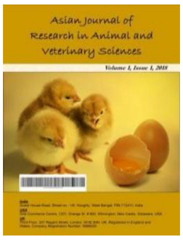

Asian Journal of Research in Animal and Veterinary Sciences

1(1): 48-55, 2018; Article no.AJRAVS.39893

Effect of Sweet Orange (*Citrus sinensis*) Fruit Waste (SOFW) with Acidomix®AFG on the Coliform Count in the Small Intestine of Weaner Rabbits

L. A. Ademu[1*], R. J. Wafar[1] and L. O. Ademu[2]

[1]Department of Animal Production and Health, Federal University, Wukari, Taraba State, Nigeria.
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author LAA designed the study, performed the statistical analysis and wrote the protocol. Authors LOA and RJW wrote the first draft of the manuscript and managed the analyses of the study. Author RJW managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRAVS/2018/39893
Editor(s):
(1) Sameh M. Farouk, Associate Professor, Department of Cytology and Histology,Faculty of Veterinary Medicine, Suez Canal University, Egypt.
(2) Fabio da Costa Henry, Associate Professor, Technology of Animal Products, State University of Northern of Rio de Janeiro, UENF, Brasil.
Reviewers:
(1) Richard Uwiera, University of Alberta, Canada.
(2) F. O. Adebayo, Nasarawa State University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/23706

Original Research Article

Received 8th January 2018
Accepted 13th March 2018
Published 19th March 2018

ABSTRACT

An experiment was conducted to determine the effect of sweet orange (*Citrus sinensis*) fruit waste (SOFW) with Acidomix®AFG on the coliform count in the small intestine of weaner rabbits. It was conducted using thirty-five weaner rabbits. There were four dietary treatments, each having five replicates consisting of graded levels of SOFW at 0, 10, 15, and 20% respectively treated with Acidomix®AFG at two levels (0.5% and 0.7%) for each dietary treatment containing SOFW. The main effect of the experimental diets was significant *(P<0.05)* with a decrease in coliform bacteria in the intestine of the rabbits observed with increasing levels of SOFW for total coliform and lactose fermenting bacteria. Effect of Acidomix®AFG levels was significant *(P<0.05)* with levels of 0.7% having lower *(P<0.05)* counts for total coliform, lactose fermenting bacteria and non-lactose fermenting bacteria. The interaction effect between orange and acidomix levels was also significant *(P<0.05)* with rabbits fed 20% SOFW having the lowest coliform counts for total coliform and

**Corresponding author: E-mail: lawrenceademu@gmail.com;*

lactose fermenting bacteria at both levels of acidomix®AFG. It was concluded that the inclusion of sweet orange (*Citrus sinensis*) fruit waste (SOFW) with Acidomix®AFG has an effect on the coliform count in the small intestine of weaner rabbits.

Keywords: Sweet orange; coliform; small intestine; bacteria; acicifiers.

1. INTRODUCTION

The continuous rise in the demand for cereal grains and oilseeds by both man and animal has led to a steady rise in the cost of these feed sources with a consequent rise in the cost of animal feeds. In order to increase farm incomes from livestock in developing countries, an adequate low-cost feeding system must be developed. Large quantities of agricultural by-products which are regarded as non-conventional feed sources are produced in Nigeria [1]. The use of these agricultural by-products for livestock has been reported to reduce the dependence of livestock on grains that can be consumed by humans and eliminates the need for costly waste management programmes which have become very important in recent years. Waste is an inevitable consequence of the food industry. Appropriate handling of waste has become an essential part of modern processing management. As concerns over the environment have increased, processes which generate lucrative products, but have attendant unmanageable waste problems, can become unaffordable because of prohibitive disposal costs [2].

Sweet orange (*Citrus sinensis*) production is significant and it is available all year round with higher production from the month of October through to March. Oranges account for 61% of the world citrus production (82 million tonnes) [3]. Nigeria produces 3% of fresh citrus in the world, and Africa produces 3,741, 000 tonnes of different varieties of citrus fruits of which Nigeria contributes 3,240,000 tonnes [4]. Heavy crop losses usually occur while the citrus fruit is being transported along its marketing chain. The loss in transit is great for citrus fruit because the fruits are fragile and juicy as well [5].

