



Intestinal Microbial Contents of Grower Pigs (*Sus scrofa domesticus* *L.*) Given Wet and Fermented Commercial Ration with Varying Levels of Wood Vinegar

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Abstract: *This study aimed to evaluate the intestinal microbial content from crossbreed-grower pigs given wet and fermented commercial hog ration with varying levels of wood vinegar in the treatments as follows: T0 (Control) 100% Plain Water (PW), T1 (PW+ 2%WV) and T2 (PW+ 5%WV). A completely Randomized Design (CRD) design was adopted. A total of twenty-seven (27) samples composed of three (3) samples per animal from three gastro-intestinal tract sites (5-10 cm sections each of the ileum, caecum and top of spiral colon) which were taken right after evisceration placed inside zip lock plastic bags and inside ice bucket transported to the Microbiology Laboratory, College of Veterinary Medicine (CVM), Visayas State University (VSU), Visca, Baybay, City, Leyte. Data gathered were analyzed using analysis of variance (ANOVA) under STAR (Statistical Tool for Agricultural Research version 2.0.1) version. Results revealed a no significant difference ($p>0.05$) on Total Plate Count (TPC) of microbial contents in the Gastro Intestinal Tract (colon, caecum and ileum) of crossbreed-grower pigs given wet and fermented commercial ration with varying levels of WV. However, different types of bacteria were found in T0 (Streptococcus, Lactobacillus, Bacillus sp., Coccus), 2% and 5% (Streptococcus, Lactobacillus sp.) of crossbreed-grower pigs fed with commercial hog ration fermented with varying levels of WV. 2% and 5% wood vinegar inclusions helped in proliferation of beneficial bacteria such as Streptococcus and Lactobacillus and reduction of undesirable microorganisms in the intestine in this study.*

Keywords: *Gastro-intestinal tract, microbial content, wet and fermented commercial ration, wood vinegar & grower-pigs.*

1. INTRODUCTION

Nature and importance of the Study

Antibiotic resistance and environmental pollution are serious concerns in the poultry and livestock industry worldwide. The use of antibiotics in animal production is a practice to be restrained in the forthcoming years, and the misuse of antibiotics resulted to the spread of multi-antibiotic-resistant bacteria, some of which able to hold out to the last-resort antibiotics (Nachman, 2016). Thus, the urgent need for the research community to persist in finding alternative to the conventional antibiotic.

The wood vinegar (WV) known as pyroligenous acid (PA) was successfully used to substitute a conventional antibiotic (apramycin) in weanling pig feeding (Choi *et al.*, 2009). They reported that a concomitant reduction in the population of harmful coliforms but higher populations of *Lactobacillus* in the intestines of pigs fed with PA. Popularly known as natural organic acids (Sasaki *et al.*, 1999), it can maintain a low pH of gastric contents and subsequently modify or decrease the intestinal microflora (Thomlison and Lawrence, 1981; Kirchegessner and Roth, 1982; Burnell *et al.*, 1988). Literatures cited several benefits of WV like: inhibit growth of bad bacteria, reduce alkaline carcinogen absorption, and enhances calcium and magnesium absorption, increase blood circulation by promoting acidity in the large intestine (Rakmai, 2009). Currently, organic acids, probiotics, prebiotics, and phytochemical substances have shown potential as antibiotic substitute and feed additives as revealed by Kamel (2001). However, the effect of WV in the fermenting solution for hog ration on the intestinal microbial content on grower-pigs should be investigated. Hence, this study.

Objectives of the Study

Generally, this study evaluated the intestinal microbial contents of grower-pigs given wet and fermented commercial ration with varying levels of wood vinegar.

Specifically, this study:

1. Determined the Total Plate Count (TPC) of intestinal microbial contents in specifically in the colon, caecum and ileum of grower-pigs given commercial hog ration fermented with varying levels of wood vinegar; and
2. Identified the kinds of microorganisms present specifically colon, caecum and ileum of the Gastro Intestinal Tract (GIT) tract of grower-pigs given commercial hog ration fermented with varying levels of wood vinegar

Time and Place of the Study

The experimental pigs were raised from July 20 to September 17, 2018 at the Piggery Project of the Department of Animal Science-College of Agriculture and Food Science, Visayas State University-Main Campus, Visca, Baybay, City, Leyte.

Scope and Limitation of the Study

This study was limited to the Total Microbial Plate Count (TPC) and Differential Identification of microbes present specifically in the colon, caecum and ileum of the Gastro Intestinal Tract (GIT) tract of grower-pigs given wet and fermented commercial hog ration with varying levels (0%, 2% and 5%) of wood vinegar.

2. REVIEW OF LITERATURE

History and Domestication of Pigs

Pig's ancestors can be traced to the genus and species, *Sus scrofa*, which is commonly known today as pigs, hogs and swine that can be used interchangeably. As many as 6 genera and 31 species, archaeological evidences showed that swine first domesticated in the East Indies and Southern Asia (Ensminger and Parker, 1997). In around 4, 900 B.C, the Chinese people are the first people to tame pigs. Meanwhile, pigs in the United States where majority of the commercial breeds in the Philippines came from were brought by Columbus during his journey from Cuba. The breeds of pigs are Landrace, characterized with a large body and dropping ears and Large white which is almost similar to Landrace but with erect ears commonly intended for lard and meat production, respectively (Gillespie, 1998).

Status of the Swine Industry in the Philippines

According to the Philippine Statistics Authority (PSA, 2017) in their inventory as of July 1, 2017, the total population of swine is 12.52 million heads. This number was 0.16 percent higher compared to the previous year's inventory of 12.50 million heads. It may be due to the increase in the stocks of the backyard farms that increased by 0.62 percent. However, the number of stocks produced in the commercial farms reduces by 0.67 percent as compared to 2016. Overall, about 64.0 percent of the total stocks were raised in backyard farms and the rest were in commercial farms (PSA, 2017).

During the months of January to March 2017, the livestock subsector agriculture in the Philippines displayed a 3.22 percent growth in output during this period. It accounted for 16.85 percent of total agricultural output in which hog is the major contributor to this sector in which production increases 3.50 percent than the previous records. The subsector's gross value of output amounted to P65.4 billion at current prices, representing an increase of 9.37 percent compared to the same period last year (Performance of Philippine Agriculture, 2017).

Fermentation of Animal Feeds

Fermentation is an enzymatically controlled anaerobic breakdown of an energy-rich compound (as carbohydrate to carbon dioxide and alcohol or to an organic acid as defined by Merriam-Webster Dictionary (2015). Broadly, it can be defined as an enzymatically controlled transformation of an organic compound just like drying and salting. By definition fermented liquid feed is a feed that has been mixed with water, at a ratio ranging from 1:1.5 to 1:4, for a period long enough to reach steady state conditions (Missotten *et al.*, 2015). Fermentation was already practiced long time ago. It was developed through the years by women in order to preserve food in times of scarcity as well as to add flavor to the food and reduce its toxicity (Rolle and Satin, 2002). Historically, fermentation has already existed since the Neolithic age dating from 7000-6600 BCE in Jiaho, China (McGovern *et al.*, 2004). In the present, fermentation is still widely practiced in most households in some countries but only few invested in an industrialized level (Holzapfel, 2002).

