Effects of Charcoal and Wood Vinegar Dietary Supplementation to Diarrhea Incidence and Faecal Hydrogen Sulfide Emissions in Pigs

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Abstract- Antibiotic resistance is now a big problem in livestock industry worldwide. Environmental pollution caused by the livestock industry has been becoming increasingly serious. Therefore, finding solutions to solve these problems simultaneously has become more necessary. Charcoal and wood vinegar has been used as fertilizer supplements and water filtration and treatment for humans and animals. This study aims toassess the applicability of charcoal and wood vinegar in prevention of diarrhea and reduction of environmental pollution in pig production. The experiments were conducted on pigs from weaned to finisher. The diets for pig were added different dosage of charcoal and wood vinegar (0.10; 0.20; 0.30% wood vinegar; 0.60; 0.80; 1.00% charcoal and 0.20 wood vinegar + 0.80 charcoal). The concentration of H_2S was measured by KITAGAWA-AP.20 kit. Results showed that charcoal and wood vinegar is effective in diarrhea prevention in pigs, reducing the percentage of pig's diarrhea. Moreover, the concentrate of H_2S in pig houses decreased in the experiment groups of adding charcoal and wood vinegar (0.30% wood vinegar, 0.60% and 0.80% charcoal, and 0.20% wood vinegar+0.80% charcoal) to diets Therefore, the study results confirm the important roles of both charcoal and wood vinegar used as additives to diet for pigs.

Index Terms- charcoal, diarrhea, wood vinegar

I. Introduction

Diarrhea is widespread disease causes great economic loss in the swine industry worldwide [2, 19]. Diarrhea can occur at any developmental stages of pigs, from suckingpigletsto slaughter pigs. Diarrhea canbe caused by nutrition, bacteria, viruses, parasites or combination of those agents. This disease is responsible for economic losses due to high mortality, morbidity, and cost of medicine application as well as decreasing growthperformance in culturing pig. Previously, *Salmonella*spp., *Clostridiumperfringen*,*Serpulinahyodysenteriae*have been known as diarrhea-related pathogens[7].*Escherichia coli*isevidenced as the main pathogen causing diarrheain cultured pig. The factors that contribute to the increasing of number of diarrhea outbreaks associated with *E. coli* are not yet fully understood[10].

Antibiotics had widely used in animal culture industry, throughout the contributing the great success of animal agriculture after World War II. However, the application of antibiotics in livestock has stimulated the resistant ability with antibiotics inbacterial pathogens[1, 17, 33]. To control this scenario supplementation of exogenous additives, including egg yolk from immunized chickens, zinc and/or spray-dried plasmaor probiotics to diets as well as dietary acidification and phage therapy have been intruded and previously applied. To date, a single control strategy has unsuccessful to provethe total effectiveness; a combination of diet change and other preventive measures are necessary to apply[10].

Hydrogen sulfide(H_2S) emission from pig culture system associates with several problems, including biogenic corrosion of concrete, release of obnoxious odors to the urban atmosphere and toxicity of sulfide gas to farmers [23, 44]. Environmental pollution caused by livestock production has been much research to show.Animal waste containing some compounds have been confirmed causing environmental pollution [29, 43], especially, concerning the pollution caused by nitrogen in fecesof pig culture industry[24]. Due to their physical attributes where pigs only consume about 50% components of nitrogen, phosphorus and sulfur in the diets[28, 34], decreasing H_2S emissions from pig production is a globally considered matter. The chemical composition of livestock waste, volatile gases emitted into the environment depends on the control of diets [19]. The impact on crude protein, amino acids in the diet are able to restrict odor intensity and emissions from pig manure[12, 15, 32]. The sulfur-containing compounds are the most important agents caused stench from manure. Changing diets in culturing system through controlling the crude protein content, composition and proportion of fiber can reduce the emission of H_2S from manure [31]. Accordingto Bindelle, *et al.*[4], the changes of the fiber content in the diet for pigs could reduce NH_3 elimination of shelters by reducing the amount of nitrogen in the urine.

