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Original Article

Effects of Fulvic Acid and Probiotic on Growth Performance, Nutrient Digestibility, Blood Parameters and Immunity of Pigs

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Abstract

This study was conducted to determine the influence of fulvic acid (FA) and probiotic (PB) supplementation on performance, nutrient digestibility, blood parameters and immunity of pigs. The trial 1 evaluated the effect of FA and probiotic on nutrient digestibility of pigs at approximately 20 kg. The trial 2 examined the effect on performance, blood parameters and immune response of 80 weanling pigs. Five dietary treatments consisting of control, antibiotic (200 ppm oxytetracycline), 200 ppm FA, 10^9cfu/g probiotic and combination of 200 ppm FA and 10^9cfu/g probiotic (FA+PB). Results from the trial 1 indicated that FA and FA+PB improved the total phosphorus, gross energy and ash digestibility compared with the antibiotic and control group ($P<0.05$). In trial 2, weanling pigs fed antibiotic showed a significantly higher ($P<0.05$) average daily gain (ADG) than pigs fed the control diet. Pigs fed FA+PB had a significantly ($P<0.05$) higher SRBC antibody titers, IgG and phytohemaglutination (skin challenge) level than pigs fed the control diet, as well as of IgG in FA group. In conclusion, FA and FA+PB supplementation increased the gross energy, total phosphorus and ash digestibility of pigs, as well as improved the immune capacity of weanling pigs.

Key words: Fulvic acid, nutrient digestibility, performance, immune response, pigs

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Introduction

The embargo by the European Union placed on those of in-feed antibiotics has been one of the biggest challenges faced by pig producers. Accordingly, a range of non-antibiotic feed additives have been investigated with the aim of avoiding economic losses. Various non-antibiotic feed additives such as probiotic (lactic acid bacteria), oligosaccharides, and plant extracts have been shown to have the same beneficial effect as antibiotics. Numerous studies have been conducted on the use of these natural substances (Haijto *et al.*, 1989).

As an alternative feed additives, humic substance (including humates, humifulvates, humic acid and fulvic acid) has been used in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, improving feed efficiency and immunity, diminishing the risk of disease (Islam *et al*., 2005; Vucskits *et al.*, 2010). However, dietary supplementation with humic substances in pig diets has not been widely studied (Kim *et al.*, 2004).

Humic substances are a group of naturally occurring heterogeneous organic substances of high molecular weight (Aiken *et al.*, 1985). The bacterial and chemical degradation of lignin and other structural carbohydrates in plants are responsible for forming the intermediate products of humic acid and fulvic acid (Wersahw, 1989; Gramss *et al.*, 1999). These intermediate products are then polymerised in the presence of polyphenols, which are leached by rain, from the leaves and other plant components. They can be oxidized to quinones either spontaneously in the presence of molecular oxygen or enzymatically, mediated by a wide variety of microorganisms (Aiken *et al.*, 1985).

Fulvic acid can be extracted from humic substances (Aiken *et al.*, 1985). Fulvic acid is soluble in water and not influence by pH (Islam *et al.*, 2005). Fulvic acid contains many reactive functional groups, including carboxyls, hydroxyls, carbonyls, phenols, quinones and semiquinones (Aiken *et al.*, 1985). These reactive groups make fulvic acid a candidate for both metal chelating and antioxidant activity (Glynn, 1995; Plaza *et al.*, 2005). Sekaly *et al.* (1999) indicated that fulvic acid can bind heavy metals such as nickel (Ni), lead (Pb), copper (Cu) and aluminum (Al). Fulvic acid was found to affect the uptake of cadmium in intestinal segments of rats (Glynn, 1995). Sanmanee and Areekijseree (2010) indicated that Cu combined with fulvic acid (Cu-FA) was significantly absorbed into the cells higher than Cu^{2+}, and showed less damage than tested with Cu^{2+}. TEM and optical studies showed fulvic acid helped binging to the nuclear surfaces, and modified the effect of Cu toxicity.

Information on the safety and toxicology of fulvic acid is minimal. Nonetheless, fulvic acid has been subjected to toxicology and mutagenicity studies and have justified the safety (Humet Product Documentation and Technical Information, 1999). Antal (1990) conducted a study to evaluate the acute toxic using 84 Wistar rats were given different doses of humifulvate concentrates for two weeks. Both male and female rats were given humifulvate up to 10 gm/kg of body weight. Results obtained did not show any death in rats given the highest dose, nor were there any signs of toxicity reported based on macroscopic alterations seen in the organs of the test animals. In addition, recent investigations point to an interesting of *Shilaji* use for medical application toward the control of cognitive disorders associated with aging, and cognitive stimulation. Fulvic acid was the main active principle of *Shilaji*, it could blocks self-aggregation, opening an avenue toward the study of Alzheimer's therapy. Thus, it is demonstrated benefits for human health (Carlos *et al.*, 2012).

