Biotec.Res.J.2016; Vol 2(1):53-57
eISSN 2395-6763

ORIGINAL RESEARCH

Variation of microbial population in the gut (ileum and caecum) and faeces of rabbits fed with pawpaw and Alchonea leaf meals

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• Received: 30 September 2015 • Revised: 17 October 2015 • Accepted: 29 October 2015 • Published: 14 February 2016 •

ABSTRACT
Rabbit is an herbivorous animal habitually practicing caprophagy which synergistically works along with the large bacteria population in the gut to efficiently digest roughages and other plant materials especially green vegetables. This study was conducted to assess the effect of *Alchornea cordifolia* leaf meal (ACLM), Pawpaw leaf meal (PLM) and a mixture of these leaf meals (ACLM+PLM) on the microbial contents of the gut of rabbits. Twenty four rabbits were used for this study and randomly allocated to four treatments. The treatment were designated as T_1 (control), T_2 (*Alchornea cordifolia*), T_3 (Pawpaw leaf meal) and T_4 (mixture of *Alchornea cordifolia* and Pawpaw leaf meal). Results showed that total heterotrophic bacteria (THB) counts and coliforms were respectively recorded in the range of 1793 – 2720 cfu/g and 111.67 – 189.00 cfu/g (Ileum), 13600 -18700 cfu/g and 1706.67 – 2140.00 cfu/g (caecum) and 69666 – 250666 cfu/g and 11700.00 - 24333.33 cfu/g (fecal materials). The microbial populations fluctuate between the control and the various treatments in both THB and coliform. The microbial counts increased from the proximal (ileum) to the distal (caecum) sections of the rabbits' gut.

KEY WORDS: *Rabbit, Feaces, Bacteria, Coprophagy*

Introduction

The importance of animal protein in the nutrition of man can never be over emphasized, because of the high value of animal protein over other sources of protein like that of plants. In a bid to satisfy protein need by humans, several animals are raised and managed on the farm and such animals are therefore referred to as farm animals. Popular among these farm animals are cattle, sheep, goats, swine, poultry and rabbits. Of these animals, rabbit is known to have a better production advantage over others because of its size, prolificacy and feeding characteristics or behavior among others.

Rabbit is a pseudo-ruminant that feeds largely on forage and makes efficient use of nutrients from these roughages (Oruseibio 2002); it is therefore characterized as an herbivore. However, rabbit feed voraciously on kitchen waste which is an added advantage in its feeding habit. Being a non-ruminant herbivore, rabbits are endowed with large hindgut that facilitates efficient digestion of feed particularly forages because of the heavy presence of microorganisms. It is therefore considered as a hindgut fermentor. The hindgut consist of the caecum and colon and a large intestine heavily loaded with microorganism enabling the rabbits to utilise fibrous feed efficiently by courtesy of its feeding and digesting strategy (Iribeekel, 2001). The domestic rabbit being primarily herbivorous, feeds on most green vegetables, hay, grains, tuber and roots thriving excellently on rations with ingredients almost entirely from

plant sources (Aduku and Olukosi, 1990; Nodu et al., 1999; Oruseibio, 2002). *Alchornea cordifolia* (Christmas bush) and *Carica papaya* (pawpaw) are plants found in the West African sub-region and most commonly found as ever green vegetation of the tropical zone of southern Nigeria. *Alchornea cordifolia* is a browse plant especially used by livestock farmers to feed their animals as well as pawpaw leaf. The combination of the leaves of these two common plants (Alchornea Cordifolia and Pawpaw) makes for good forage as feed to animals. This is most probable in respect of nutritional and medicinal values of these plants. *Alchornea cordifolia* leaf is often considered bacteriocidal and used as herb to treat/cure several ailments of man (Lamikara et al., 1990; Iwu, 1993).

Rabbits habitually practices coprophagy, which in conjunction with fermentation in the large intestine supplies necessary amount of most B-Vitamins, prevent amino acid deficiency, facilitates digestion of fiber and makes available other nutrient for the animal (Aduku and Olukosi, 1990). This is made possible by the high population of bacteria in the hindgut of the animal. Therefore, consideration should be given to whatever feed including green plants fed to rabbit in order not to negatively alter the bacteria population in the gut and eventual interference with the digestive mechanism or process of the rabbit. It is against this background therefore that this study was conducted to ascertain the effects of feeding the leaf meals of *Alchornea cordifolia* and pawpaw on the microorganism (bacteria) content in the guts of rabbits.

Materials and methods

Animal procurement

Twenty four weaner rabbits (mixed breed of New Zealand white, Chinchilla and Dutch) comprising of both males and females aged 3 – 4 months old were used for this study. The average weight of the rabbits is 1.05kg. The rabbits were procured from Korama town, Gbarian in Yenagoa Local government area of Bayelsa state, Nigeria

Pre-experimental management of animals

The rabbits were pre-conditioned (acclimatized) for two weeks under the same environmental condition. The same types of water and feed (growers mash and forage) were administered to them during the period. Also, the rabbits were administered with the same medications (antibiotics and ivomec injection).