Wood vinegar also called pyroligneous acid or liquidsmoke is the water condensate of smoke produced during wood carbonization. To completely exploit the sawdust, branchand shell of the forestry industry, most of these wood materialsare used to produce charcoal[5].Wood vinegar is a complex mixture of water, phenols, guaiacols, vanillins, catecols, syringols, furan carboxaldehydes, isoeugenol, pyrones, acetic acid, formic acid and other carboxylic acids [20].Charcoal had been known as a good adsorption material because of easy to linking to different types of molecules [6]. Charcoal has been previously proved to be very efficient in removal of bacteria and bacterial toxins in both *invivo*and*in vitro* studies[8-9, 11, 26].Naka, *et al.*,[21]demonstrated thatcharcoalcould remove the toxins of*E. coli*. Other authors used wood vinegar to inhibit the growth of bacteria involving in intestinal disease odd[3, 13, 22], as well as to control the presence of*Cryptosporidiumparvum* in the digestive tract of ruminants [14].Yanyong and Sukhumwat, [40] concluded that wood vinegar is effective in inhibiting thegrow and pathogenicity ofpathogens (fungi and bacteria) on plants.

II. MATERIALS AND METHODS

Animals and diets

A total of 32 weaned breed F1 pigs (Landrace x MongCai) were allocated to 8 groups. Each of one pigwas housed in a cage of 0.8×0.6×0.5mequiped automatic drinking valves. The similarity of body weight of pigs between treatments was ensured. The initial weight of pigs in each treatment is presented in Table 1.All pigs were vaccinated before conducting experiment.

Charcoal and wood vinegar was produced as described below. Wood vinegarliquid obtained after cooling charcoal smoke from wood of *Acacia auriculiformis*trees by dry distillation at 350 to 450^{o}C was kept for 1 to 2 years. Then the filtrate solution was distilled to removeharmful substances such as tar.The experimental diets were formulated to meet all nutrition requirements for pigs from weaned to 30kg-weight period and from 30kg-weight period to finisheraccording to PHILSAN’s recommendation (2003).The ingredients in the diets include rice bran, maize, fish meal, cassava meal,rice bran and mineral-vitaminpremix. A total of 7 test diets were established based on control diets by adding 3 dosages of wood vinegar (0.10%, 0.20% and 0.30%), 3 dosages of charcoal (0.60%, 0.80% and 10%), and mixture of 0.20% wood vinegar and 0.80% of charcoal. The experiments were conducted in three replicate. The composition and nutrient content of experimental diets are presented in Tables 2 and 3.

Table 1: The initialbody weight of pigs

Treatment	Weight of pigs	Number of pigs per cage	Number of repeats
Control	8.87 ± 0.61	1	4
0.1% wood vinegar	9.35 ± 0.73	1	4
0.2% wood vinegar	8.65 ± 0.74	1	4
0.3% wood vinegar	8.48 ± 0.35	1	4
0.6% charcoal	9.15 ± 0.65	1	4
0.8% charcoal	8.65 ± 0.18	1	4
1.0% charcoal	9.20 ± 0.27	1	4
0.2% wood vinegar + 0.8% charcoal	9.18 ± 0.66	1	4

Experimental procedure

Pigs werefed *ad libitum* twicea day (7:00 and 17:00) and had free access to water during experiment. Data of pigs with diarrhea had also daily recordedin the morning.The diarrhea cases recorded based on the symptoms was described by Will [39]. The data of diarrhea of pig was recorded from start to finish experiment. Before concentration H_2S wasmeasuring began, a small test was done to determine the sample weight and timing of suitable with kit. Over a period of 5 days (from 45th to 49th day of experiment), faecesfrom each pig in both phases (weaning to 30kg-weight period, and 30kg-weight to finisher period) werecollected immediately after spontaneous defecation.Faeces of 4pigs within a replication were pooled. Then 200 grams of faecal samples was subsampled and placed in two 7.5 cm diameter × 15cm height plastic bottle for measuringof H_2S. All the bottles had been predrilled a hole of 0.5mm diameter on the capto create aerobic conditions. Then, concentration ofH_2S wasmeasured using KITAGAWA-AP.20 kit (Kitagawa, Japan).

