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Effect of dietary chloroacetic acid as antibiotic replacer on the gastro-intestinal micro flora and gut morphology of weanling pigs

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Abstract

Adding organic acid to piglets' diets is known to be helpful in overcoming post weaning syndrome and chloroacetic acid is known to be the main energy source for the epithelial cells of the large intestine. This study investigated the effect of chloroacetic acid on the vivo swine micro flora and gut wall morphology. Thirty-six weanling pigs were allotted to four treatments i.e., T_1, T_2, $T_{3,}$ T_4. Each treatment was replicated three times with three pigs per replicate. Treatments 2, 3, and 4 received diets containing 0.3%, 0.6% and 0.9% of the basal diet, respectively while T_1 served as control. On the 42nd day, one animal per replicate were slaughtered and samples from the gut and mucosa were collected and analyzed. The pH of caecum and rectum were increased while the pH of stomach, duodenum and jejunum were decreased. The gut intestinal micro flora was highest in T_1 followed by T_2, T_3 and in T_4 and varied significantly (P<0.05). Morphological evaluation of the gut mucosa did not show any significant difference among treatment. This study showed that feeding chloroacetic acid as antibiotic replacer at 0.3% influenced the composition and activity of the gut micro flora, reduced the pH and may improved the gut wall morphology after weaning.

Keywords: Antibiotic replacer; dietary chloroacetic acid; guts morphology; intestinal micro flora; weanling pigs

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Introduction

Newly weaned pigs are very susceptible to developing digestive disturbances and gastro-intestinal diseases, resulting in stresses on the pig and consequently inability to meet their growth potential (Hedemann and Jensen, 2004). These problems were counteracted with widespread use of antibiotics substances that may as a side-effect select antibiotic-resistant genes in the intestinal flora with the possibility of a transfer to human pathogens (Philips et al., 2004).

The potential risk and the consumer demand for a food chain that is free of drugs has resulted in the decision of the European Union to completely ban antibiotics used as growth promoters as of January 2006. Therefore, pharmaceutical feed supplements must be sought to control microbial activity in the gastro intestinal tract of non-ruminant animals. Among the alternatives are organic acids (Roth and Kirchgebner, 1998), probiotics (Gibson, 1998), botanicals (Great head, 2003) and prebiotics (Gibson, 1998). Most of these alternatives have effects on micro flora, directly or indirectly (Richards et al., 2005).

Acidification with various weak organic acids to diets have been reported to decrease colonization of pathogens and production of toxic metabolites, improve digestibility of protein and of Ca, P, Mg and Zn and serve as substrates in the intermediary metabolism (Roth and Kirchgebner, 1998). Chloroacetic acid which is prepared industrially from chlorination of acetic acid effectively reduced the levels of *Escherichia coli* in the duodenum and jejunum in weanling pigs (Cole et al., 1968). Piglets fed diets supplemented with acetic acid showed changes in gastro intestinal (GIT)

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characteristics (Partanen and Mroz, 1999). The present study was conducted to determine the effect of using a single commercially made organic acid (chloroacetic acid) as a suitable replacement of dietary antibiotic growth promoters on gut micro flora and morphology of weanling pigs.

Materials and Methods

Thirty-six cross bred weanling pigs (large white x Duroc) weaned at 28 days were transported to College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Nigeria, where they were housed in individual pens for a 42 days trail period. During four days adaptation period all piglets received the same base diet. They were divided into four groups, (9 animals per group replicated trice). Group one received the basal diet, group two received 0.3%, group 3 received 0.6% while group 4 received 0.9% of chloroacetic acid of the basal diet. All diets were formulated to provide the same amount of energy, protein, amino acids, calcium and phosphorus. Feed and water were provided *ad lubitum*. The gross compositions of the experimental diets are reported in Table 1. Routine management was carried out throughout the trial.