Probiotics are defined as viable micro-organisms, which provide beneficial effects on modulate GIT micro-ecology conditions by reducing harmful bacteria and favoring beneficial bacteria (Fuller, 1989; Havenaar and Huis, 1992). A healthy GIT microflora may provide an optimal precondition for effective protection against pathogenic microorganisms, ultimately resulting in improved performance in animals (Flickinger *et al.*, 2003; Niba *et al.*, 2009).

Using fulvic acid as an alternative feed additives in animal production is still at its infant stage, and therefore we also tested the hypothesis that fulvic acid combined with probiotic may have positive effectives for pigs, since both of them are

beneficial for intestinal health, as well as the fact that fulvic acid may absorb toxic materials or bad bacteria in the intestine and probiotic can implant good bacteria. Therefore, this study evaluated the influence of fulvic acid and probiotic on nutrient digestibility, performance, serum traits and immune capacity of pigs.

Materials and Methods

Experiment 1: digestion trial

For determine the response of adding fulvic acid and probiotic on nutrient digestibility, a total of eight pigs of 20 ± 1.8 kg were used in this trial. Using the Latin square design, pigs were randomly assigned to five dietary treatments which changes after each period. The five treatments consisted of: Control group (basal diet, Table 1, experiment 1), antibiotic group (200 ppm oxytetracycline) (positive control), fulvic acid group (200 ppm), probiotic group (PB)(10^9 cfu/g) (includes 30% *Lactobacillus acidophilus, 30% Bifidobacterium lactics, 20% Bacillus subtilis* and *20% Bacillus natto*) and fulvic acid (200 ppm) + probiotic (10^9 cfu/g) (FA+PB) group. Pigs were housed in individual metabolic cages for the duration of the trial. The first week was an acclimation period and the pigs were fed the grower feeds (Table 1). During the trial period, pigs were fed 5% of their body weight daily and adjustment was made when necessary, while water was supplied *ad libitum*. Left-over feed was collected, weighed and deducted from the feed given on a daily basis. Chromium oxide (Cr_2O_3) was used as a marker and added to all diets at 1% on the first and last feeding of each period. The collection time was five days each period. After all marker feces (those collected) completely disappeared (about 2-3 days after meal), a rest period of 5 days was allotted wherein all pigs were fed a basal diet, five periods were performed. Total fecal collection was done and feed weight was recorded daily. Fecal sub-samples (200 grams) from each pig were collected daily and oven drying. After drying, re-weighed and ground for chemical analysis including crude protein, gross energy, crude fat and ash

Chemical analysis

Feeds and feces samples were both analyzed. Calcium and phosphorus concentrations in samples were analyzed using a flame atomic absorption spectrometry (Perkin-Elmer Analyst 100: Flame Atomic Absorption Photometry).

Crude Protein (7.015) analysis was done using the Kjeldahl method while crude fat (7.056) percentage was done using the Soxhlet extractor for 3-4 hours. Ash (7.010) was determined by dry incineration at 600 ℃ for 3-4 hours (AOAC, 1990). Gross Energy was analyzed by combustion in a bomb calorimeter. Dry matter was analyzed by oven-drying at a temperature of 110℃ for overnight.

For each nutrient, digestibility was calculated using the following formula (McDonald et al., 2002):

$$\text{Nutrient digestibility} = \frac{\{(\text{Feed intake} \times \% \text{ nutrient}) - (\text{Feces} \times \% \text{ nutrient})\}}{(\text{Feed intake} \times \% \text{ nutrient})} \times 100\%$$