Fermentation often results in the production of nutritionally enriched, very stable food products from low-value carbohydrate and protein substrates according to Food and Agriculture Organization/World Health Organization (FAO/WHO, 2002). In addition, according to Battcock (1992), fermentation also provides farmers a variety of products by selling different products with different flavors. It also adds value to the farmer's products and its by-products to improve the flavor, aroma, texture and appearance of food as well as making food more palatable. While Holzapfel (2002) revealed that the importance of fermentation in the modern-day life was the preservation and safety as well as the enhancement of the sensory attributes of the foods being marketed in both developed and developing countries. He also added that fermented products are highly appreciated as major components of diets in some developing countries under normal conditions that contribute to food safety, nutritional quality and digestibility.

Description and Composition of Wood Vinegar

Wood vinegar also known as pyroligneous acid a liquid collected from burning wood. Also called wood acid, it is a dark liquid produced by the destructive distillation of wood and other plant materials. The wood vinegar contains more than 200 constituents, with principal components such as acetic acid, methanol, phenol, ester, acetals, ketone, formic acid and many others. It was once used as a commercial source for acetic acid. In addition, the vinegar often contains 80-90% water along with some 200 organic compounds. Other materials may also be used in making wood vinegar such as coconut shell, bamboo, grass and other plants. The term is synonymous and used to define the aqueous fraction obtained from carbonization or slow pyrolysis of wood and other lingo-cellulosic raw materials. Typically, carbonization of wood produces charcoal, non-condensable gases (NCGs), tar and PA. Yields can vary widely depending on the type of wood and the process conditions, such as final temperature and heating rate (Yoshimoto 1994; Santos *et al.*, 2013). PA is recovered from carbonization process by trapping the pyrolysis gases through a proper condensing unit. After some time, which might vary from days to even a few months, wood tar, which is a heavier fraction, decants at the bottom of the container and separates from PA. Several research efforts have been made to better

know the chemical composition of PA, and more than 200 major compounds have been identified in variable concentrations.

Wood vinegar is also known as natural organic acids (Sasaki *et al.*, 1999) that can maintain a low pH of gastric contents and subsequently modify or decrease the intestinal microflora (Thomlison and Lawrence, 1981; Kirchegessner and Roth, 1982; Burnell *et al.*, 1988). Kim (1996) reported that wood vinegar also shows strong acid activity at pH 3 and contains 280 different components, the major ones being acetic and propionic and antioxidant substances like phenolic compounds (Loo *et al.*, 2008). Chemical composition and concentration of compounds intrinsically depend on which original material is charred (Pimenta *et al.*, 2000; Nakai *et al.*, 2007; Rakmai 2009; Souza *et al.*, 2012). According to these authors, major compounds present in pyroligneous extract include formic, acetic, propionic and valeric acids; methanol, butanol and amylic alcohol; phenol and cresols besides guaiacol and syringol derivatives; neutral compounds such as formaldehyde, acetone, furfural and valerolactone; among several others, as maltol, cyclotene, etc.

Furthermore, another research about PA is focused on its usage as a supplement in ruminant and monogastric animals feeding, favouring ruminal and intestinal flora additionally improving digestibility and nutrient absorption (Li and Ryu 2001; Kook and Kim 2002; Kook *et al.*, 2003). PA was successfully used to substitute a conventional antibiotic (apramycin) in weanling pig feeding, as reported by Choi *et al.* (2009). According to these authors, higher populations of *Lactobacillus* were noted in the intestines of pigs fed with PA and a concomitant reduction in the population of harmful coliforms was verified as well. Such research line is rather important because the addition of antibiotics on animal feed is considered a practice to be restrained in the forthcoming years. Nachman (2016) pointed out that the misuse of antibiotics in feeding of pigs is accredited to be responsible for a crescent spread of multi-antibiotic-resistant bacteria; some of them were able to hold out even with some of the last-resort antibiotics.

Uses of Wood Vinegar

Wood vinegar was reported to have an inhibiting effect on the growth of bad bacteria, reduction of alkaline carcinogen absorption, enhancement of calcium and magnesium absorption and increase blood circulation by promoting acidity in the large intestine (Rakmai, 2009). It has a variety benefits such as adjust bacterial levels in the digestive tract and improved meat quality when mixed with animal feed (Burnette, 2010). It was reported to have an inhibiting effect on the growth of bad bacteria, reduction of alkaline carcinogen absorption, enhancement of calcium and magnesium absorption and increase blood circulation by promoting acidity in the large intestine (Rakmai, 2009). The good point is, it is considered as probiotic added to the feeds and drinking water of livestock and poultry to enhance proper digestion for easy nutrient absorption and to remove pigpen odor (Sarian, 2017). There are a lot of microbial products supplemented to feeds that may exert beneficial effects to the animals (Lettat *et al.*, 2012 and Chiquette 2009).

It also promotes digestion, nutrition adsorption and reduction of diarrhea. It reduces the number of *Cryptosporidium parvum*oocyst (Watarai and Tana, 2008). Currently, organic acids are considered as one of the attractive feed additives for weanling pigs (Jensen, 1998; Partanen and Mroz, 1999). In animal production, wood vinegar can be used as feed additive that served as an alternative for antibiotics (Wang *et al.*, 2012): induction of increased production rate and efficiency of chicken (Yamauchi *et al.*, 2010). It also improved growth performance of ducks (Ruttanavut *et al.*, 2009) and pigs (Wang *et al.*, 2012) and inhibits the action of harmful coiliforms in pigs (Choi *et al.*, 2009). Watarai and Tana (2005) observed that wood vinegar has two major effects against intestinal bacteria. First, it inhibits the growth of pathogenic bacteria like *S. enteriditis*. Second, it stimulates the growth of normal bacterial flora which serves as probiotics such as *E. faecium* and *B. thermophilum* in the intestine. Watarai *et al.* (2008) found out that the use of bamboo vinegar combined with bamboo charcoal was an efficient treatment for cryptosporidiosis in calves. However, there are no reports about bamboo vinegar used as feed additive in animal production. The evaluation of toxicological safety by Sprague Dawley (SD) rats and mice has proven that the bamboo vinegar was safe to animals as oral medicine (Chen *et al.*, 2007). Therefore, bamboo vinegar can be regarded as a secure feed additive as an alternative to antibiotics in food animal production. Pigs fed antibiotic showed higher (Pb0.001)

ADG and better feed efficiency followed by pigs fed 0.2% wood vinegar and 0.2% organic acid diets while those fed the control diet had lowest ADG and poorest feed efficiency. Kim (1996), reported that wood vinegar also shows strong acid activity at pH 3 and contains 280 different components, the major ones being acetic and propionic and antioxidant substances like phenolic compounds (Loo *et al.*, 2008).

According to Luo *et al.*, (2004) reported that the used of bamboo vinegar had antimicrobial activity on *E. coli* even at dilution of 1:100. Previous works (Lin and Shiah, 2006; Lin *et al.*, 2006; Shiah *et al.*, 2006) had established a clear positive relationship between bamboo vinegar and fungi resistance. The variation of bacterial communities observed in the high concentration treatment of bamboo vinegar may be attributed to the inhibitory effect of the bamboo vinegar on the fecal bacteria. The acetic acid concentration in vinegar was 2% in the vinegar, this computes to a concentration of 0.004–0.016% acetic acid in the diets containing bamboo vinegar in the trial. Although, the acetic acid in the diets might influence pH of feed, the action on pH in the intestine was insignificant due to the buffering capacity of the body (our unpublished data), thus acetic acid from bamboo vinegar in diets may not affect the fecal bacterial communities of piglets. It is inferred that the effect of bamboo vinegar on bacterial communities attributes to the active components such as phenolic compounds in bamboo vinegar. Higher populations of lactobacillus were noted in the ileum of pigs fed the wood vinegar diet, while the population of coliforms in the ileum and cecum was higher (Pb0.001) in pigs fed the control diet when compared with pigs fed antibiotic, 0.2% organic acid or 0.2% wood vinegar diets (Choi *et al.*, 2009). These results indicated that wood vinegar could reduce harmful intestinal coliforms, but increase the probiotics (Choi *et al.*, 2009). It is inferred that the action mode of bamboo vinegar like wood vinegar most probably differs from antibiotic.