A major limitation to using fruits and vegetables in the formulation of animal diets is that their composition may be extremely variable depending on the area of production and the period of the year when they are produced [6]. Another drawback is the high moisture content that may make the handling difficult and favour microbial contamination [7]. Heat treatment of these types of wastes is necessary not only to ensure microbial quality [8] but also to reduce the moisture content in order to facilitate their inclusion in animal diets. Animal feed, due to its composition, provides a favourable environment for the microbial growth. Microorganisms found in the feedstuffs can be saprophytic, pathogenic, conditionally pathogenic and toxic. Their growth and proliferation in the feed depend on numerous factors, such as moisture, temperature, type of feed, aerobic and anaerobic conditions, chemical and physical properties of raw material feed pH value, the presence of feed supplements, storage periods and conditions as well as feed decomposition products [9, 10]. The feed may be contaminated during processing, storage or transport. Contaminated feed frequently causes zoonozes [9].

Organic acids, marketed as acidifiers have a long history in the food and feed industry. Acidomix®AFG which is a blend of organic acids (formic acid, propionic acid, ammonium formate and ammonium propionate) improves feed hygiene due to strong antimicrobial effects. The inclusion of Acidomix®AFG in feed for rearing animals results in the stabilization of the gastrointestinal microflora. Organic acids and salts exert their growth inhibiting effects on stomach and gut microbes through pH reduction and anion and proton effects in the microbial cell. Studies have demonstrated a reduction in the bacterial count in the duodenum [11,12]. Low pH also provides a barrier against microbes ascending from the ileum and large intestine. The use of Acidomix®AFG is a preventive measure to control salmonella [13]. The aim of the study was to determine the effect of sweet orange (*Citrus sinensis*) fruit waste (SOFW) with Acidomix®AFG on the coliform count in the small intestine of weaner rabbits.

2. MATERIALS AND METHODS

2.1 Location of the Study

The experiment was conducted at the Teaching and Research farm, Animal Science Department, Ahmadu Bello University, Samaru Zaria in the

Northern Guinea savannah zone of Nigeria. The farm is located at latitude 11° 9´ 45´´ N and longitude 7° 38´ 8´´ E, at an altitude of 610 m above sea level [14]. The wet season occurs between early May and early October. The dry season occurs between the middle of October and early May. Zaria is located in a region with a mean annual rainfall of about 1000 mm (mean of 1976 - 2005). August has the highest mean value (267 mm) while October has the least (19 mm). Daily maximum temperatures may rise to between 32 and 34°C. Relative humidity is fairly high (60 – 65 percent but could rise as high as 85% during the rainy season [15].

2.2 Source and Processing of the Sweet Orange Fruit Waste (SOFW)

The sweet orange fruit waste used in the study consisted of discarded sweet oranges gathered from sweet orange traders at the Railway station market in Kaduna, Kaduna State. The oranges consisted of mixed varieties of sweet orange. The oranges collected were washed with water, split open, then sun-dried until they became brittle. The oranges were however used unpeeled. After sun drying, they were then stored in polythene bags to prevent moisture absorption until they were milled using a hammer mill and incorporated into the experimental diets.

2.3 Microbiological Enumeration

This was carried out at the Department of Microbiology, Ahmadu Bello University Zaria, to enumerate the bacteria and fungi from the sweet orange fruit waste using the Spread Plate technique as described by [16]. Twenty-five grams of ground sweet orange fruit waste was weighed and dissolved in 225 ml of 0.1% peptone water. The stock solution was obtained by taking out 10 ml in a test tube. Ten-fold dilution of the sweet orange fruit waste was prepared by withdrawing 1 ml from the stock solution into 9 ml of the sterile peptone water to obtain 10^1 dilutions with the aid of a sterile pipette from this dilution.1ml was also taken into another 9 ml of sterile peptone water; this procedure was repeated until 10^5 dilutions were obtained. 0.1 ml of 10^3, 10^4, and 10^5 dilutions were aseptically transferred into sterile plates of plate count agar for bacteria and sabouraud dextrose agar for fungi in duplicate plates. With the aid of sterile bead glass rod, the innoculum was spread on the culture media. The inoculated plates were then incubated at 37°C for 24 hours (bacteria growth) while the plates of sabouraud dextrose were incubated at room temperature for 5 days (fungi growth). The number of bacterial and fungal colonies was counted by using a colony counter and the result was recorded as colony forming units per gram (cfu/gm). Total colony forming unit per gram was expressed as total number of colonies counted multiplied by the dilution factor and then divided by the volume of the inoculums.