Housing

The rabbits were housed in wooden conventional hutches. The hutches were constructed with wood and wire mesh in the Niger Delta University Teaching and Research farm. The hutches had three compartment each and spacious enough to accommodate about 3 – 4 rabbits.

Feeding

All the rabbits on arrival were pre-conditioned for two weeks and fed with the same feed and water (growers mash and forage from grasses and legumes such as avarlake, *Peureria* sp, *Aspirilla africana* (Bush marigold), elephant grass, *Mucuna utilio* etc). The rabbits were fed *ad-libitum*.

The feeders used were constructed from India bamboo while silver stainless plates were used as drinkers. At the commencement of the experiments, the rabbits were divided into four groups (treatments). Table 1 presented the various feed used to feed the rabbits. While Table 2 presents the proximate composition of the various feeds/forage.

Medication

During pre-conditioning, all the animals were administered broad spectrum antibiotics (pantex-oxytetra 200 water soluble powdered % concentration) for five days. On the further day of administration of antibiotics 0.2ml of Albiomee injection was given simultaneously. Subsequently, as the experiment progressed, the antibiotics were repeated on the 3rd and 6th week to prevent and protect the animals from some common rabbit diseases that would have likely occurred and interfered with the experimental period.

Experimental design

The completely Randomized Design (CRD) experimental method was used for this study. The twenty four rabbits used for this study were randomly divided into four groups/treatments (Table 1) of six rabbits per group/treatment representing one control group (T_1) and three treatment groups (T_2, T_3 and T_4). The experiment diets were given in the morning between 8:00 – 9:00 am and evening from 4:30 – 5:30pm. The feed and water were given both ad-libitum. But the supplements (concentrate) were restricted to 50g per treatment a day. The duration of the experiment was 8 weeks.

The source of the feeds used for the study

In the control diet/group, the concentrate (growers mash) used was bought from Jakes feed in Yenagoa, Bayelsa

State, Nigeria, while the forage was harvested from the environment of the farm. Also in the treatment/ group 2, pawpaw leaves were harvested form Polaku town in Gbarian, Yenagoa local Government Area, Bayelsa state, Nigeria. The harvested green leaves were chipped into smaller bit and sun dried for 4 days. The *Alchornea cordifolia* leaves were harvested within the Niger Delta University premises were the experiment was conducted. Variegated colour leaves with hole were removed and the good leaves were chopped into smaller size and sun dried for 3 – 4 days. This was done to prevent spoilage.

Sample collection from the experimental rabbits

The rabbits were immobilized by placing cotton wool soaked in chloroform in a carton jar containing the experimental animals. The rabbits were dissected on a dissecting table using dissecting kit. The digestive system was carefully handled and the ileum and caecum were identified and the content collected aseptically into a sterile bottle and labeled accordingly. The fecal materials from the test rabbits was randomly collected for three consecutive days prior to the end of the experiment. They were mixed based on the test animals of the same group/treatments for the three days. Samples were analyzed for microbial counts immediately after collection.

Enumeration of microbial counts from gut and fecal samples of rabbits

The populations of microorganisms in the samples were enumerated using modified serial dilution pour plate techniques of Pepper and Gerba (2004). About 1g of the sample(s) was serially diluted in 9ml sterile normal saline. Aliquots of the dilutions were ascetically plated into growth media (Nutrient Agar and MaConkey Agar for total heterotrophic bacteria and coliforms respectively) using pour plating techniques. The agar plates were incubated inverted at 37°C for 24 - 48 hours. After incubation, the colonies that grew on the medium were counted with the aid of hand lens and expressed as colony forming units (cfu)/g of the samples.

Results and discussion

The microbial counts of ileum, caecum and faecal samples from the rabbits few with different forage used in this study are presented in Table 3. In the samples from the ileum, the total heterotrophic bacteria (THB) ranged from 1793 – 2720 cfu/g, being not significantly different among the treatments. However, T1 (control) have a high microbial counts. Coliform counts ranged from 111.67 – 189.00 cfu/g. No significance difference (P>0.05) exist between T1 and T3 and T2 and T4. Also in the caecum samples, the THB ranged from 13600 - 18700 cfu/g being significantly different (P<0.05) among the treatment. However, population in the control (T1) was higher than other treatments.

Table 1: Feeds and concentrations used for the study

Treatments	Feed/ supplements	Supplements/feeds composition
1 control	Concentrate growers mash and forage	100% concentrate
2	*Carica papaya* leaf meal (CPLM) using concentrate feed as supplement	50 % concentrate: 50 % ACLM
3	*Alchonea cordifolia* leaf meal (ACLM) using concentrate feed as supplement	50 % concentrate: 50 % CPLM
4	*Carica papaya* leaf meal and *Alchonea cordifolia* leaf meal	50 % concentrate: 25 % ACLM 25 % CPLM