In addition, the study conducted by Choi *et al.*, (2009), the overall ADFI was highest (Pb0.001) in pigs fed wood vinegar and lowest in pigs fed the control diet. In this study, adding 0.4% bamboo vinegar in feed was effective in improving the performance of piglets compared with the pigs fed diets containing antibiotics. Due to the higher feed intake of pigs in BV4 and antibiotics, the final weight and daily weight gain for pigs in both treatments were significantly higher than those in control, therefore feed to gain ratio was not significantly different among treatments. The physical condition was good for animals on bamboo vinegar, similar to animals treated with antibiotics during the whole experiment. The results manifested that the bamboo vinegar has the potential to replace antibiotics.

Many countries have been using wood vinegar as pesticides in where commercial pesticides are not available or too expensive especially for small scale farmers. The need to minimize the environmental impact caused by using pesticides that leached to the ground water and waterways has been encouraged across the globe through the use of wood vinegar as biocide and pesticide (Tiilikkala *et al.*, 2010). It also showed potential biological activities as larvicidal, pupicidal and adult deformities against *M. domestica* by reducing the growth, development and metamorphosis as well as decreasing the life span of the adult insect (Pangnakorn *et al.*, 2012).

According to De Guzman (2009), wood vinegar must be blended with water in a ratio of 1:50 (1 liter wood vinegar and 50 liters water), or up to a ratio of 1:800 (1 liter wood vinegar and 800 liters water). He also cited that as feed additive, it has the effect of adjusting the bacteria in the intestines and facilitating absorption of nutrients. In milk cows, wood vinegar helps prevent mastitis. Another study on the effect of wood vinegar on the performance, nutrient digestibility and intestinal microflora in weanling pigs revealed that wood vinegar could improve the performance of weanling pigs by improving the nutrient digestibility and reducing harmful intestinal coliforms (Choi *et al.*, 2009). Generally, the performance of pigs fed with wood vinegar was superior to those fed with organic acid. In cattle, wood vinegar also improved meat quality, fertility rate, milk production, feed efficiency and prevented mastitis. In chicken egg production, farmers also claimed improved egg-laying performance in hens, better rearing characteristics, and better hatchability. Moreover, better taste of eggs, reduced cholesterol content, and thicker eggshells were also noted (De Guzman, 2009). In duck, growth performance tended to be improved with increasing SB from 0 to 1% supplemented in basal commercial diet (Ruttanavut *et al.*, 2009).

Description of Prebiotics

Prebiotic is defined as a ‘non-digestible’ compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host” as revealed by Bindels *et al.* (2015). Another definition of prebiotics is a non-digested food component that, through stimulation of growth and/or activity of a single type or a limited amount of microorganisms residing in the gastrointestinal tract, improve the health condition of a host” according also to Gibson and Roberfroid, (1995). Many different nutrients, such as pectins, cellulose and xylanes, favoured the development of various intestinal microorganisms. Prebiotics should not be extensively metabolized, but should induce targeted metabolic processes, thus bringing health benefits to the host’s ecosystem. The best documented benefits are associated with the use of indigestible oligosaccharides, such as fructans and galactans as revealed by Rastall and Gibson (2015).

Mode of Action of Prebiotics

When prebiotics reaches the large intestine, those substances become nutritional substrates for beneficial intestinal bacteria (Grajek, 2005). In terms of properties that determine a favorable effect on the host’s health, prebiotics may be divided into following groups: not digested (or only partially digested), not absorbed in the small intestine, poorly fermented by bacteria in the oral cavity, well fermented by seemingly beneficial intestinal bacteria and increase of *Bifidobacterium* count and of acetic acid level, with simultaneous reduction of intestinal pH, compared to the control group and the diet with an addition of inulin. That phenomenon is explained by, among others, easy degradability of bonds presents in the structure of fructo-oligosaccharides (FOS) and galactooligosaccharides (GOS) by certain enzymes, such as β -fructanosidase and β -galactosidase, commonly occurring in *Bifidobacterium* genus bacteria. Some types of nutritional fibre may be considered prebiotic. Prebiotics play a significant role in nutrition of both livestock and home pets. Some commonly used prebiotics are: FOS, oligofructose, trans-galacto-oligosaccharides (TOS), gluco-oligosaccharides, glico-oligosaccharides, lactulose, lactitol, malto-oligosaccharides, xylo-oligosaccharides, stachyose and raffinose (Gajeck *et al.* 2005; Monsan and Paul, 1995; Orban *et al.*, 1997; Patterson *et al.*, 1997; Collins and Gibson, 1999). And according to Patterson and Burkholder (2005), when they reach the large intestine, those substances become nutritional substrates for beneficial intestinal bacteria

Meanwhile, Xu *et al.* (2003) checked effects of FOS used in doses: 0, 2, 4 and 8 g/kg feed on the activity of digestive enzymes and on intestinal morphology and microbiota. It was found that the administration of FOS at the dose of 4 g/ kg feed had a positive effect on the mean daily growth of studied animals, and on the growth of *Bifidobacterium* and *Lactobacillus* bacteria, with a simultaneous inhibition of growth of *Escherichia coli* in chickens’ gastrointestinal tract. On the other hand, in the study by Juśkiewicz *et al.* (2006) carried on turkeys for 8 weeks, no effect of FOS used at concentrations of 0.5, 1 and 2% was found on animal growth and productivity. However, reduction of the intestinal pH was noted in case of FOS administration at the concentration of 2%. Supplementation of broiler chickens’ diet with prebiotics results in reduction of gastrointestinal pH and increased *Lactobacillus* and *Bifidobacterium* counts, caused by increased amount of volatile fatty acids (Ziggers, 2000).

Description of Probiotics

Probiotics can be defined as live microbial feed supplement, which beneficially affects the host animals by improving its intestinal balance as described by Fuller (1989). Probiotic comes from a Greek word means “for life” (Gibson and Fuller, 2000). Fermentation or nutrient digestion also influences some systemic functions such as lipid homeostasis (Clydesdale, 1997 and Roberfroid, 1996). Probiotic foods have been consumed as a part of food components for centuries. Functional foods usually targeted the gastrointestinal functions including those that control transit time, bowel habits, and mucosal motility as well as those that modulate epithelial cell proliferation. Bellisle *et al.*, (1998) stated that foods can be functional if they contain components that affect certain functions in the body with positive effects on health or if it has a physiology or physiologic effects beyond the traditional nutritional effects (Clydesdale, 1997).