2.4 Experimental Design, Diets and Management

The diets consisted of sweet orange fruit waste replacing maize at graded levels of 0, 10, 15 and 20%. The diets containing sweet orange fruit waste were treated with Acidomix®AFG at 0.5% and 0.7% levels, respectively (Table 1). The acidifier used in the study was Acidomix®AFG and the diets were formulated to meet the nutritional requirements for weaner rabbits. A total of 35 weaner rabbits were used for the study. The experimental design was a completely randomized design in a 3 × 2 factorial arrangement (SOFW × acidifier) with a control with 5 rabbits per treatment. The animals were kept individually in cages equipped with feeders and drinkers. The cages had wire screen bottoms; this allowed faeces and urine to pass into a collection grid. The rabbits were allowed a two-week adjustment period before the feeding trial commenced. The initial weights of the animals were taken before the experimental diets were introduced to the animals. The feed was supplied daily and water administered *ad lib*. The experiment lasted for a period of 56 days.

2.5 Coliform Count

To evaluate the effect of dietary treatment on coliform bacteria population in the small intestine, 3 rabbits were randomly selected from each of the seven dietary treatments. The rabbits were fasted overnight prior to slaughter. They were bled skinned and eviscerated. This was carried out at the Animal Product Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria. Immediately after slaughter, swabs were taken from the duodenum for total coliform count, lactose fermenting and non-lactose fermenting bacteria using the Spread Plate technique according to [16]. Mac-Conkey agar was used for the culture of the swabs. The analysis was carried out at the Clinical Pathology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Table 1. Composition and calculated analysis of Acidomix®AFG treated sweet orange fruit waste based diets fed to weaner rabbits

	SOFW levels (%)						
	0	**10**		**15**		**20**	
Acidomix (%)	0	0.5	0.7	0.5	0.7	0.5	0.7
Ingredients							
Maize	45.95	35.26	35.26	29.90	29.90	24.55	24.55
Soyabean meal	25.20	25.39	25.19	25.75	25.55	26.10	25.90
Sweet orange fruit waste	-	10.00	10.00	15.00	15.00	20.00	20.00
Palm kernel cake	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Rice offal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Acidomix®AFG	-	0.50	0.70	0.50	0.70	0.50	0.70
Vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
ME kcal/kg	2604	2651	2651	2675	2675	2699	2699
Crude protein, %	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Ether extract, %	4.52	5.86	5.86	6.53	6.53	7.21	7.21
Crude fibre, %	10.15	10.46	10.46	10.62	10.62	10.78	10.78

**0.25 kg of premix will supply the following: Vitamin A 1500 IU, Vitamin D 300 IU, Vitamin E 3.00, Vitamin K 0.25 g, Thiamine 0.2 mg, Riboflavin 0.6 mg, Pantothenic acid 1.00 mg, Pyridoxine 0.4999 mg, Niacin 4.00 mg, Vitamin B12 0.002 mg, Folic acid 0.10 mg, Biotin 0.008 mg, Choline chloride 0.05 g, Antioxidant 0.012 g, Manganese 0.0096 g, Zinc 0.0060 g, Copper 0.0006g, Iodine 0.006 g, Iodine 0.00014 g, Selenium 0.024, Cobalt 0.004 mg*

2.6 Data Analysis

All data collected were subjected to analysis of variance (ANOVA) using the general linear model of [17] according to the following model:

$$Y_{ij} = \mu + O_i + A_j + OA_{ij} + e_{ij}$$

Where Y_{ij} is the individual observation, µ is the overall mean of the population, O_i is the effect of the ith SOFW level, A_j is the effect of the jth acidifier level, OA_{ij} is the interaction effect of the ith and jth levels of factors O and A and e_{ijk} is the random error.