Table 2. Composition and nutrient content of experimental diets for pigs from weaned to 30kg

Ingredients	Treatments							
	Control	0.1% wood vinegar	0.2% wood vinegar	0.3% wood vinegar	0.6% charcoal	0.8% charcoal	1.0% charcoal	0.2% wood vinegar + 0.8% charcoal
Ingredients								
Maize (%)	29.55	29.55	29.55	29.55	29.55	29.55	29.55	29.55
Cassava meal (%)	14.62	14.62	14.62	14.62	14.62	14.62	14.62	14.62
Fish meal (%)	17.91	17.91	17.91	17.91	17.91	17.91	17.91	17.91
Rice bran (%)	35.70	35.70	35.70	35.70	35.70	35.70	35.70	35.70

Mineral –vitamin premix (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Charcoal (%)	0.00	0.00	0.00	0.00	0.60	0.80	1.00	0.80
Wood vinegar (%)	0.00	0.10	0.20	0.30	0.00	0.00	0.00	0.20
Chemical composition								
Crude protein (%)	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
ME (kcal/kg)	3265	3265	3265	3265	3265	3265	3265	3265
Lysin (%)	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Met + Cys (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58

One hundred grams of mineral – vitamin premix contains 10,800 Mn, 2,160 mg Fe, 7,200 mg Zn, 1,260 mg cu, 144 mg Iodine, 21.6 mg Co, 14.1 mg Se, 40 mg acid folic, 4,800 mcg biotin, 20,000 mg choline chloride

Table 3. Composition and nutrient content of experimental diets for pigs from 30kg to slaughter

Ingredients	Treatments							
	Control	0.1% wood vinegar	0.2% wood vinegar	0.3% wood vinegar	0.6% charcoal	0.8% charcoal	1.0% charcoal	0.2% wood vinegar + 0.8% charcoal
Ingredients								
Maize (%)	21.01	21.01	21.01	21.01	21.01	21.01	21.01	21.01
Cassava meal (%)	17.33	17.33	17.33	17.33	17.33	17.33	17.33	17.33
Fish meal (%)	10.82	10.82	10.82	10.82	10.82	10.82	10.82	10.82
Rice bran (%)	50.36	50.36	50.36	50.36	50.36	50.36	50.36	50.36
Mineral –vitamin premix (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Charcoal (%)	0.00	0.00	0.00	0.00	0.60	0.80	1.00	0.80
Wood vinegar (%)	0.00	0.10	0.20	0.30	0.00	0.00	0.00	0.20
Chemical composition								
Crude protein (%)	15.50	15.50	15.50	15.50	15.50	15.50	15.50	15.50
ME (kcal/kg)	3265	3265	3265	3265	3265	3265	3265	3265
Lysin (%)	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Met + Cys (%)	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39

One hundred grams of mineral – vitamin premix contains 10,800 Mn, 2,160 mg Fe, 7,200 mg Zn, 1,260 mg cu, 144 mg Iodine, 21.6 mg Co, 14.1 mg Se, 40 mg acid folic, 4,800 mcg biotin, 20,000 mg choline chloride.

Statistical analysis

The data obtained from experiment were analyzed using general linear model (GLM) in SPSS software version 18.0. Significant differences among means were separated by Tukey test or $\chi^2$with a 5% level of probability.The frequency of diarrhea was expressed as percentage of days with diarrhea for each pig. The frequency of diarrhea of pig was calculated by the following formula. (%day/pig=(total days with diarrheaof pig/total days of the experiment)*100).