Experiment 2: Feeding trial

Animals, feeding and sampling

Eighty Landrace x Yorkshire x Duroc weaned piglets (4 weeks-old) at 8.8 ± 0.13 kg body weight were blocked by weight, sex and litter origin, then randomly divided into 5 dietary treatments: control (basal diet, Table 1, experiment 2), antibiotics group (200 ppm oxytetracycline) (positive control), fulvic acid group (200 ppm), probiotic (10^9 cfu/g) group (includes 30% *Lactobacillus acidophilus, 30% Bifidobacterium lactics, 20% Bacillus subtilis* and *20% Bacillus natto*) and 200 ppm fulvic acid + 10^9 cfu/g probiotic (FA+PB) group. Each treatment had 4 pens (replicates) and each pen had 4 pigs. During the entire experimental periods (56 days), pigs had free access to water and the diets. Body weight was measured at the beginning and the end of the trial. Feed intake was calculated by deducting the leftover feeds from the total consumed to determine the average daily feed intake (ADFI), average daily

gain (ADG) and feed efficiency (FE). All pig blood sample collection was made through the jugular vein on the eighth week of experiment and taken for chemical analysis. Animals used in this experiment were cared for under the guidelines stated in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999)*;* and this study was approved by our institution's Animal Care and Use Committee.

Table 1: Composition of basal diets on as fed basis

Ingredients (g/kg)	Trial 1	Trial 2
Extruded corn meal	-----	465.3
Yellow corn meal	662.3	----
Full fat soybean meal	----	313.7
Soybean meal	218.8	----
Fish meal (60%)	40	60
Whey	----	100
Calcium phosphate, dibasic	11.9	21.3
Limestone, pulverized	2	----
Lard	50	26.7
Iodized salt	5	3
Vitamin premix[a]	5	5
Mineral premix[b]	5	5
Total	1000	1000
Calculated value		
Crude protein (%)	18.0	20.9
Metabolizable energy (MJ/kg)	13.38	13.60
Lysine (%)	0.95	1.15
Methionine + Cystine (%)	0.54	0.65
Calcium (%)	0.6	0.7
Total phosphorus (%)	0.5	0.6
Analysis value		
Crude protein (%)	18.2	20.5
Gross energy (MJ/kg)	14.71	14.79

[a.]Vitamin supplied the following per kilogram of premix: vitamin A, 5000 IU ; vitamin D_3, 1500 IU ; vitamin E, 40 mg ; vitamin K, 3 mg ; vitamin B_1, 2.6 mg ; vitamin B_{12}, 4 mg ; niacin, 35 mg ; pantothenic acid, 23 mg.

[b.]Mineral supplied the following per kilogram of premix: Fe ($FeSO_4.7H_2O$, 20.09%Fe), 217 mg; Cu ($CuSO_4.5H_2O$, 25.45%Cu), 125 mg; Mn ($MnSO_4.H_2O$, 32.49%Mn), 40 mg; Zn ($ZnSO_4$, 80.35%Zn), 110 mg; Se ($NaSeO_3$, 45.56%Se), 0.36 mg; Co ($CoSO_4.H_2O$, 32%Co), 0.7 mg.

Blood parameters analysis

Blood cells counting

All blood cells analysis was using fresh blood sample with the Hematology Analyzer (MEK–6318, Nihon Kohden Corporation, Japan) and according to the operation manual to determine. This includes white blood cells (WBC) and red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemotocrit (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), granulocytes, monocytes (MO), lymphocytes (LY), red blood cell distribution width (RDW) and procalcitonin (PCT).

Immunoglobulin G (IgG) analysis

IgG was measured using the procedure described by Banotai (1999) with an ELISA procedure. Each sample was tested and replicated thrice. Ninty-six well microtitre plates were coated with rabbit anti-porcine IgG (Sigma Chemico Co., USA) (diluted 1: 1000 with coating buffer) was used as the primary antibody, while rabbit anti-

porcine IgG conjugated with horseradish peroxidase (diluted 1: 1000 with PBS buffer) was used as the secondary antibody. Purified porcine IgG was adopted as the standard. The coated plates were then washed three times with 0.05% phosphate buffer saline (PBS) buffer. After washing, 100μL 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) (as substrate, avoiding light) and 40 μL H_2O_2 were added into each well and allowed to stand for 15 mins at room temperature. The substrate was then measured at a wavelength of 450 nm by an ELISA reader (Multiskan Ascent, Helsinki, Finland).

