



RESEARCH ARTICLE

Korean Beechwood Creosote as a Substitute to an Antibiotic for Post Weaning Diarrhea in Piglets

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ABSTRACT

Post weaning diarrhea (PWD) effects the economy of pig industry and extensive use of antibiotics to treat PWD led to the development of antibiotic resistance. To counter the issue of antibiotic resistance, role of Korean Beechwood creosote has been studied. Six weeks old, 12 piglets were divided into four groups (3 in each). Diarrhea was induced with *S. typhimurium* in three groups. Piglets treated with creosote have shown 11.52 and 3.12% higher growth rate than control group (C₂) and antibiotic treated groups respectively. Creosote treated group has shown 78% reduction in the fecal score of as compare to C₂ on 5th day after induction. No negative impact is imposed by Korean Beechwood creosote on hemogram. Significantly (P<0.05) high values of complete blood count (WBCs, RBCs, PCV, MCH and MCHC) have been observed in the diarrheic animals and persisted up to 10th day after induction whereas these parameters were in normal range in animals treated with creosote after recovery. Significantly (P<0.05) lower platelet count and total protein values have been observed in C₂ as compared to treatment groups and similar decreasing trend has been observed for albumin, globulin and A:G ratio. The blood urea nitrogen (BUN) levels in treatment groups were in normal range while it was significantly higher in C₂. Significantly low and high values of sodium (Na⁺) and potassium (K⁺) respectively, have been observed in C₂. Parallel findings between the antibiotics and Korean Beechwood creosote strongly suggest its use as an antidiarrheal agent and growth promoter for weaning piglets.

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INTRODUCTION

Post weaning diarrhea is the major cause which affects the economy of pig industry (Mudronova *et al.*, 2012). Infectious agents, lack of immunity and improper management practices are the major causes of PWD (Shoemaker *et al.*, 2001). Disruption of intestinal epithelium by microbial or viral pathogens is among the common causes which lead to insufficient absorption of water and result into diarrhea. Infections with *Salmonella* serotypes are source of food borne diseases in the worldwide (Santos, 2003) and pigs are the host for *Salmonella* serotypes. In pigs, outbreaks mostly occur in 2 to 4 months of age. Antibiotics in therapeutics and as

growth promoter led to their extensive use as feed additives. Extensive use of antibiotics led to the development of resistance and antibiotic residues in animal products. In new diarrheic syndrome, no response to antibiotics and common management practices have been reported (Kongsted *et al.*, 2013). It made the governing bodies to put scrutiny/ ban on the use of antibiotics in animal feed (Han *et al.*, 2007). Since 2011, South Korea Ministry for food, agriculture, forestry and fisheries has too imposed ban on the extensive use of antibiotics as feed additives.

To counter the issue of antibiotic resistance, medicinal herbs/plants are catching importance (Jung *et al.*, 2011). Remedies from herbal origins have been in

regular use for the treatment and management of various ailments since the beginning of human civilization (Hussain *et al.*, 2012). Although use of wood creosote as an herbal medicine against diarrhea in rats and mice has been reported (Ataka *et al.*, 2007; Hiramoto *et al.*, 2012) but still meager work has been done on the anti-bacterial and anti-diarrheal role of Korean traditional herbs (Bae, 2005) against PWD in piglets. There are few references which support the anti-diarrheal properties of wood creosote (Arteaga *et al.*, 2005). The current study was planned to assess the effect of Korean beechwood creosote as a substitute to antibiotics with an emphasis on its anti-diarrheal effect, to identify an alternative to maintain growth performance and control economic losses.

MATERIALS AND METHODS

Experimental design: Post weaned, six weeks old 12 piglets of Landrace breed with initial mean body weight of 11.88 ± 0.80 kg were used for current study. Piglets were procured from a commercial supplier (Chilsung Farm, Seogwipo-si, Jeju-Do, South Korea). The experiment was approved by the animal ethics committee of the School of Applied Life Sciences. Experimental animals were kept in pig farm of Jeju National University with the ambient temperature maintained at $25 \pm 1^\circ\text{C}$. Piglets were housed in pens with concrete flooring, a nipple bowl drinker and feeder. Piglets were offered the commercial feed (Seoul Feed, Jeju- Si, South Korea) and water *ad libitum*.