III. RESULTS AND DISCUSSION

Growth performance

Table 4. Growth performance of pigs in the experiment

	Initial BW (kg)	Finish (before 30kg phase) BW (kg)	Final BW (kg)	ADG from weaned to 30kg (g/day)	ADG from 30kg to slaughter (g/day)	ADG from weaned to slaughter (g/day)
Control	8.87	37.25	71.50	378.33^{a}	456.67^{a}	417.50^{a}
0.10%WG	9.35	42.93	82.93	447.78^{b}	533.33^{a}	490.56^{b}
0.20%WG	8.65	40.40	79.15	423.33^{ab}	516.67^{a}	470.00^{bc}
0.30%WG	8.48	39.98	77.86	420.00^{ab}	505.00^{a}	462.50^{bc}

0.60%C	9.15	37.82	78.48	382.22^{a}	495.17^{a}	438.69^{ac}
0.80%C	8.65	37.90	73.03	390.00^{ab}	468.33^{a}	429.17^{ac}
1.00%C	9.20	37.33	73.08	375.00^{a}	476.67^{a}	425.83^{a}
0.20%WG+0.80%C	9.18	38.93	75.18	396.67^{ab}	483.33^{a}	440.00^{ac}

$^{a\text{-}c}$Means with different superscripts within a row differ significantly (p < 0.05).
CT: Control; WG: Wood *vinegar*; C: Charcoal; BW: body weight; ADG: Average daily gain

The growth performance results show that, the daily gain of 0.10% wood vinegar group was highest (490.56g/day), control group was lowest (417.50g/day). The daily gain of 0.10; 0.20 and 0.30% wood vinegar group were higher control group and 1.00% charcoal group (*P<0.05*). With increasing dietary wood vinegar levels, daily gain tendedto decrease, but no significant (*P>0.05*).Meanwhile, daily gain of dietarycharcoal levels lower dietary wood vinegar levels and mix charcoal and wood vinegar.

Effects of charcoal and wood vinegar to diarrhea incidence in pigs

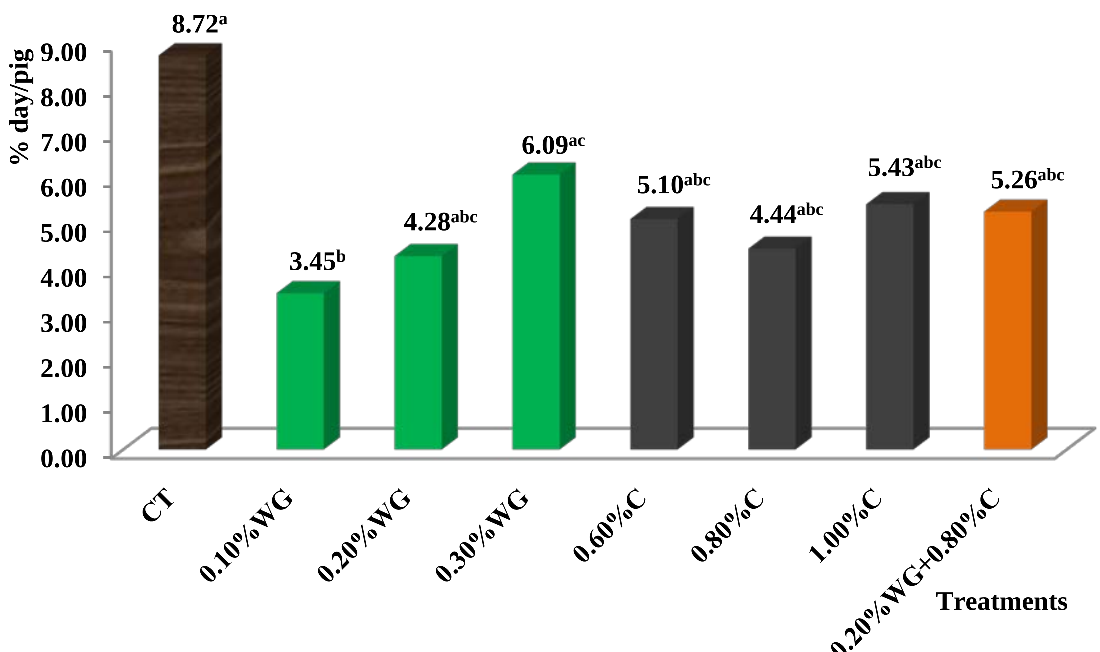

Figure 1: Frequency of diarrhea (% day/pig)

