordifolia* (christmas bush) root bark, seeds and pod husks on the gonads, serum level of testosterone, estrogen, serum enzymes and blood corpuscles of rabbits. *International Journal of Current Research in Life Sciences*. 3 (5): 46-50
- Timibitei, K. O., Alikwe, P. N. C., Ayakurai, J. I. and Ohimain, E. I. 2014a. Haematological Characteristics and Organ Weights of Rabbits Fed *Alchornea cordifolia* Leaf Meal. *International Journal of Farming and Allied Sciences* 3 (7) 754-759.
- Timibitei, K., O., Alikwe, P. C. N., Wekhe, S. N. and Ohimain, E. I. 2013. Effects of *Alchornea cordifolia* root bark, seeds and pod husks on the growth response and visceral organs of rabbits. *Nigerian Journal of Agriculture, Food and Environment*. 9 (3): 23-27
- Ukpabi, C.F., Agbafor, K.N, Ndukwe O.K., Agwu A and Nwachukwu S.N (2012) Phytochemical composition of *Costus afer* and its alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity *International journal of modern Botany*. 2(S0):120-126.