Sheep red blood cell (SRBC) antibody titer

Sheep red blood cell titer was determined following the procedures of Van Heugten *et al.* (1996). Blood samples (10 mL) were collected from sheep via jugular vein containing 10% EDTA-2Na (disodium dihydrogen ethylenediamine tetraacetate dehydrate-2Na) (blood: EDTA, 10:1). 5mL isotonic sodium chloride solution was added and then mixed gently. This was followed by centrifugation at 3000 rpm for 10 min, removal of the suspension and repeated three times. The red blood cells were collected and diluted with sodium chloride solution to 0.25% sheep red blood cell. Each pig was injected 2mL sheep red blood cell intramuscularly on the 5^{th} and 6^{th} week of the experiment period. Blood was collected on the 7^{th} week of experiment, and then sheep red blood cell antibody titer was measured by hemagglutination test. 50 μL sodium chloride was put into the U-type 96-well followed by dilution 2-fold afterwards, then added 50 μL of 0.9 % sheep red blood cell into each well. Hemagglutination was read after 1 hour.

Phytohemagglutinin (PHA) skin challenge test

Phytohemagglutinin (Sigma Chemicals Co. USA) skin challenge test was measured following the procedure described by Kegley and Spear (1995). 150 μg/mL (in PBS) PHA was injected intraperitoneally in the ear skin (last week of experiment). Micrometer was used to gauge the degree of swelling from the injection site after 24 hr of phytohemagglutinin injection.

Serum cholesterol and triacylglycerol (TG) determination

A commercial kit (Roche Cobas, Miras, Switzerland) was used to determine the serum cholesterol and triacylglycerol concentration with a serum autoanalyzer (Roche Cobas, Miras, Switzerland).

Statistical Analysis

Mixed linear model procedure was used to analyze the data. Tukey's tests were used to test significantly difference between the treatments means (SAS, 1998). Main effect of fulvic acid and probiotic were analyzed, according to the following model:

$$Y=\mu+T_i+P_j+e_{ijk}$$

Where Y is the dependent variable, μ represents the mean, P is the pen (replicate) effect and e is the random residual error term.

Results

Effect of fulvic acid and probiotic supplements on nutrient digestibility of pigs

The effects of fulvic acid and probiotic on nutrient digestibility are showed in Table 2. The average crude protein, crude fat, calcium and dry matter digestibility's of pigs from the different treatment were not different among the groups.

Fulvic acid, probiotic and the combined FA+PB group had significantly ($P<0.05$) higher energy, phosphorus and ash digestibility than the control group.

Effect of fulvic acid and probiotic on performance of weanling pigs

Table 3 shows the effects of fulvic acid and probiotic on performance of weanling pigs. Pigs fed diet supplemented with antibiotics had greater ($P<0.05$) average daily gain (ADG) than pigs in the control group. However, there was no significant difference in the ADG of pigs fed fulvic acid and probiotic in comparison to the antibiotics group. The average daily feed intake (ADFI), feed efficiency for the pigs among the different treatment showed no significant difference.

Effect of fulvic acid and probiotic on the blood parameters in weanling pigs

Table 4 lists the effects of fulvic acid and probiotic on blood parameters in weanling pigs. The fresh blood analysis which includes red blood cell (RBC), white blood cell (WBC), hemoglobin (HG), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), monocytes (MO), lymphocytes (LY), granulocytes (GT) and procalcitonin (PCT) concentrations of pigs supplemented with treated diets did not show any significant difference compared with the control group.

Effect of fulvic acid and probiotic on immune capacity of weanling pigs

Table 5 shows the effects of fulvic acid and probiotic on the immune capacity of weanling pigs. The data indicated that SRBC of pigs fed with antibiotic, probiotic and the FA+PB diets had a greater response ($P<0.05$) than the control group. Phytohemagglutinin of pigs fed diets treated with the combine FA+PB and antibiotics attained a significant effect ($P<0.05$) than the control group. The IgG level of pigs in all the treatment diets showed higher ($P<0.05$) than the control group. The treatments did not significantly influence serum cholesterol and triacylgyceride concentrations.

Discussion

Nutrient digestibility

In this study supplementation with fulvic acid, probiotic and FA+PB in combination showed significantly higher energy, phosphorus and ash digestibility than the control group.

The structure and chemical properties of fulvic acid are thought to be responsible for chelating mineral ions (Plaza *et al.*, 2005), and therefore indirectly affecting nutrient uptake and utilization of these minerals (Aiken *et al.*, 1985; Sanmanee and Areekijseree, 2010). Kastelan-Macau and Petrovic (1996) found that fulvic acid increased phosphorus retention at approximately 10-20%. Wang *et al.* (1996) studied the influence of commercial fulvic acid by abdominal injection on Wistar rats. Results of the study indicated that fulvic acid was incorporated into the bones and cartilages of the rats. The above study correlates to the positive effect on phosphorus digestibility in this study.