Mode of Action of Probiotics

Probiotics act as gut-beneficial bacteria that create a physical barrier against unfriendly bacteria, help offset the bacterial imbalance caused by taking antibiotics, and may help breakdown protein and fat in the digestive tract ([www health Harvard edu](http://www.health.harvard.edu), The benefits of Probiotic Bacteria). Depending on the kind of feeds being fed to piglets, the ratio between the population of lactic acid bacteria and coliforms differs in the lower gut (Moran *et al.*, 2006). When fed with fermented liquid feed, the ratio favors to the lactic acid bacteria while when fed dried feeds favors the coliforms. Using fermented liquid feeds; the composition of the microbial population in the gastrointestinal tract can be altered. It is usually due to the increase in the concentration of the lactic acid bacteria in the stomach and small intestine (Canibe and Jensen, 2003). The same authors also reviewed the value of fermented liquid feed in reducing enteric diseases in pigs, and a lot of studies support that fermented liquid feed can reduce the incidence of *Salmonella spp.* (Tielen *et al.*, 1997; Lo Fo Wong *et al.*, 2004; Vander Wolf *et al.*, 1999; and Vander Wolf *et al.*, 2001). Based on the study conducted by Canibe and Jensen (2003) the results showed there are changes in pH in the different parts of the GI tract when the pigs are feed fermented liquid feed or dried feed. Lalles *et al.*, (2007) added that the most significant change is a decrease in the pH of the stomach which serves as a barrier against pathogens and by lowering the pH may strengthen the stomach and prevent scouring due to coliforms. The low pH especially in newly weaned piglets is attributed to their incapacity of producing sufficient amounts of gastric acid (Easter *et al.*, 1993 as cited by Partridge *et al.*, 1993).

The significant change is affected also by the fermentation conditions. For example, in the study conducted by Canibe and Jensen (2003), they found out that there is no significant difference in the population of lactic acid bacteria in the distal small intestine of growing pigs when the GI content was 37 °C. however, with an incubation temperature of 20 °C, the population of the lactic acid bacteria in the same part of the intestine has significantly higher when pigs are fed with fermented liquid feed compare to fed with dried feed or liquid feed. Yeast cells also have significantly increased with the change in the population of microbes in the intestine. There are some studies shows that yeast has the ability to bind enterobacteria to their surface thus preventing them to bind in the gut epithelium (Mul and Perry, 1994). Increasing the number of lactic acid bacteria and yeasts can be a good method of reducing the enteropathogens such as *Salmonella spp.* and *E. coli*.

Uses of Probiotics

Different strains of probiotic bacteria has different effects based on their specific capabilities and enzymatic activities even they belong to single species (Ouwehand *et al.*, 1999; Bernet *et al.*, 2003) and also different microorganisms express preference in their habitat which may defer in various host species (Freter, 1992). Most of the bacterial colonies adhere to the intestinal wall and so does the probiotic. Thus, the colonies are not swept away due to the peristalses along the intestinal wall and prevent the pathogenic bacterial colonization along the intestinal wall and prevent disease development (Fuller, 2000). Among the indigenous flora colonizing the chicken's crop, stomach of mice and rats, and the lower ileum in man are the Lactobacilli. Bacteria colonizing such high-transit-rate sites must adhere firmly to the mucosal epithelium (Savage, 1972). Proliferation of useful bacteria facilitates fermentation in all kinds of animals including humans and this fermentation has nutritional significance in most of the animals (Ahmad, 2004). Probiotics have effect on the main physiological functions of the gastrointestinal tract, which are digestion, absorption and propulsion (Fioramonti *et al.*, 2003). Ahmad (2004) reported that there is an increase of crypt cells proliferation on small intestines with the use of probiotics as compared to control. This mechanism of useful bacteria is very important especially in the ruminants and to some non-ruminants because it gives substantial amount of energy to the host.

Probiotics has been commonly studied in poultry and livestock production. In poultry, chicks administered with probiotics shows reduction in colonization and invasions of *Salmonella* (Higgins *et al.*, 2008; Vila *et al.*, 2009), decreased mortality (Timmerman *et al.*, 2006), and increased body weight (Mountzouris *et al.*, 2007). In the study conducted by Vicente *et al.*, (2007) on the effects of probiotics in the growth performance of broilers, there is a significant difference in the reduction of mortality rate

among treatments. However, there is no significant difference in the feed conversion ratio and body weight.

In pigs, probiotics supplementation on swine ration has been demonstrated to decrease the pathogen load (Taras *et al.*, 2006; Collado *et al.*, 2007). Ameliorate gastrointestinal diseases symptoms (Zhang *et al.*, 2010b) and improved weight gain (Konstantinov *et al.*, 2008). There are some studies reveal that the microorganisms use in the probiotic products are the same in animal and humans. The most common species used is the lactobacillus species followed by the bifido bacteria. Since most of this species are lactic acid producing bacteria, they inhibits the growth of coliforms in the G.I tract in piglets through the reduction of pH of the said part since acidic environments are detrimental to some pathogens according by Fuller (1989) and as cited by Chiquette (2009).

Several studies show beneficial effects of probiotics in ruminant's health as well to its milk production. That is why there are a lot of microbial products supplemented to feeds that may exert beneficial effects to the animals. In a recent study conducted by Lettat *et al.*, (2012) and Chiquette (2009), their results showed that probiotic bacteria (propionic bacteria and lactobacilli) were ineffective in ameliorating lactic acidosis but some of the probiotics maybe effective in reducing occurrence of butyric and propionic SARA in sheep (Lettat *et al.*, 2012). In adult ruminants, it is about 100 liters, and it harbors bacteria (1011 cells/ml), protozoa (105 cells/ml), fungi (103 cells/ml), and methanogens (109 cells/ml) in volume (Chiquette, 2009). These products are not attributed with specific nutritional roles and are given the term 'probiotics' lactobacillus acidophilus, for example, appears to endowed with the ability to reduce scouring and increase live weight gain in calves, but the effects are not consistent between trials. Yeast cultures may also be used as Probiotic (Wallace and Newbold, 1992).

Pork Quality

Meat quality is a highly subjective topic which the industry and consumers agree on a number of important quality indicators like tenderness, juiciness, appearance (color and structure), fat and protein content, drip and cooking loss, fat quality, and off-odors (Borggaard and Andersen, 2004). As a major source of food, pork provides a significant portion of the protein intake in the diets of a large proportion of the people, particularly in developing countries. Pork is cheap and highly acceptable, which gives it an advantage over poultry or beef (Eyo, 2001). Pork has essential sulphur-containing amino acids such as cysteine, methionine and lysine which are limiting in some legumes and most cereal-based diets (Borgstrom, 1962). However, pork is highly perishable, being a high-protein food with typically high levels of free amino acids of which microbes metabolize, producing ammonia, biogenic amines (putrescine, histamine, and cadaverine), organic acids, ketones, and sulphur compounds (Delgaard *et al.*, 2006).

Pork is the culinary name for meat from the domestic pig (*Sus domesticus*). The word pork often denotes specifically the fresh meat of the pig, but can be used as an all-inclusive term that includes cured, smoked, or processed meats (ham, bacon, prosciutto, etc.). Pigs are found throughout the world especially in areas where no religious edicts prevent their rearing. They are raised for various reasons ranging from social to economics, but the ultimate purpose of rearing pigs is to provide human food in the form of fresh or processed pork to satisfy the protein needs of human beings (Raloff, 2003).