3. RESULTS AND DISCUSSION

3.1 Microbial Count of Sweet Orange Fruit Waste

The result of the microbial count of sweet orange fruit waste is presented in Table 2. The total count of bacteria and coliform count at 10^4 dilution factors was 6.8 x 10^6 and 2.8 x 10^5 cfu/gram respectively. Of the coliform counted, all were identified as lactose fermenting. The total fungal count was 1.6x10^6 cfu/gram. None of the bacteria counted was identified as non-lactose fermenting bacteria. The total bacterial and fungal counts of sweet orange fruit waste were found to exceed levels allowed for dried fruit materials [18]. Low numbers of coliforms are usually permitted in raw vegetables and raw fruits at numbers ranging from 1 to 200/g or ml [19]. No colony count was obtained for non-lactose fermenting bacteria. However, it is not uncommon to find no non-lactose fermenting bacteria such as salmonella in materials of plant origin as their primary habitat is the intestinal tract of animals. Fungal count obtained from the sweet orange fruit waste sample exceeded the limit set for yeast and mould counts (<100 000 cfu/gm) [19]. Fruits containing high levels of nutrients with low pH values make them particularly desirable to fungal decay [20], which could cause infections or allergies [21].

3.2 Effect of SOFW on the Coliform Count at the Small Intestine of Weaner Rabbits

The result showing the effect sweet orange fruit waste diets on the coliform count at the small intestine of weaner rabbits is presented in Table 3. Total coliform and lactose fermenting bacteria counts were observed to decrease significantly *($P<0.05$)* across the dietary treatments with increasing levels of SOFW. Rabbits receiving 20% SOFW had the least *(P<0.05)* total coliform and lactose fermenting bacteria counts while the control had the highest *(P<0.05)* count. Non-lactose fermenting bacteria counts was also significant *(P<0.05)* with the control group having significantly higher counts compared to the SOFW containing groups which were similar *(P>0.05)*. The effect of levels of Acidomix®AFG on the coliform count at the small intestine of weaner rabbits is presented in Table 4. Significant differences (<0.05) were observed between the two levels of Acidomix®AFG with the 0.7% inclusion level having a lower count than the 0.5% inclusion level of Acidomix®AFG for total count, lactose fermenting bacteria and non-lactose fermenting bacteria. No colony count was obtained for non-lactose fermenting bacteria at 0.7% inclusion level of Acidomix®AFG. The interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the coliform count at the small intestine of weaner rabbits is presented in Table 5. There was significant *(P<0.05)* difference across dietary treatments with higher coliform counts obtained for the control group and a decrease in the coliform count with increasing levels of sweet orange fruit waste. No significant *(P>0.05)* difference was observed between the two levels of Acidomix®AFG for each dietary treatment groups. No colony count was obtained for non-lactose fermenting bacteria for the sweet orange fruit waste-containing diets which were similar *(P>0.05)*.

Although coliforms are normally found in the gastrointestinal tract of animals, it is pertinent to mention that most of the lactose fermenting bacteria and non-lactose fermenting bacteria found in this study are *E. coli* and *Salmonella spp.* suspects respectively. *Salmonella* growth requires warmth (35-37ºC is optimal), a moisture content greater than 12% and a pH between 4.5 and 9.0 [22]. It is no coincidence that the rabbit's gut can provide *salmonella* everything they need to thrive. [23] reported a low count of 100 to 10000 cfu/gram for coliform bacteria in the intestinal tract of healthy rabbits. The sweet orange fruit waste diets showed the effectiveness of the acidifier in sanitizing the feed with a gradual decline observed across treatments as the replacement level of SOFW increased up to 20% irrespective of level of Acidomix®AFG inclusion in the diets. Organic acid blends based on formic acid and propionic acids are most effective in *salmonella* inhibition [22]. In an *in vitro* carried out by [22], a dose of 0.3% of Acidomix® AFG was reported to be used for feed

Table 2. Microbial count of sweet orange fruit waste

Total aerobic count	
Bacteria, cfu/gram	6.8×10^6
Coliform bacteria count	
Lactose fermenting bacteria, cfu/gram	2.8×10^5
Non-lactose fermenting bacteria, cfu/gram	-

cfu – colony forming unit; (x10^4) - dilution factor

Table 3. Sweet orange fruit waste effect on the coliform count in the small intestine of weaner rabbits

Coliform indices	**SOFW inclusion levels, %**				**SEM**
	0	**10**	**15**	**20**	
Total coliform	9.00^d	3.17^c	1.83^b	0.17^a	0.18
Lactose-fermenting bacteria	4.33^d	3.17^c	1.83^b	0.17^a	0.17
Non-lactose fermenting bacteria	4.67^b	0.00^a	0.00^a	0.00^a	0.13