$^{a\text{-}c}$ Means with different superscripts within a column differ significantly (*P < 0.05*).
CT: Control; WG: Wood vinegar; C: Charcoal

The results in Fig. 1 show the frequency of diarrhea in pigs duringexperiment period. The highest frequency of diarrhea was observed in the control group(8.72%day/pig).Meanwhile, the lowest percentage days(3.45%) with diarrhea of pigs was found in the group of fed on the diet added 0.10%wood vinegar. The difference in frequency of diarrhea in pigs fed on control diet and diet added 0.10% wood vinegar was significant (*P<0.05*). There was no significant difference in frequency of diarrhea in pigs between the 6 remain treatments (Fig. 1). The increasing tendency was observed for the frequency of diarrhea in pigs fed on wood vinegar with increasing concentrations. The percentageof 3.45, 4.28 and 6.09% day of pigs got diarrhea was observed when fed on dietsadded 0.10, 0.20 and 0.30% wood vinegar, respectively.Compared to pigs fed control diet, the pigs fed on diets supplemented 0.20% wood vinegar and 0.80% charcoal showed the decrease of 39.7% in occurrences of diarrhea.In the treatments use charcoal as an additive to the diets of experimental pigs, the percentage (% day/pig) of diarrhea odd was lower than the controls; in the treatment of 0.6%, 0.8% and 1.00% charcoal was 5.10, 4.44and 5.43, but in the control group was 8.72 (% day/pig).This study results are consistent to previous study results.

The usefulness of activated charcoal was reported in reducing the effects of toxins in diets by preventing their absorption through the intestine [25-27, 37].Watarai and Tana, [36]indicated the mixture of wood vinegar and charcoal reduce the virulence and numbers of *Salmonella*and increase the number of useful bacteria (*E. faecium* and*B. thermophilum*) in the digestive tract of poultry.Wood vinegar compound liquid was induced acetic acid, propanoic acid, butanoic acid, dimethylphenol and methoxyphenol[42][30]. Which organic acids included in wood vinegar solutions controlled the balance of intestinal microflora and pathogens [18, 27, 36].Yanyong and Sukhumwat, [40]evaluated the effect of adding a mixture of charcoal and wood vinegar to feed to the gastrointestinal mucosa of chickens and resulted in at the 1% mixture of food, less change mucosal surfaces; butat the 5%, microvilli eroded. Naka, *et al.*,[21]recommended charcoal could be used to remove the toxins excreted from*E. coli* O157:H7 after 5 minutesmixing 10 mg charcoal into the liquid of bacterial-growing media. The protective efficacy of charcoal and wood vinegar against intestinal infection with some of bacterial, fungal and parasites was proven [5, 8-9, 16, 21, 36], some of which are causes of diarrhea in pig. Furthermore,Paraud*et al.*,[25]found that charcoal was able to use for preventing disease caused by *C. parvum*on goats. Combined these above results, it is obviously that charcoal and wood vinegar added to diets for pigs are good and validableto prevent porcine diarrhea. Charcoal could well adsorpt toxins and other components excreted by pathogen bacteria; wood vinegar could improve the digestibility and nutritional absorption, inhibit the growing of pathogenic bacteria and stimulate the growing of potentially useful bacteria harboredin the gastrointestinal tract of pigs.