Previous studies on the effect of probiotic supplementation on nutrient digestibility in pigs indicated beneficial effects. Kil *et al.* (2004) studied the effect of continuous feeding of probiotic on the performance, nutrient digestibility, blood urea nitrogen (BUN), and immune response in pigs. During the overall period, apparent digestibility of dry matter, ash, protein, fat and calcium in pigs fed probiotic supplemented diets were greater than that in pigs fed the control diet.

Growth performance

The use of humic substances in pig diets is a rather novel approach (Kim *et al.*, 2004). The functional groups and minerals in humic substance may benefit animal performance even though the actual mechanism is not yet understood (Ji *et al.*, 2006). Ji *et al.* (2006) reported that pigs fed humate supplemented diets increased ADG. Kim *et al.* (2004) observed a significantly higher result in the ADG for pigs fed supplemental humate than pigs fed control diets. Kocabagli *et al.* (2002) and Ozturk et al. (2012) also reported that the body and carcass weights and feed efficiency increased by adding 1.5 or 2.5 humate. However, this study found no such effects in fulvic acid group.

For the probiotic group, previous studies indicated the supplementation of *Streptococcus* species to swine diets was reported to improve growth rate and survival in weanling, growing, and finishing pigs (Korniewicz *et al.*, 1992). Kil *et al.* (2004) also found out that there was a significant improvement in the ADG of pigs fed probiotic. However, this study no significant difference in ADFI, ADG and gain/feed ratio was observed.

Blood parameters

Not many studies on the effects of fulvic acid on blood parameters in domestic animals have been observed. Yoshino and Murakami (1998) observed no significant difference on the effect of humifulvate complex supplements in the haematological parameters of rat pups studied.

Table 2: Effect of fulvic acid and probiotic supplements alone and in combination on nutrient digestibility of growing pigs

Parameters	Control	Antibiotic	Probiotic	Fulvic acid	Fulvic acid + Probitic	SEM	P value
		----------%----------					
Dry matter	75.48	75.17	75.65	75.22	75.21	0.25	0.08
Crude protein	73.46	78.74	79.18	77.72	75.04	2.96	0.18
Crude fat	86.87	85.75	90.65	86.00	86.50	1.95	0.39
Ash	53.72[b]	52.32[b]	61.67[a]	61.67[a]	67.16[a]	2.12	0.03
Gross energy	59.80[b]	60.52[b]	71.8[a]	70.52[a]	67.64[a]	2.01	0.01
Calcium	63.48	61.32	66.71	61.97	63.90	2.87	0.71
Phosphorus	44.86[b]	47.38[b]	56.52[a]	57.61[a]	56.71[a]	2.09	0.03

[a,b]Means with the same letter are not significantly different (P<0.05). n=8.

Table 3: Effect of fulvic acid and probiotic supplements alone and in combination on growth performance of weanling pigs

Parameters	Control	Antibiotic	Probiotic	Fulvic acid	Fulvic acid + Probiotic	SEM	P value
Feed intake (Kg/day/head)	0.88	0.90	0.84	0.84	0.86	0.07	0.18
Weight gain (kg/day/head)	0.37[b]	0.42[a]	0.39[ab]	0.40[ab]	0.39[ab]	0.015	0.05
Feed efficiency	2.38	2.14	2. 15	2.11	2.21	0.17	0.12

[a,b]Means with the same letter are not significantly different (P<0.05). n=5x4.

Table 4: Effect of fulvic acid and probiotic supplementation alone and combination on blood parameters of weanling pigs

Parameters	Control	Antibiotic	Probiotic	Fulvic acid	Fulvic acid + Probiotic	SEM	P value
Red blood cells (10^6/µL)	6.82	6.56	6.63	6.79	6.63	0.14	0.67
White blood cells (10^3/µL)	17.48	20.48	20.71	19.17	17.78	1.79	0.07
Hemoglobin (g/dL)	11.56	10.99	11.26	11.45	11.22	0.22	0.61
Hematocrit (%)	36.74	34.80	35.61	36.24	35.59	0.66	0.67
Mean corpuscular volume (fL)	54.33	53.00	53.90	53.35	53.62	0.56	0.78
Mean corpuscular hemoglobin (pG)	16.88	16.76	16.94	16.90	16.93	0.17	0.87
Mean corpuscular hemoglobin concentration (g/dL)	31.54	31.61	31.48	31.49	31.45	0.13	0.15
Lymphocytes (%×10^3/µL)	11.09	10.13	10.45	10.52	10.24	0.37	0.07
Monocytes (%×10^3/µL)	2.51	2.75	2.61	2.44	2.16	0.18	0.07
Granulocytes (%×10^3/µL)	2.74	3.27	3.16	2.91	2.50	0.42	0.36
Procalcitonin (%)	0.28	0.35	0.29	0.35	0.30	0.13	0.07

n=5x4.