^{a,b,c,d} Means within rows with different superscripts are significantly different (P<0.05)
SEM- Standard error of means; x 10^4- dilution factor

Table 4. Acidomix®AFG effect on the coliform count in the small intestine of weaner rabbits

Coliform indices	**Levels of Acidomix®AFG, %**		**SEM**
	0.5	**0.7**	
Total coliform	3.58^a	1.67^b	0.18
Lactose fermenting bacteria	2.42^a	1.67^b	0.17
Non-lactose fermenting bacteria	1.17^a	0.00^b	0.13

^{a,b} Means within rows with different superscripts are significantly different (P<0.05)
SEM- Standard error of means; x10^4- dilution factor

Table 5. Interaction effect of sweet orange fruit waste based diets treated with Acidomix®AFG on the coliform count at the small intestine of weaner rabbits

	SOFW Inclusion levels,%							SEM
	0	10		15		20		
Levels of Acidomix®AFG,%	0	0.5	0.7	0.5	0.7	0.5	0.7	
Coliform Indices								
Total coliform	9.00^d	3.33^c	3.00^c	1.67^b	2.00^b	0.30^a	0.00^a	0.75
Lactose fermenting bacteria	4.33^d	3.33^c	3.00^c	1.67^b	2.00^b	0.30^a	0.00^a	0.25
Non-lactose fermenting bacteria	4.67^b	0.00^a	0.00^a	0.00^a	0.00^a	0.00^a	0.00^a	0.13

^{a,b,c,d} Means within rows with different superscripts are significantly different (P<0.05)
SEM- Standard error of means
$x10^4$- dilution factor

sanitation against *Salmonella spp.* The results obtained also showed a clear reduction in lactose fermenting organisms, mainly *E. coli.* The use of organic acid can reduce *E. coli* colonization in the gut [24,25,26] as well as the incidence of diarrhoea [27]. Other studies have reported a reduction in the bacterial count in the duodenum [11,12]. Reduced bacterial counts have been associated with intestinal tract thinning and a declining mucosal surface area [28], both of which are correlated with improved performance and basal metabolic rate [29].

4. CONCLUSION

To minimize the costs of rabbit production in hot climate countries, low-priced discarded sweet orange fruit wastes can partially replace maize up to 20% when treated with Acidomix®AFG. The study also demonstrated the potential of using acidifiers in achieving higher inclusion levels of sweet orange fruit waste up to 20%. *Salmonella* contamination is recognized as one of the most important food safety issues worldwide. Thus the use of acidifiers in the diet of weaner rabbits has shown its effectiveness in maintaining feed hygiene and to checking *salmonella* colonization of the gastrointestinal tract.

ETHICAL CONSIDERATION

The study was conducted with permission from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC). Approval number ABUCAUC/2012/VET.MED/ APP/017.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chedly K, Lee S. Silage from by-products for smallholders. FAO Electronic Conference on Tropical Silage; 1999.
2. Rodríguez A, Fuentes R, Díaz H. Anaerobic digestion and direct acidification of poultry processing sludge for animal diets. Journal of Agricultural Universities P.R. 2003;87(1-2):81-86.
3. United States Department of Agriculture/Foreign Agricultural Service (USDA/FAS) Citrus: World Markets and Trade. July 2010 Citrus Update. Foreign Agriculture Service - USDA; 2010.
4. Adeyemi SA, Ogazi PS. Export Potentials for fruits Grown in Nigeria National Horticultural Research Institute (NIHORT) Bulletin. Ibadan. 1998;1-56.
5. FAO FAOSTAT data. Food and Agricultural Organization of the United Nations, 00100, Rome, Italy; 2004.
6. Westendorf ML. Food waste as animal feed: An introduction. In: Westendorf, M.L. (Ed.), Food Waste to Animal Feed. Iowa State University Press, Ames. 2000;3–16: 69–90.
7. García AI, García J, Corrent E, Chamorro S, García-Rebollar P, De Blas C, Carabaño R. Effect in age-rabbit, the protein source and use of enzymes on apparent digestibilities of dry matter and crude protein on a rabbit food.

Proceedings of the 11th Research Days Cunicole, Paris, France. 2005;197-200.