The properties of fresh meat according Aberle and Forrest (2001) indicate its usefulness to the merchandiser, its appeal to the purchaser or consumer and its adaptability for further processing which particular importance are water-holding capacity, colour, structure, firmness and texture. Water-holding capacity (WHC) is the ability of meat to retain naturally occurring or added water during application of external forces such as cutting, heating, grinding or pressing (Aberle and Forrest, 2001). Many of the physical properties of meat, including colour, texture and firmness of raw meat and the juiciness and tenderness of cooked meat are partially dependent on water holding capacity (Aberle and John, 2001). In addition, the freshness of meat is generally indicated by its smell. The smell of fresh meat should be slightly acidic, increasing in relation to the duration of the ripening period because of the formation of acids such as lactic acid (Hui and Wai-Kit, 2001). On the other hand, meat in decomposition generates an increasingly unpleasant odor owing to substances originating from the bacterial degradation of the meat proteins, such as sulphur compounds, mercaptane, etc. (Hui and Wai-Kit, 2001).

The appearance of meat, either as a carcass or as boneless meat cuts, has an important impact on its objective or subjective evaluation. Although in modern grading procedures, more and more technical equipment has been incorporated, visual methods are still in use. They can be of special value in most developing countries where no extremely sophisticated methods are needed. The way the consumers or the processors check the appearance of meat is subjective. Differences will be registered in the relation of lean meat and fat including the degree of marbling or in the relation of bones and lean meat. Furthermore, unfavorable influences can be detected such as unclean meat surfaces, surfaces too wet or too dry, or unattractive blood splashes on muscle tissue. It was noted that evaluating the appearance, special product treatment (chilling, freezing, cooking, curing, smoking, drying) and quality of portioning and packaging (casings, plastic bags, and cans) would be recognized (FAO, 1999).

Under normal circumstances, the color of meat is in the range of red and may differ from dark red, bright red to slightly red; but also pink, grey and brown colors may occur. In many cases, the color indicates the type and stage of the treatment to which the meat has been subjected, as well as the stage of freshness. In judging meat color, some experience is needed to be able to distinguish between the colors which is typical for a specific treatment or which is typical for specific freshness. Furthermore, meat derived from different species of animals may have rather different colors, as can easily be seen when comparing beef, pork and poultry meat (FAO, 1999). Remarkable changes in the meat color occur when fresh meat has been boiled or cooked. It loses its red color almost entirely and turns to grey or brown (FAO, 1999). The reason for this is the destruction of the myoglobin through heat treatment. This is the typical color shown in sausages of all types, raw and cooked hams, corned beef, others (FAO, 1999). Cured products with a decreasing keeping quality can be recognized when the red color becomes pale or changes to grey or green. Meat color is mainly determined by the incident light reflectance that is dependent upon the concentration and chemical state of myoglobin pigments and the physical structure of meat. The first 30-60 minutes immediately after muscle tissue is exposed to air are critical to myoglobin oxygenation and “bloom” of muscle colour from the typical colour of reduced myoglobin (purple) to that typical oxymyoglobin (red) (Brewer *et al.*, 2001). Myoglobin is the principal protein responsible for meat color, although other proteins such as hemoglobin and cytochrome C may also play a role in beef, lamb, pork and poultry color (Harold and Hedrick, 1994). Metmyoglobin (brown color) formation is associated with discoloration or color fading due to oxidation of both ferrous myoglobin derivatives to ferric iron (Harold and Hedrick, 1994). Metmyoglobin formation depends on numerous factors including oxygen, partial pressure, temperature, pH, meat reducing activity and in some cases, microbial growth (Harold and Hedrick, 1994).

Structure, firmness and texture are meat properties that are generally evaluated by consumers with visual, tactile and gustatory senses. Many factors within muscles such as rigor state and associated water-holding properties, intramuscular fat content, connective tissue content and bundle size contribute to these physical properties (Aberle and Forrest, 2001). The texture of meat can be defined as the composite of the structural elements of meat (Varnam and Sutherland, 1995). As the meats are consumed in the cooked state, the texture of cooked meat is interpreted as tenderization (Hui and Wai-Kit, 2001). The tenderization of meat occurs in two steps; a rapid phase that is mainly due to the structural weakening of myofibrils and a slow phase caused by the structural weakening of the intramuscular connective tissue (endomysium and perimysium) (Pearson and Gillet, 1999). Meat prepared for the consumer should be tender and juicy. Meat tenderness depends on the animal species from which the meat originates. Lamb, pork and poultry meat are sufficiently tender after slaughter, but beef requires a certain period of maturation to achieve optimal eating quality (FAO, 1999). Texture and consistency, including juiciness, are an important criterion, still neglected by many consumers, for the eating quality of meat. The meat should be cooked to become sufficiently tender, but cooking should not be too intense otherwise the meat becomes dry, hard and with no juiciness (FAO, 1999). The simple way to check the consistency of foods is by chewing. Although this test seems easy, in practice it is rather complicated. Taste panelists need experience, particularly when the different samples have to be ranked, for example, which sample is the toughest, the second toughest or the tenderers. On the other hand, inappropriate processing methods (too intensive cooking, curing, comminuting) may cause losses in the desired consistency and juiciness, and the best way to check this is by chewing (FAO, 1999).

The flavor of fresh meat can also be checked by putting small samples (approx. 10 pieces of 1 cm³ each) in preheated water of 80°C for about five minutes (boiling test). The odour of the cooking broth and the taste of the warm meat samples will indicate whether the meat was fresh or in deterioration or subject to undesired influences (FAO, 1999). When processing the meat, the smell and taste of the meat products can differ a great deal owing to heat treatment and the use of salt, spices and food additives. Every meat product has its typical smell and taste, and the test person should know about it (FAO, 1999). Panelists should not smoke or eat spicy meals before starting the test and should rinse their mouth frequently with water during the test (FAO, 1999).

Nutritive Value and Quality of Pork

Every serving (0.1 kg) of pork contains 242 kcal, 13.92 g fat (5.23 g saturated fat, 6.19 g monounsaturated fat, and 1.2 g polyunsaturated fat), and 27.32 g protein. It also contains 0.464 mg vitamin B6, 0.70 µg vitamin B12, 93.9 mg choline, 0.6 mg vitamin C, 53 IU vitamin D, and trace minerals such calcium (19 mg), iron (0.87 mg), magnesium (28 mg), phosphorus (246 mg), potassium (423 mg), sodium (62 mg), and zinc (2.39 mg) as found on the USDA Nutrient Database (USDA Handbook, 1989). Pork has up to 72.96% moisture, 21.52% crude protein, 3.42% crude fat, and 1.10% ash (USDA Handbook, 1989). Warris *et al.* (2003) approximated pork to have a chemical composition of 60% water, 4% minerals, 20% proteins, 15% fats (lipids), and 1% carbohydrates. There are several factors affecting the characteristics of meat. As for consumers to decide during purchase, eating and physical qualities are important considerations, and are relative across cultures and individual preferences (Sanudo *et al.*, 1998). According to Hambrecht (2004), the key factors of most quality assurance schemes are food safety and ethical aspects. Warris *et al.* (1996) stated that the major factors affecting meat quality are: yield and gross composition; appearance and technological characteristics; palatability; wholesomeness, and ethical quality.

Meanwhile, the amount of marbling, fat texture, color, chemical composition, and Water Holding Capacity (WHC) of the lean describes the quality of meat in terms of appearance and technological characteristics (Monin, 1998; Warris, 2000). Moreover, palatability can be defined through tenderness, juiciness and flavor or odor whereas wholesomeness considers nutritional quality, chemical safety, and microbiological safety (Gunenc, 2006). Lastly, ethical quality is related to people's belief that the animals where the meat come from should be of good animal husbandry, and should be bred, reared, handled and slaughtered in ways that are sympathetic to animal welfare (Forian, 2006).