H_2S emissions from pig's manure

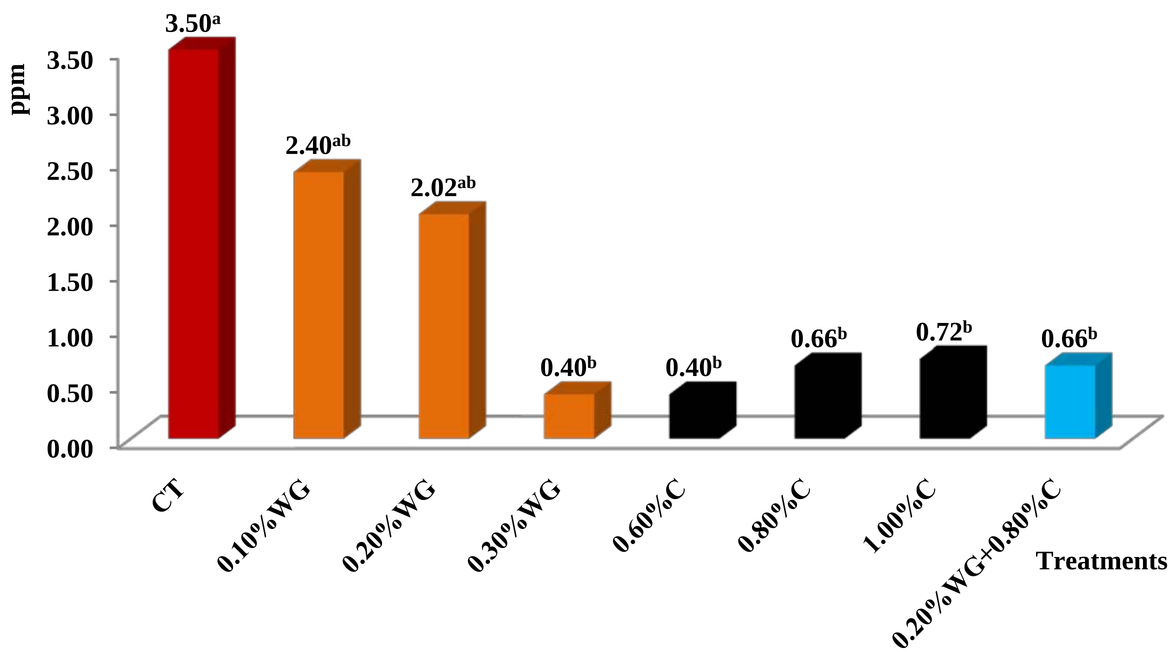

Figure 2: The concentration of H_2S emissions from manure of pig from weaned to 30kg

[a-c] Means with different superscripts within a column differ significantly ($P < 0.05$).

CT: Control; WG: Wood vinegar; C: Charcoal

Concentrations of H_2S were measured after 4 hours post sampling. The results showed that the highest concentration of H_2S was found in the control group 3.50ppm, and at 0.10% and 0.20% vinegar of the treatments 2.40and 2.02ppm respectively (Fig. 2).In contrast, the lowest concentration of H_2S was determined in the treatments of 0.30% vinegar and 0.60% charcoal, sharing a similar concentration of 0.40ppm. Statistically, the average concentration of H_2S in treatments, excepting for 0.10% and 0.20%vinegar treatments, was significantly different ($P<0.05$) in comparison with the control groups. The results also show that an opposite relation between the doses of vinegar to the concentrations of H_2S measured in faeces of pigs. However, it is found to be different in the treatment of supplementation of charcoal, the increase of dose uses, the high concentrations of H_2S. Additionally, in the treatment of 0.20% vinegar + 0.80% charcoal, the concentration of H_2S was lower than the controls, it also lower than treatments 0.20% vinegar, but higher treatments 0.80% charcoal.

The results of H_2S concentration emitted in the second phase of the experiment (from 30kg-weight to ending) show that it was the highest(2.82ppm) in the control group and the lowest(0,98ppm) in the treatment of 0.80% charcoal (Fig. 3). The concentrations of H_2S tended to decrease with the increasing percent of vinegar added to the diets. The concentrations of H_2S in all treatments were lower than the control group, but not statistically significant ($P<0.05$). In the treatment of 0.20% vinegar + 0.80% charcoal, the average concentration of H_2S (1.40ppm) was lower than the control (2.82ppm). Moreover,the average concentration of H_2S in the treatments of 0.20% vinegar(1.96ppm) was higher than such in the treatments 0.80% charcoal (0.98ppm). Theresults suggest the existence of interaction between the use of charcoal and wood vinegar when added to diets to decrease the H_2S emissions from manure pigs.