Piglets were divided into 4 groups with 3 piglets in each group. The groups were designated as C₁, C₂, T₁ and T₂. In group C₁, three healthy piglets without diarrhea were grouped. These animals were fed normal feed. Diarrhea was induced in the nine piglets by one shot of *S. typhimurium* mixed in their feed. Diarrheic piglets were grouped in C₂, T₁ and T₂ with three piglets in each group. Animals in groups C₂, T₁ and T₂ were offered normal feed, mixed with antibiotic and creosote, respectively. SAMU CPS (Chlortetracycline, Procainpenicilline-G and Sulfathiazole, SAMU, Median, South Korea) was supplemented to group T₁ piglets at a dose rate of 0.25% of feed for 10 days. Group T₂ piglets were supplemented with Korean beechwood creosote at the rate of 0.34 gm per 10 kg BW/day without any supplementation of antibiotic for 2 months. Creosote and antibiotic were administered orally by mixing in feed. Pyroligenous liquor being the active ingredient was used for the preparation of Korean beechwood creosote in the lab. Other herbal components like algenic acid, chitosan, guar gum, levan, locust bean gum and wheat flour were also used to prepare the creosote.

Body weight gain and feces score: The body weight of individual piglets was measured at day zero and subsequently at weekly intervals up to two months of supplementation. In the end of the study per cent increase in body weight for respective groups were calculated.

The severity of diarrhea was noted visually and scores were given on the basis of consistency of the feces on a standardized scale of 0-3 as described by Cox *et al.* (1987). Watery feces was awarded a score- "3" followed

by loose feces with score- "2", semi-solid feces was scored "1" and the condition when there was no diarrhea was scored "0".

Blood biochemistry: Blood samples were collected with K2E (K2EDTA) (Ref. 365974, BD Microtainer[®]) from all of the piglets for complete blood count CBC. The samples were collected from the jugular vein on "0, 3rd, 5th, 7th and 10th day" after the induction of diarrhea. Samples were analyzed using a hematology analyzer (pocH-100 iV, Sysmex, Hong Kong) for white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit test (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT).

At the same time, blood from each animal was also collected in the plasma separator tubes with lithium heparin (Ref- 365958, BD Container[®]). The heparinized samples were centrifuged at 2000g for 10 min. After centrifugation, plasma was collected and analyzed by automatic chemistry analyzer (VetScan, VS2, Abaxis, USA). Plasma samples were analyzed for total protein (TP), albumin (Alb), globulin (Glob), glucose (Glu), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), phosphorus (Phos), Sodium (Na⁺) and potassium (K⁺). Albumin to globulin ratio (A: G), serum total protein (STP) and fibrinogen were calculated manually.

Statistical analysis: Data were analyzed through one way analysis of variance (ANOVA) and the results were expressed as mean \pm SD. The means were compared for significance by Student's *t-test* at $P < 0.05$.

RESULTS

Effect of creosote on body weight gain and feces score: Each individual animal was weighed weekly from day "0" to till completion of treatment. Piglets treated with creosote have shown 11.52 and 3.12% higher growth rate than control group (C₂) and antibiotic treated group (T₁) respectively (Fig. 1). The lowest rate of body weight gain has been recorded in C₂ group which may be due to persistent dehydration and reduced appetite for 7 days.

During the experiment severe diarrhea had been observed in C₂, T₁ and T₂ groups up to 3rd day after induction (Fig. 2). The significant recovery ($P < 0.05$) was observed in T₁ and T₂ groups from 5th day onwards, whereas in C₂ group severity of diarrhea was observed up to 7th day after induction.

Complete blood count: No negative impact has been imposed by Korean beechwood creosote on hemogram (Table 1). Parallel trends between the groups fed with the antibiotic and creosote has been observed. Both the treatment groups did not show any significant difference ($P < 0.05$) in hemogram during the recovery phase, which indicated about the efficacy of creosote with respect to antibiotic. A sharp increase has been observed in the number of WBCs on the 3rd day of induction of diarrhea in C₂, T₁ and T₂ groups (Table 1). From 5th day onwards level of WBCs started decreasing in T₁ and T₂ groups, which were in normal range by 10th day in all the groups.