Table 1: The gross composition of the experimental diet

Ingredient (%)	T_1	T_2	T_3	T_4
Maize	25	25	25	25
Maize offal	10	10	10	10
Brewer dry grain	15	15	15	15
Groundnut cake	8	8	8	8
Palm kernel cake	6	6	6	6
Wheat offal	27.3	27.3	27.3	27.3
Soya meal	5	5	5	5
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Bone meal	3	3	3	3
Sale	0.25	0.25	0.25	0.25
Vit./premise	0.25	0.25	0.25	0.25
Total	100	100	100	100
Crude protein	19.421	19.421	19.421	19.421
M.E. k/cal	2486.11	2486.11	2486.11	2486.11

On the 42 days, 4 animals per treatment were killed followed by complete bleeding. Within 20 minutes after death, the contents and the mucosa from the stomach, duodenum, jejunum, caecum and rectum were sampled for pH determination, intestinal mucosa morphology and viable bacteria counts.

pH Determination

1g of samples from different segment of the intestine were collected aseptically in 9ml sterilized physiological saline (1:10 dilution) (Al-Natour and Alshawobkeh, 2005) and pH was determine by reading the pH meter.

Morphological Evaluation

Mucosa samples were collected from the different segment of the intestine, fixed in 10% buffered formalin and embedded in paraffin. Histological sections were obtained from tissue block, cut perpendicular to the mucosal surface and stained with haematoxylin and eosin. The heights of villi and depth of crypts on mucosal samples of the intestine were assessed as described by Biagi et al. (2006).

Bacterial Counts

Immediately after collection of faeces and chyme samples, 1g of sample was diluted with 9ml of sterile saline (0.9g sodium chloride in 100ml distilled water). Serial dilutions of collected samples from different part of gastro-intestinal (GIT) contents were made up to the fifth dilution with sterile saline and different bacterial loads of the gut contents were enumerated by the pour plate method (Quinn et al., 1992).

Statistical analysis

The experiment was carried out in a completely randomized design (CRD). The data collected were subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980) while significance in means was carried out sing Duncan Multiple Range Test as described by Duncan (1955).

Results and Discussion

Table 2 showed the pH of chyme from GIT of pigs fed different levels of chloroacetic acid. The pH of stomach, duodenum, jejunum, caecum and rectum were not significantly different among all the treatments ($P<0.05$). These findings are consistent with the observations of Matthew et al. (1991) and Hernandez et al. (2006) who reported no effect on intestinal pH with the use of luprosol-NC (a product containing 53.5% propionic acid), acetic acid and formic acid. The caecum and rectum recorded the highest pH values because appreciable amount of bacterial fermentations takes place there (Sukie, 1970) thereby increasing their alkalinity. Furthermore, the lowest pH value were recorded in the stomach and in the duodenum because undissociated organic acid can cross membrane and be absorbed in the small intestine and hardly reach the large intestine (Piva et al., 1997). The presence of HCl in the stomach and chloroacetic acids will cause reduction of the pH to low levels (Dibner and Butin, 2002).

The effects of dietary treatments on GIT micro flora of pigs are shown in Table 3. The highest microbial count was recorded in Treatment 1, because there was no inclusion of chloroacetic acid that will break down the DNA structure of the bacteria cell

Table 2: pH of chyme from GIT of pigs fed different levels of chloroacetic acid

Treatments	Stomach	Duodenum	Jejunum	Caecum	Rectum	SEM
T 1 (0.0g)	5.37[c]	5.28[c]	6.37[b]	7.22[a]	7.17[a]	0.25
T2 (0.3g)	4.50[c]	4.48[c]	5.47[b]	6.20[a]	6.10[a]	0.20
T3 (0.6g)	4.33[c]	4.50[c]	5.47[b]	6.40[a]	6.17[a]	0.23
T4 (0.9g)	4,53[c]	4.50[c]	5.63[b]	6.37[a]	6.33[a]	0.22

a,b,c,d in the same row with different superscript differs significantly

Table 3: Effect of dietary treatments on GIT bacteria in pigs

Diets	Stomach	Duodenum	Jejunum	Caecum	Rectum	SEM
T 1 control	6-8.4 x 10^{4b}	5-7x10^{4c}	3-4.2x10^{4d}	4-5.6x10^{4d}	8-9.1x10^{4a}	0.50
T2 (0.3%)	3-4.2x10^{4bc}	4-5.6x10^{4b}	2-2.8x10^{4b}	4-5.6x10^{4d}	6-8.4x10^{4a}	0.41
T3 (0.6%)	3-4.2x10^{4b}	3-4.2x10^{4b}	2-2.8x10^{4b}	3-4.2x10^{4b}	5-7x10^{4a}	0.34
T4 (0.9%)	2-2.8x10^{4c}	4-5.6x10^{4ab}	2-2.8x10^{4b}	3-4.8x10^{4b}	5-7x10^{4a}	0.38