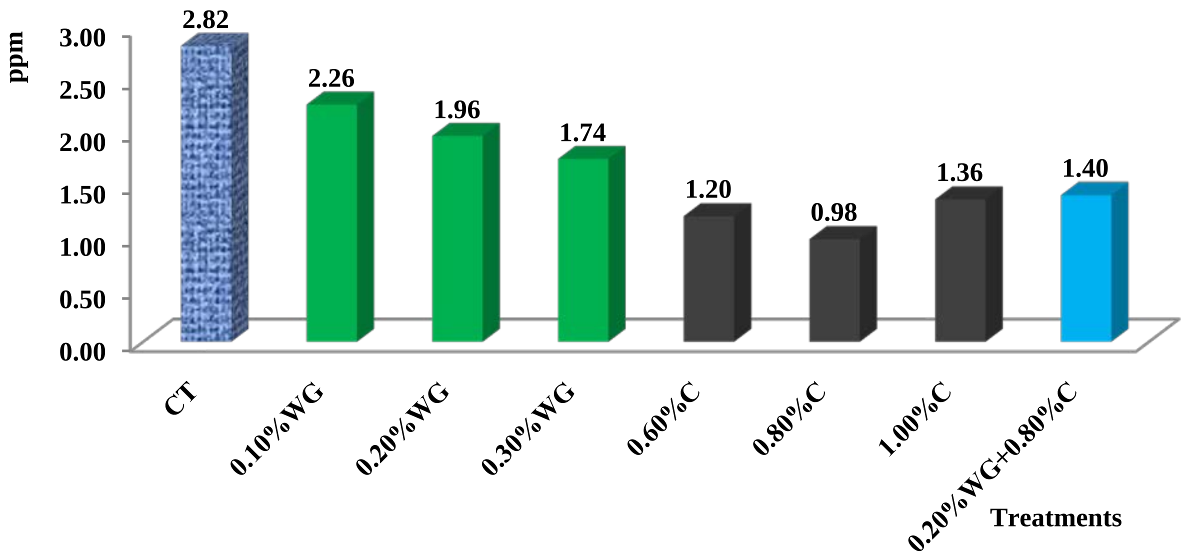

Figure 3: The concentration of H_2S emissions from manure of pig from 30kg to finisher

[a-c] Means with different superscripts within a column differ significantly ($p < 0.05$).

CT: Control; WG: Wood vinegar; C: Charcoal

Whitehead, *et al.* has reported that quebracho tannins added to diets of swinereduced overall gas, hydrogen sulfide, and methane production by greater than 90% after 7days of treatment and continued to at least 28days post treatment as well as decreased the total bacterial population in the gastrointestinal tract. In our study, charcoal added to pig diets could reduce the concentration of H_2S from 2-8.5 times, being accordant to previous study results that a removing of 98% H_2S gas produced by pig manure using bamboo charcoal as an additive to diets[41]. Additionally, Wang, *et al.*, were conducted to evaluate the effects of the dietary withBioPlus 2B® supplemented on the concentration of H_2S emitted from growing pigs;however, in this study, the H_2S emissions were not affected by supplementation with BioPlus 2B®.

IV. CONCLUSION

Thisstudy provides basic information on the advantages of application of charcoal and wood vinegar as additive components in diets of pig during life stages. The current study confirmed these additives functioning in the prevention of porcine diarrhea as well asreducement of H_2Semissions excrectedfrom pig products. However, there should be more experiment to clarify the mechanism of action as well as expand the applications of preparations towards this. In this study only evaluated some doses of charcoal and wood vinegar for purposes of prevention of diarrhea and reduce emissions of H_2S. So, researcheson alarge-scaleand other dose of charcoal and wood vinegar addition to the diets need to further perform.

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