8. Sancho P, Pinacho A, Ramos P, Tejedor, C. Microbiological characterization of food residues for animal feeding. Waste Management. 2004;24:919–926.

9. Radanov-Pelagić V, Jurić V, Kunc V, Ristić M, Koljajić V. Relationship of the microflora and mycotoxins in animal feeds. Agriculture, Novi Sad. 1999;48(1-2):281-284.

10. Dordević N, Dinić B, Hrana Za Životimje, Cenzone Tech-Europe, Aranđelovac; 2007.

11. Hebeler D, Kulla S, Winkenwerder F, Kamphues J, Zentek J, Amtsberg G. Influence of a formic acid-potassium formate-complex on chyme composition as well as on the intestinal microflora of weaned piglets. Proceedings of the Society of Nutrition Physiology. 2000; 9:63.

12. Hellweg P, Tats D, Männer K, Vahjen W, and Zentek J. Impact of potassium diformate on gut flora of weaned piglets. Proceedings of the Society of Nutritional Physiology. 2006;15:63.

13. Novus Schwanheit 10. 34281. Gudensberg, Deutschland; 2007.
Available: www.novusint.com

14. Ovimaps Map version 01.28.107. Nokia® Corporation; 2012.

15. Sawa BA, Adebayo AA. Climate Change and the Yield of Grain Crops at Samaru, Zaria. Nigerian Journal of Geography and the Environment. 2011; 2(1):255–265.

16. Seeley HW, Vandemark PJ, Lee JJ. Microbes in Action: A Laboratory Manual of Microbiology. W. H. Freeman & Co., New York. ISBN: 0716721007.

17. SAS (2008). SAS Institute Incorporated. Cary, USA; 1990.

18. Food Administration Manual Microbiological reference criteria for food, microbiological Criteria, Version 2.0. Ministry of Health, New Zealand; 1995.

19. FCD Regulations Governing Microbiological Standards for Foodstuffs and related Matters (R.692 of 16 May 1997). Department of Health Directorate: Food Control; South Africa; 1997.

20. Singh D, Sharma RR. Postharvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), Sustainable Pest Management. Daya Publishing House, New Delhi, India; 2007.

21. Monso EM. Occupational asthma in greenhouse workers. Curr. Opin. Pulm. Med. 2004;10:147-150.

22. Joardar D, Lichtenstein DL, Garner C. Salmonella control in feed: can organic acids application be an important part of the solution? 17th Annual ASAIM SEA Feed Technology and Nutrition Workshop; Imperial Hotel, Hue, Vietnam; 2009.

23. Cortez S, Brandenburger H, Greuel E, Sundrum A. Investigations of the relationships between feed and health status on the intestinal flora of rabbits. (in german) Tierärztl. Umschau. 1992;47:544-549.

24. Mathew AG, Sutton AL, Scheidt AB, Forsyth DM, Patterson JA, Kelly DT. Effects of a propionic acid containin additive on performance and intestinal microbial fermentation of the weanling pigs. ln: Proc. 6 /nt. Symposium on the Digestive Physiology in pigs, PUDOC, Wageningen, The Netherlands. 1991;464-469.

25. Eckel B, Kirchgessner M, Roth FX. Effects of formic acid on daily gain, feed uptake, feed conversion and digestibility. 1st communication: Studies on nutritive effects of organic acids in piglet rearing. Journal of Animal Physiology and Animal Nutrition. 1992;67:93-100.

26. Isobe Y, Shibata F, Komaki H, Kamada A. Influence of fumaric acid administration on intestinal microrganism in weanling pigs. Animal Science technology. 199465:59-66.

27. Eidelsburger U, Roth FX, Kirchgessner M. Effects of formic acid, Ca-formate and Na-hydrogen-carbonate on daily gain, feed uptake, feed conversion and digestibility. 7th communication: Studies on nutritive effects of organic acids in piglet rearing. Journal of Animal Physiology and Animal Nutrition. 1992;67:258-267.

28. Gordon HA, Bruckner-Kardoss E. Effect of normal microbial flora on intestinal surface area. American Journal of Physiology. 1961;201:175–178.

29. Belay T, Teeter RG. Virginiamycin and caloric density effects on live performance, blood serum metabolite concentration, and carcass composition of broilers reared in thermo neutral and cycling ambient temperatures. Poultry Science. 1996;75: 1383–1392.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23706