Each in colony forming units/ml = cfu/ml; a,b,c,d, in the same row with different superscript differ significantly

Table 4: Effect of dietary chloroacetic acid on the villous height of small intestine

Diets	Duodenum (µm)	Jejunum (µm)	Ileum (µm)
T_1 (0.0g)	278	235	245
T_2 (0.3g)	347	267	260
T_3 (0.6g)	365	309	301
T_4 (0.9g)	387	337	319

Table 5: Effect of dietary chloroacetic acid on the depth of crypts of small intestine

Diets	Duodenum (µm)	Jejunum (µm)	Ileum (µm)
T_1 (0.0g)	215	276	246
T_2 (0.3g)	248	305	287
T_3 (0.6g)	267	319	298
T_4 (0.9g)	296	341	320

nucleus and cause the bacteria cell not to divide and die (Dibner and Butin, 2002). The inclusions of chloroacetic acid at 0.3% (T_2) had increasingly controlled pathogen in the gastro intestine tracts in the pigs. Tsiloyanism et al. (2001) reported that several organic acids reduced the severity of diarrhea in weaned piglets. Treatments 3 and 4 recorded the lowest bacteria counts because the chloroacetic acid inclusion (0.6% and 0.9%) respectively was high. This was as a result of reduction of pH value, the bacteria will try to get rid of the proton (H^+ion) released, which will eventually exhaust the bacterial metabolism and subsequently leads to the death of the bacteria.

A similar observation was also recorded by Byrd et al. (2001) who reported that the addition of acetic acid, formic acid, lactic acid and proponic acid to the diet and water effectively reduced *Escherichia coli*, coli forms and *salmonella* in monogastric animals.

Morphological evaluations of intestine mucosa samples from duodenum, jejunum and ileum did not show any significant differences among treatments. From the result it was observed that the villous height of T_4 was highest (P<0.05). But no significant difference was found in villous height between T_2 and T_3. In gut morphology, it is well known that weaning has a dramatic negative impact on the intestinal mucosa morphology of piglets (Gu et al., 2002). Feeding chloroacetic acid may reduce some of the negative effects of weaning by providing the ilea and the hindgut mucosa with the preferred energy source. In a trial with weaned piglets, van Beers-Schreurs et al. (1998) observed that weaned piglets had shorter intestinal villi than unweaned animals.

Average depth of crypts in duodenum, jejunum and ileum was 270 µm, 321 µm and 302 µm respectively. Wang et al. (2005) reported that feeding weaning piglets with 1,000 ppm of sodium butyrate (SB) increased the height of the intestinal villi. In another study, when weaned piglets were fed tributyrin and lactitol as precursors of chloroacetic acid (Piva et al., 2002) animal growth was improved, whereas ceacal crypt depth was reduced. In the present study, one reason for the absence of major effects of chloroacetic acid on intestinal morphology could be that the chloroacetic acid concentrations along the gut were not increased appreciably by the tested dose, suggesting a rapid metabolization of chloroacetic acid prior to reacting with the intestine. Furthermore, intestinal mucosa samples have been collected at the end of the study, 6 weeks after weaning; therefore the piglets must have a fully developed digestive system (Gabert and Sayer, 1994).

This study showed that chloroacetic acid modulates the gut micro flora, reduced the pH value with no significant effect on the gut morphology. This implied that chloroacetic acid at 0.3% in 25kg feed is recommended for weanling pig diet. It suggest that chloroacetic acid is worthy of further investigation as a potential alternative to antibiotic in improving growth performance and gut health status of piglets in the post weaning period.

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