While the coliform counts ranged from 1706.67 – 2140.00 cfu/g. Again, there was significance difference (P<0.05) among the various treatments. In the fecal samples, the THB ranged from 169666 – 250666 cfu/g, being not significantly different (P>0.05) apart from T3. While the coliform counts ranged from 11700.00 - 24333.33 cfu/g. Basically, there was significant difference (P<0.05), but no difference (P>0.05) exist between T1 and T2 and T3 and T4. The ratio of coliform /THB ranged from 4.18 – 10.35, 10.22 – 15.80 and 5.22 – 13.24 for ileum, caecum and feaces respectively. Generally, microbial population was THB increased from the ileum, through the caecum to the faeces i.e. from the proximal to the distal parts of the GIT. This trend has been reported in poultry broilers by Ohimain and Ofongo (2013). The microbial counts between the ileum and caecum were in the trend presented by Combes et al. (2012). However, the microbial counts were far lesser than estimated values of 10^{10} to 10^{12} bacteria /g and 10^{6} to 10^{8} bacteria / g found in

Table 2: Proximate composition of the concentrate feed and forage used during the experiment

Nutrients	Treatment T_1	Treatment T_2	Treatment T_3
(%)	**Concentrate feed (growers mash)**	**Pawpaw leaf meal**	*Alchonea cordifolia* **leaf meal**
Carbohydrate	25.11	-	-
Crude protein	15.10	8.85	12.66
Crude fiber	5.75	1.85	22.60
Phosphorus	0.45	-	-
Fat (lipid)	3.34	-	-
Ash	-	6.38	8.14
Dry matter	-	17.26	17.75
Ether extract	-	6.24	8.30
Lysine	0.41	-	-
Methionine	0.91	-	-
Dry matter	-	22.10	17.75

Table 3: Microbial counts (cfu/g) of ileum, caecum and faecal samples from the rabbits

Tmt	Ileum			Caecum			Feaces		
	THB	Coliform	Coliform/ THB Ratio	THB	Coliform	Coliform/ THB Ratio	THB	Coliform	Coliform/ THB Ratio
T1	2720±308a	111.67±5.04a	4.18±0.34a	18700±1044b	1983.33±77.96ab	10.63±0.19a	236000±31817b	24333.33±1820.56b	10.49±0.68b
T2	2610±403a	189.00±2.65b	7.64±1.27b	13600±642a	2140.00±11.55b	15.80±0.65c	169666±2027a	22466.67±517.47b	13.24±0.19c
T3	2400±327a	115.33±1.45a	4.99±0.70a	16900±2250ab	1706.67±152.46a	10.22±0.57a	250666±4910b	16533.33±2955.41a	6.65±1.30a
T4	1793±12a	185.67±0.88b	10.35±0.02c	14000±173a	1913.33±24.04ab	13.68±0.34b	224333±3929b	11700.00±230.94a	5.22±0.03a

the caecum-colon and ileum of rabbits as reported by Combes et al. (2012). The general low population of microbial counts in the control remains unknown. However, the low population in the treatment with plant supplement (T1 - T3) could be due to the role of this plant extracts in controlling some microorganisms. Kigigha and Atuzie (2012), Combes et al. (2012), Adeyemi et al. (2008), Adeshina et al. (2012), Ebi (2001), Osuagwu and Ihenwosu (2014) have reported that *Alchornea cordifolia* have antimicrobials activities. Similarly, Aruljothi et al. (2014), Nirosha and Mangalanayaki (2013), Alabi et al. (2012), Anibijuwon and Udeze (2009), Peter et al. (2014), Orhue and Momoh (2013) have reported that *Carica Papaya* have antimicrobial potentials. Antibiotics administered to the rabbits could also affect the microbial population in the rabbit's gut

The domestic rabbit (*Oryctolagus cunniculus*) is naturally an herbivorous animal, which means it feeds more on green vegetation, grains, hay, root and tubers as well as other plant resources. Although rabbit is a non-ruminant, it has the ability to digest plant ingredients, roughages, fibers, and other feed materials. This is made possible by the high population of bacterial present in the gut especially in the caecum. The habitual practice of caprophagy in rabbit which is the eating or consumption of their soft faecal pellets

directly from the anus is the feeding strategy adopted to achieve this high level of digestion of plant materials and efficient utilization of nutrients from the plant sources. The synergetic effect of the large population of microorganisms in the gut and the strategy of caprophagy has earned the rabbits its uniqueness in nutrient utilization from plant source among the simple stomach animals (monogastrics). Rabbits recycle their soft faeces to supplement protein quantity and quality, B-vitamins, improve digestibility and makes for positive nitrogen balance. It is known that bacteria in the caecum utilise urea in low protein diets (Aduku and Olukosi, 1990; Fielding, 1998).

Conclusion

This study investigated the effects of *Carica papaya* and *Alchornea cordifolia* leaf meal on the microbial population of weaner rabbits. The plant leaf meals were found to have effects on the microbial counts in the ileums samples. But, the least microbial counts were found in the treatment with supplement for caecum and fecal matters. The reasons for the fluctuation in the microbial counts in the guts may be due to the effects of the botanicals and antibiotics administered to the rabbits.

Acknowledgement

The authors wish to thank Sylvester C. Izah for the editorial works.

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