Table 5: Effect of fulvic acid and probiotic supplements alone and in combination on serum traits and immune response of weanling pigs

Parameters	Control	Antibiotic	Probiotic	Fulvic acid	Fulvic acid + Probiotic	SEM	P value
Sheep red blood cell antibody titer ($\log_2$)	2.00^b	3.50^a	3.33^a	2.73^b	3.06^a	0.25	0.01
Phytohematoglutination skin challenge, mm	3.31^b	4.00^a	3.00^b	3.13^b	3.73^a	0.18	0.0001
Immunoglobulin G (mg/dL)	121.0^b	127.0^a	126.0^a	126.0^a	127.0^a	2.08	0.18
Tricyglyceride (mg/dL)	59.97	62.68	63.85	59.96	57.81	5.89	0.17
Cholesterol (mg/dL)	70.30	74.90	71.68	70.81	74.99	4.96	0.06

[a, b]Means with the same letter are not significantly different (P<0.05). n=5x4.

This study found no significant effect in the number of blood cells analyzed among the treatment and the control groups. However, all blood parameters fall within their normal ranges. Monson and Kim (2007) also revealed that humate supplemented diets had no significant difference in the blood cells assayed (red blood cells, white blood cells, neutrophyl, lymphocytes, monocyte, basophil and eosinophil) compared with the control diets in weanling pigs.

This study of the effects of supplementing with probiotic on blood parameters in animal production is consistent with the previous studies of Alvarez *et al.* (2001). In that study, they found an increase in phagocytic activity of alveolar macrophages of the mice tested, with values two times higher than in the control mice. However, the white blood cells differential counts did not show any detectable modification of polymorphonuclear cells and lymphocytes between control and the *Lactobacillus casei*+ yogurt treated groups.

Immune capacity

No previous studies on fulvic acid or its related humic substance family employing the same immune capacity parameters has been established. According to the result, there are promising potential for fulvic acid to become an antimicrobial. However, its ability as replacement for antibiotics in pig diets remains to investigation. In addition, further studies on the exact mechanism of fulvic acid in pig diet needs a thorough study.

Fulvic acid is an important source of macro- and micronutrients, and these materials are able to stimulate oxygen transport (Visser, 1987; Osterberg and Mortensen, 1994). These observations prompted scientists to study the specific properties of humates and their possible benefits in improving health of humans and animals.

As an alternative feed additive, fulvic acid has been used in animal husbandry to diminishing the risk of disease (Islam *et al.*, 2005). However, there are literature which states that it has growth related effects as well as health protection capacity by changing some physiology and developing immunity in different species of animals (Islam *et al.*, 2005). Vucskits *et al.* (2010) reported that ovalbumine antibody titer of rats on fulvic acid supplemented diets showed dose-dependent (0.1-0.8% fulvic acid) and significant increase over the control, diameter of the 'B'-dependent lymphoid tissues in the ileum and spleen were significantly larger in the fulvic acid treated animals. This study also indicated that fulvic acid supplementation beneficial to immunity of piglets.

Results on this study indicated that sheep red blood cells antibodies titer and IgG (Table 5) of pigs fed with probiotic and the FA+PB diets had a greater response than the control fed pigs. Not only for the *lactobacilli*, other strains of probiotic organisms actually attenuated the cytokine response.

The result of the study showed that fulvic acid and FA+PB combination groups in performance and immune capacity aspects were not inferior than antibiotics group, and the energy, ash and phosphorus digestibility were better than antibiotics group. Thus, fulvic acid and FA+PB combination had the potential to be antibiotics substitution. There was no interaction observed between the fulvic acid and probiotic diet. Further study is needed to determine the fulvic acid efficacy as a growth promoter.

Conclusion

This study indicated that fulvic acid and probiotics supplementation in the diets improves the energy, ash and phosphorus digestibility and immune capacity (IgG and sheep red blood cell antibodies titer) of weanling pigs. Further research is needed to consolidate this study.

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