Change in meat pH is one of the indicators of meat quality. The ultimate pH attained after the muscle has passed the stage of rigor mortis, is one of the identifiers of meat quality which has a significant relationship with water-holding capacity (WHC) and color after the onset of rigor. Rodriguez *et al.* (2011) stated that pH readings taken at 45 minutes post-slaughter do not guarantee the final behavior of pH, water-holding capacity and color unless equal to or less than pH 5.8. At 24-hour post-mortem, most of the aerobic biochemical processes have finalized, and so pH is determinant for final characteristics of pork.

Likewise, Du (2001) stated that variations in meat pH are a result of post-mortem metabolism or glycolysis and the conversion of glycogen into lactic acid. In addition, changes in physiological parameters affect meat quality. Increase in blood lactate concentration is associated with pre-slaughter stress, and has been shown to have a detrimental effect on pork quality (Hambrecht *et al.*, 2005, Edwards *et al.*, 2010a). Hambrecht *et al.* (2004) determined that pigs exposed to aggressive handling just prior to stunning had a higher blood lactate concentration at slaughter and exhibited pork with higher drip loss and thus proposed that lactate was a potential indicator of both physical and psychological stress associated with the handling of pigs immediately before slaughter. Furthermore, the level of cortisol is an individual characteristics of each animal (Miale, 1972), and it affects the amount of fat in the body, meatiness, and thus, carcass quality (Foury *et al.*, 2007).

Technological Categories of Pork Quality

There are three important parameters to define pork quality: drip loss, ultimate pH and color (Lee *et al.*, 2000), and using these parameters, meat can be classified into the five quality categories;

PSE (pale, soft, exudative), normal or RFN (red, firm, non-exudative), DFD (dark, firm, dry), RSE (reddish-pink, soft, exudative) and PFN (pale, firm, non-exudative) meat (Kauffman *et al.*, 1992). It is well known that changes in some meat quality traits can affect many other meat quality attributes and overall pork quality (Huff-Lonergan, 2010).

The pH declines and cell membranes are disrupted during postmortem (Fortin and Raymond, 1988), and the amount of intra and extracellular fluid changes. At low pH (<5.4) proteins in meat with poor WHC do not bind to free water tightly. On the other hand, water holding capacity or WHC is the ability of the meat to retain moisture or water determined by the amount of drip loss. Loss of meat water is a result of shrinkage of muscle proteins, such as actin and myosin because water holding capacity or WHC of the meat largely depends on the rate of glycogen conversion to lactic acid and its accumulation during the conversion of muscle to meat (Meat Evaluation Handbook, 2001).

Several meat quality characteristics are correlated with pH value after 24 hours. In pigs with lower ultimate pH values, higher drip loss (Huff-Lonergan, 2010; Hambrecht *et al.*, 2004; Edwards *et al.*, 2010a) and lighter colour (Huff-Lonergan, 2010; Hambrecht *et al.*, 2005; Boler *et al.*, 2008; Edwards *et al.*, 2010b) were measured. In carcasses with rapid development of rigor mortis, ultimate pH values were significantly lower (Warriss *et al.*, 2003). Contrary to those findings, in this study, ultimate pH value was positively correlated only with marbling, as was also observed by other authors (Boler *et al.*, 2008; Czarniecka-Skubina *et al.*, 2010). This can be explained by the fact that energy reserves in muscle fibers are distributed among intramuscular fat and glycogen, and muscles with lower glycogen content have a higher intramuscular fat content.

Sensory Evaluation

Sensory evaluation has been defined as “a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the sense of sight, smell, touch, taste and hearing” (Stone and Sidel, 1993). Sensory analysis is a useful tool when evaluating the quality of foods frequently used both in the food industry and research. Sensory analysis can be used in quality control (of e.g. raw material, process and product), product development (copying competing products, product improvement) and shelf life evaluation (Sensorisk Studiegroupe, 1997). Borggaard and Andersen (2004) stated that the important meat traits include tenderness, juiciness, appearance (color and texture), fat and protein content, drip and cooking loss, fat quality, and off-odors. In addition, as regards meat quality, Risvik (1994) added that the main sensory descriptors for the perception of whole meat in relation to preferences are tenderness, juiciness and absence of off-flavor.

3. MATERIALS AND METHODS

Preparation of Experimental Pigs

A total of nine (9) newly weaned (30 days old) Landrace x Large White piglets with (3 females and 6 castrated males) from the same litter were used in the study. The males and females have been randomly and equally distributed to the different treatments to eliminate the effect of sex. All necessary deworming, iron supplementation and immunization was given as scheduled. Upon arrival at the experimental area, multi-vitamin/mineral supplement was given to prevent transport stress. The experimental piglets were given the usual commercial pre-starter feeds given at the farm, and was gradually shifted to the experimental diet after five days of feeding.

Preparation of Experimental Diets

The wood vinegar (WV) was acquired from the production area of the Department of Agriculture-Abuyog Experiment Station, Balinsasayaw, Abuyog, Leyte. The experimental rations were prepared based on the different treatments as follows: T₀ -(Control) 100% Plain Water 0% WV); T₁-(PW+ 2% WV) and T₂-(PW+ 5% WV). Based on the plain water (PW) to wood vinegar ratio, the specific amount of WV was thoroughly mixed with the plain water before adding and mixing into the commercial ration. A ratio of 3L of plain water or PW-WV solution for every kilogram of feeds was used in order to totally submerge the feeds. The feed-liquid mixture has been placed in a properly labeled and tightly covered containers and stored inside a cool dry area at room temperature at about 8-12 hours. The daily experimental ration that was based on the requirement of the animal was prepared

in the morning for afternoon feeding and in the afternoon for the morning feeding of the next day to prevent rancidity.

Preparation of Experimental Area

Existing pigpens in the piggery of the Department of Animal Science-College of Agriculture and Food Science, Visayas State University Main Campus, Visca, Baybay City, Leyte was utilized. The three large concrete pigpens were equally divided into three partitions with 1.0 m height, 1.5 m length, and 1.0 m width. Two weeks before the conduct of the study, the pigpens, facilities and surrounding areas were thoroughly cleaned and disinfected.

Feeding and Water Management

The wet and fermented commercial ration was prepared on daily basis, and was provided based on the daily requirement for crossbred-grower pigs. The feeding schedule was at 7:00 AM and 4:00 PM, thereafter, the feed refuse was recorded to account for the daily feed consumption. Fresh drinking water was available at all times during the whole duration of the study.

Health Management, Sanitation and Biosecurity Measures

The standard procedure for health management as prescribed by the Philippine National Standard has been followed. Antibiotics were not used during the study period. Simultaneous with the bathing of the pigs, the daily and regular cleaning schedule of the pens and facilities were strictly implemented from 6:30 AM and 3:30 PM. For biosecurity reason, unauthorized persons and stray animals were strictly prohibited within the research vicinity.

Experimental Treatments and Design

The wood vinegar (WV) was incorporated into the Plain Water (PW) at different levels based on the treatments as follows:

- T₀–100% PW or 0% WV (3L PW)
- T₁–PW+ 2% WV (3L: 60 ml)
- T₂–PW+ 5% WV (3L: 150 ml)

Using a Completely Randomized Design (CRD) set-up, a total of nine (9) piglets were randomly distributed to three (3) treatments and replicated three (3) times with one (1) animal per replication as shown in the lay-out:

T₀ R₁	T₁ R₂	T₀ R₂
T₂ R₁	T₁ R₃	T₁ R₁
T₂ R₃	T₀ R₃	T₂ R₂

Management of Pigs Prior and During Slaughter

After 60 days of feeding trial, the experimental pigs was weighed and fasted for 24 hours prior to slaughter. The standard ante-mortem techniques have been strictly followed to minimize stress related handling procedures. The animals were weighed and driven to the stunning area in a quiet and orderly manner without undue fuss and noise. Immediately after stunning, the animals were bled via jugular incision to drain out blood for 5 minutes. Thereafter, scalding and evisceration followed.