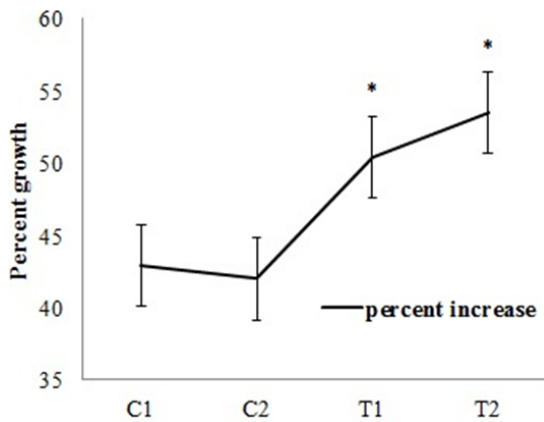


Fig. 1: Per cent increase in body weight in groups during the period of study; *significantly different from the control group ($P<0.05$)

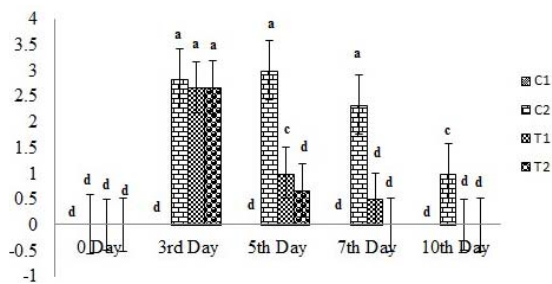


Fig. 2: Feces score in groups during the period of study; ^{abcd} Bars not sharing a common superscript letter were significantly different, ($P<0.05$).

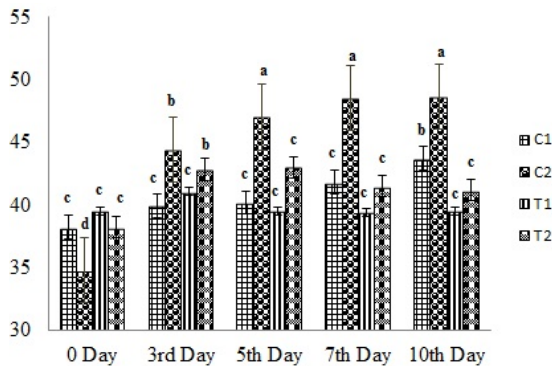


Fig. 3: Trend in hematocrit values or packed cell volume (PCV) in groups during the period of study; ^{abcd} Bars not sharing a common superscript letter were significantly different, ($P<0.05$).

A regular increasing trend has been observed for RBCs in the C₂ (Table 1). Till 5th day non-significant difference for RBCs has been observed among control and treatment groups whereas C₂ group still had significantly high RBCs till 10th day, indicating that severe loss of water during diarrhea led to hemo-concentration. High levels of hemoglobin by 10th day in negative control group indicated the dehydration in piglets due to the fluid loss from vascular compartment and confirmed hemo-concentration (Table 1).

Progressive increase in HCT with the increase in the severity of disease in C₂ group has attributed dehydration. A significantly ($P<0.05$) high values of HCT have been observed in negative control group (Fig. 3). Significantly

high values for the MCV, MCH and MCHC have been observed in diarrheic control group up to 3rd day as compare to treatment groups (Table 1). Overall decreasing trend except on 5th day has been observed for platelet count in negative control group. A significantly high values ($P<0.05$) have been observed in C₂ group up to 5th day for STP. The significant differences among control and treatment groups have been observed for fibrinogen but no regular trends have been observed among treatment groups (Table 1).

Blood/Plasma Chemistry: The significant decrease in total protein (TP) levels has been observed in C₂ group during the period of study. Although for treatment groups, TP levels decreased by 3rd day of induction but from 5th day onwards treatment groups shown improvement in the TP (Table 2). T₁ and T₂ groups have shown parallel trend for TP. Significantly ($P<0.05$) lower values have been observed in C₂ as compared to treatment groups which are indicative of loss of protein through gastrointestinal tract (GIT). Albumin, globulin and A:G ratio have also shown parallel trends with respect to TP (Table 2).

A significant recovery has been observed from 5th day onwards in the BUN levels for treatment groups but piglets from C₂ group had significantly high BUN levels (Table 2). Blood biochemical analysis of creosote treated animals with respect to healthy animals has shown no significant difference. Glucose, ALT and ALP (indicators for liver injury) were under normal range for creosote treated animals and glucose did not show much variation (Table 2). It indicated that creosote does not have any sort of ill effects on liver.

Sodium levels came to normal from 5th day onwards in treatment groups and were comparable to healthy animals. Significantly low values of Na⁺ have been observed in C₂ from 3rd day onwards and were low till 10th day (Table 2). Other electrolytes like potassium has shown significantly high values in C₂ as compare to treatment group whereas no particular trend has been observed in case of phosphorus (Table 2).

DISCUSSION

PWD causes severe morbidity and mortality in the piglets. It has been listed as one of the major mainspring which affects the economy of pig industry (Mudronova *et al.*, 2012). Housing, nutrition, immune level of the piglets and managerial practices contribute towards PWD (Shoemaker *et al.*, 2001). Pigs have been enlisted as the configured host for *Salmonella* serotypes and these are one of the most common bacteria causing diarrhea in piglets. Extensive use of antibiotics causes resistance and its residues in animal products. Therefore, the controlling authorities imposed ban on the use of antibiotics in animal feed (Han *et al.*, 2007). Even the ministry for food, agriculture, forestry and fisheries of South Korea has too strongly implemented ban on the extensive use of antibiotics as feed additives. Therefore, in the current study the anti-diarrheal effect of Korean beechwood creosote was judged.

It has been reported that pigs suffering from *S. typhimurium* infection grew poorly over a period of two to three weeks (Thomson *et al.*, 1998). Therefore, the

Diarrhea caused by *Salmonella* is associated with increased vascular permeability leading to increased influx of neutrophils (Zhang *et al.*, 2003). Actually, neutrophils cause necrosis of the intestinal epithelium which results into leakage of extravascular fluid in the form of diarrhea. While observing the pattern shown by the hemogram in the current study, creosote has shown the equal efficacy with respect to antibiotics. The normalization of leukocyte count after 5th day onwards indicates the clearance of bacterial infection with complete recovery.

Normalization of RBC count in treatment groups and significantly high number of circulating RBCs till 10th day in C₂ group indicated severe loss of water during diarrhea which might have caused hemo-concentration (Hassan *et al.*, 2013). Mavenyengwa *et al.* (2010) also reported significant increase in the number of circulating RBCs from 7th day onwards in diarrheic cattle. Even 5% increase in PCV also has been reported in case of dogs suffering from hemorrhagic diarrhea (Reineke *et al.*, 2013). A finding that hemato- biochemical change in piglet diarrhea has reported increased hemoglobin, PCV and serum potassium levels in infected animals (Das *et al.*, 2008) support our results.

The significantly (P<0.05) decreased levels of TP in C₂ with respect to treatment groups have indicated about the loss of proteins through GIT. Mavenyengwa *et al.* (2010) also reported decreased levels of total plasma protein (P<0.05) during diarrhea in cattle. Similarly, decreased levels of albumin, globulin and A:G ratio might be due to marked decrease in diet intake, mal-absorption and due to ongoing protein losing enteropathy (Bhat *et al.*, 2013). Reduced albumin levels indicate about hepatotoxicity but normal levels of albumin in the piglets reared on the diet mixed with creosote indicated that creosote does not cause any kind of hepatotoxicity (Arteaga *et al.*, 2005).

The significantly increased BUN levels in plasma have indicated towards the pre- renal uremia which may be due to decreased glomerulo-filtration rate and catabolic breakdown of epithelia occurring during diarrhea. Blood biochemical analysis of creosote treated animals with respect to healthy animals has shown no significant difference for the blood plasma chemistry. Wood creosote by blocking the Cl⁻ channel on the intestinal epithelium effectively inhibits intestinal secretions induced by enterotoxins. It is also reported to decrease the intestinal motility accelerated by mechanical, chemical or electrical stimuli by the inhibition of the Ca²⁺ influx into the smooth muscle cells. The no particular trend for phosphorus levels has left any impact, although a decreased phosphorus level during diarrhea has been reported in cattle (Mavenyengwa *et al.*, 2010). Recovery of electrolyte balance in creosote treated piglets during the current study has indicated that creosote is effective against diarrhea and the similar results have been reported by Ataka *et al.* (2003).

The incidence of resistance in *E. faecalis* against most of the antimicrobials which are used for growth promotion in livestock is more prevalent in Korea than in European countries (Hwang *et al.*, 2009). Aparamycin sulphate has also been used as feed additives to control bacterial enteritis in weanling pigs but because of

resistance, their use has been banned (Shoemaker *et al.*, 2001). It is also reported that wood creosote inhibits STA-induced fluid secretion in the jejunum (Ogata and Shibata, 2001). Acetyl choline and Ba²⁺ induced tonic contractions of longitudinal and circular muscles of ileum are also inhibited by creosote which indicates that anti- diarrheal activity of wood creosote is because of its anti-secretory and anti-motility effects.

Conclusion: The parallel findings of body weight gain, feces score, hematocrit values and blood chemistry strongly suggest the use of Korean beechwood as a substitute to antibiotics, for its use as an antidiarrheal agent and growth promoter for weaning piglets. Our results would have significant impact on the economics of pig farming/ industry, provide an antibiotic replacement option and most importantly would be helpful to control the possible antibiotic health hazards in consumer.

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