Preparation of the Intestinal Microbial Samples for Analysis

A total of twenty-seven (27) samples composed of three (3) samples per animal will be taken from three gastro-intestinal tract sites (5-10 cm sections each of the ileum, caecum and top of spiral colon) were taken right after evisceration. Each part was tied and cut-off placed inside zip lock plastic bags to the ice bucket and transported to the Microbiology Laboratory, College of Veterinary Medicine (CVM), Visayas State University (VSU), Visca, Baybay, City, Leyte.

Data Gathered

Data on Total Microbial Plate Count (TPC) and Differential Identification of microbes present specifically in the colon, caecum and ileum of the Gastro Intestinal Tract (GIT) tract of grower-pigs given commercial hog ration fermented with varying levels of wood vinegar.

Data Analysis

All data gathered were analyzed using analysis of variance (ANOVA), and comparison of treatment means were subjected to Least Significant Difference (LSD) Test. Statistical analysis was carried out using the STAR (Statistical Tool for Agricultural Research version 2.0.1).

3. RESULTS AND DISCUSSION

Colon

The Total Plate Count (TPC) of microbial contents in the colon of crossbreed grower-pigs given wet and fermented commercial hog ration with varying levels of wood vinegar are presented in Table 1. Although not significant, a higher colon TPC value at 5% WV (1.18×10^9 CFU/mL) than at 2% WV (8.99×10^8 CFU/mL) and 0% WV (1.18×10^9 CFU/mL). Results displayed that the wet and fermented hog commercial ration with wood vinegar generally favored the proliferation of microflora in the colon of grower-pigs. The intestinal microbial TPC coincided with TPC values on the wet and fermented feed also showing highest (6.3×10^6 CFU/mL) at 5% WV followed by 2.7×10^6 CFU/mL at 2% WV and 1.0×10^6 CFU/mL on 100% PW or 0% WV

Caecum

Although not significant, highest microbial TPC value was observed in the caecum of crossbreed grower-pigs at 2% WV (2.02×10^8 CFU/mL) than at 5% WV (1.74×10^8 CFU/mL) and at 0% WV (1.29×10^8 CFU/mL) as shown in Table 1. Result agreed with higher TPC values on the wet and fermented feed at 5% WV and 2% WV that implies the same microbes were ingested by the grower-pigs. Moreover, it signifies that the wet and fermented hog commercial ration with wood vinegar generally favored the proliferation of microflora in the caecum grower-pigs.

Ileum

In the ileum, the same trend showing highest TPC value of 7.85×10^8 CFU/mL at 2% WV, followed by 5% WV (6.60×10^8 CFU/mL) and 0% (9.84×10^7 CFU/mL) as reflected in Table 1. Despite insignificant difference, results also suggest that the wet and fermented hog commercial ration with wood vinegar generally favored the proliferation of microflora in the ileum of grower-pigs. Similarly, results agreed with higher TPC values on the wet and fermented feed at 5% WV and 2% WV that implies the same microbes were ingested by the grower-pigs. Consequently, wood vinegar probably caused favorable changes in physiological conditions in the gut subsequently modified or decreased the intestinal microflora especially the non-beneficial species (Thomlison and Lawrence, 1981; Kirchegessner and Roth, 1982; Burnell *et al.*, 1988).

Table 1. Total Plate Count (TPC) of intestinal microbial contents (colon, caecum and ileum) of crossbreed grower-pigs given wet and fermented commercial ration with varying levels of wood vinegar

Treatment	Colon (million/ mL)	Caecum (million/mL)	Ileum (million/mL)
T ₀ –Plain Water (PW)	3.06 x 10 ⁸	1.29 x 10 ⁸	9.84 x 10 ⁷
T ₁ –PW+ 2% WV	8.77 x 10 ⁸	2.02 x 10 ⁸	7.85 x 10 ⁸
T ₂ –PW+ 5% WV	1.18 x 10 ⁹	1.74 x 10 ⁸	6.60 x 10 ⁸
<i>p</i> -value	0.4429	0.6208	0.1357

TPC-Total Plate Count

Microbial Intestinal Content Identified

The microbial contents identified in the colon, caecum and ileum of crossbreed grower-pigs given wet and fermented commercial hog ration with varying levels of wood vinegar are presented in Table 2 and Figures 2, 3 and 4). Specifically, the wet and fermented commercial hog ration with WV added revealed the same microbial content present as follows: *Lactobacillus* and *Streptococcus*. But, in the 0% WV other bacterial species (*Bacillus sp.* and *Coccus* were present. The lactic acid bacteria and other beneficial species seemed to reduce enteropathogens like *Bacillus sp* and *Coccus* that favors development of various beneficial intestinal microorganisms (Rastall and Gibson, 2015). Watarai and Tana (2005) reported that wood vinegar inhibits the growth of pathogenic bacteria and stimulates the growth of normal bacterial flora which serves as probiotics such as *E. faecium* and *B. thermophilum* in the intestine.

The higher TPC values on the wet and fermented feed at 5% WV and 2% WV than 0% WV. Anadon (2006) revealed that bacteria belonging to Genus *Lactobacillus* and *Streptococcus sp.* are potentially beneficial to the host because they are lactic acid producing bacteria. The amount of lactic acid bacteria naturally present on the feed or the amount of lactic acid bacteria added to the feed, determine the extent of lactic acid production that favors a healthy gut. The reasons behind this phenomenon are because WV has major effects against intestinal bacteria: it inhibits the growth of pathogenic bacteria (Rakmai, 2009) and stimulates the growth of normal bacterial flora which serves as probiotic in the intestine (Watarai and Tana, 2005). The wood vinegar was also reported to have an inhibiting effect on the growth of bad bacteria, reduction of alkaline carcinogen absorption, enhancement of calcium and magnesium absorption and increase blood circulation by promoting acidity in the large intestine (Rakmai, 2009). These authors stated that higher populations of *Lactobacillus* noted in the intestine of pigs fed with wood vinegar resulted to concomitant reduction in the population of harmful coliforms was verified as in the study. It was also cited revealed that wood vinegar could reduce harmful intestinal coliforms, but increase the probiotic bacteria (Choi *et al.*, 2009).

Moreover, Canibe and Jensen (2003) stated that fermented liquid feed can alter the composition of the microbial population in the gastrointestinal tract, usually due to the increase in the concentration of the lactic acid bacteria in the stomach and small intestine extending the beneficial effect to the whole GI tract (Roberfroid, 1998). The feeding fermented feeds also increase nutrient digestibility, improved intestinal morphology, a reduce content of various anti-nutritional factors in feeds and a reduction in dust levels in the farm.

Table 2. Microbial contents identified in the Gastro Intestinal Tract (colon, caecum and ileum) of crossbreed grower-pigs given wet and fermented commercial ration with varying levels of wood vinegar

Treatment	Colon	Caecum	Ileum
0	<i>Streptococcus, Lactobacillus, Bacillus sp., Coccus</i>	<i>Streptococcus, Lactobacillus, Bacillus sp., Coccus</i>	<i>Streptococcus, Lactobacillus, Bacillus sp., Coccus</i>
1	<i>Streptococcus, Lactobacillus sp.</i>	<i>Streptococcus, Lactobacillus sp.</i>	<i>Streptococcus, Lactobacillus sp.</i>
2	<i>Streptococcus, Lactobacillus sp.</i>	<i>Streptococcus, Lactobacillus sp.</i>	<i>Streptococcus, Lactobacillus sp.</i>

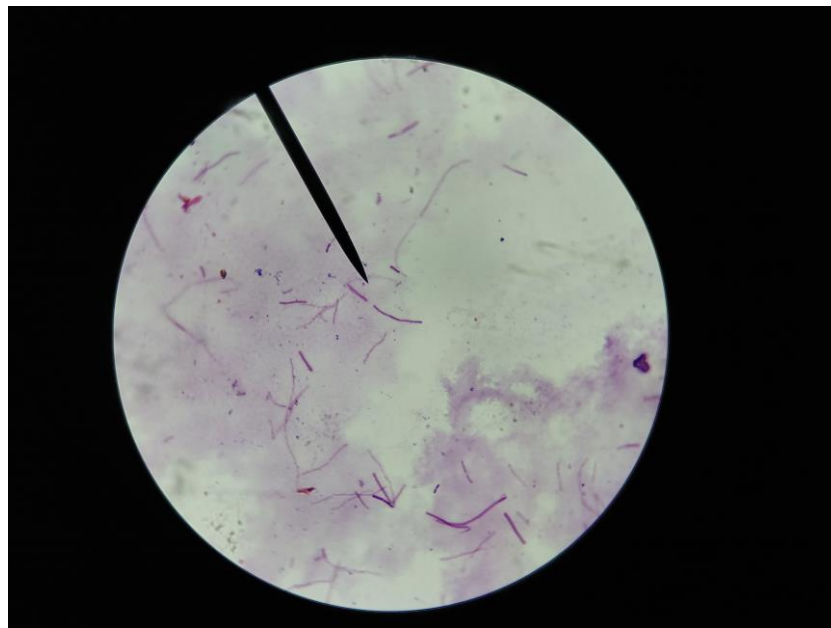


Figure 2. Mixed microbial content of *Streptococcus, Lactobacillus, Bacillus sp.,* and *Coccus* were found (10x OIO) in colon, caecum and ileum of crossbreed grower pigs (Table 2) given wet and fermented commercial ration with 0 % WV

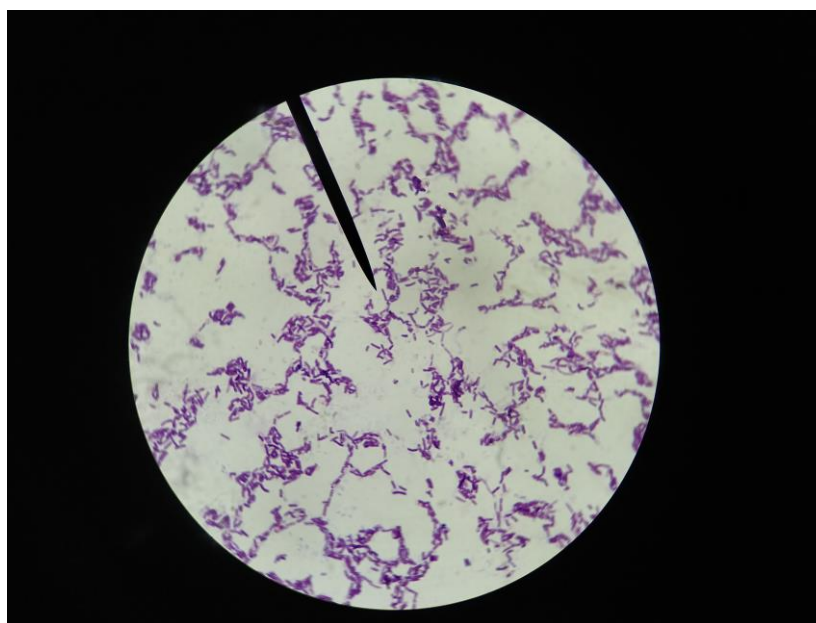


Figure 3. *Streptococcus* and *Lactobacillus sp.* were found in colon, caecum and ileum of crossbreed-grower pigs (Table 2) (10x OIO) given wet and fermented commercial ration with 2% WV

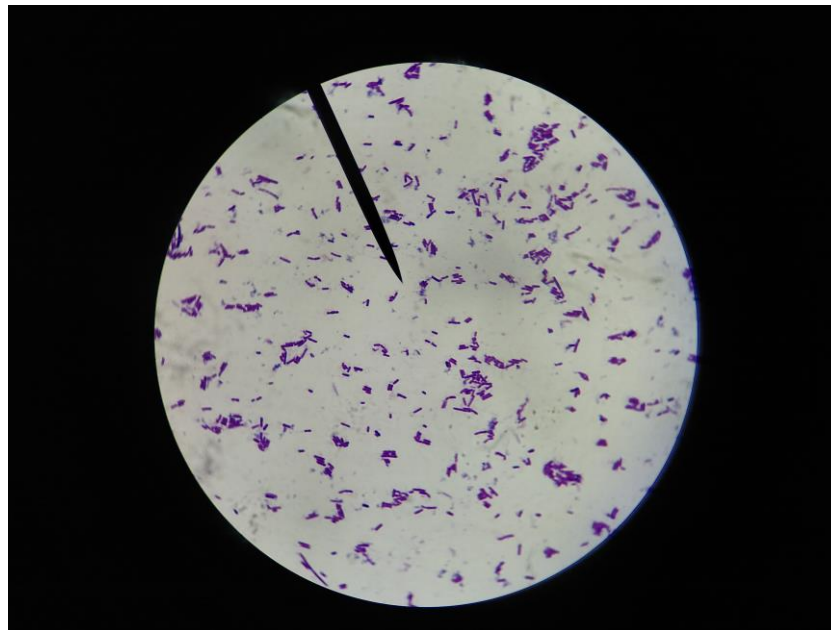


Figure 4. *Streptococcus* and *Lactobacillus sp.* were found in colon, caecum and ileum of crossbreed-grower pigs (Table 2) (10x OIO) given wet and fermented commercial ration with 5% (T₂) levels of wood vinegar

4. CONCLUSION AND RECOMMENDATION

Conclusion

This study concluded that wood vinegar helped in the proliferation of beneficial bacteria such as *Lactobacillus sp.* and *Streptococcus sp.* and reduction of undesirable microorganisms in the intestines of grower-pigs given wet and fermented commercial ration with varying levels of wood vinegar. The wet and fermented diets with WV added disclosed its capacity to maintain a low pH in the GIT, that subsequently modified or decreased the intestinal microflora reducing harmful intestinal coliforms and increase the growth of normal bacterial flora which serves as probiotics.

Data revealed that 2% and 5 % Wood Vinegar inclusions into the Plain Water used to ferment the commercial hog ration resulted to a healthy intestines that displaced non-beneficial bacteria instead favored the beneficial microflora in the intestines of grower-pigs.

Recommendations

Further study be conducted in finisher pigs and sows to evaluate the long term effects of varying levels of wood vinegar. It is also recommended to establish the optimum WV inclusion level, include dry-non-fermented commercial ration as treatment and a differential count of the identified microflora on both the feeds and GIT is highly advisable.

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