

Effects of probiotics on growth performance, blood parameters, and antibody stimulation in piglets

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Abstract

The study investigated the effects of probiotic bacteria (*Lactobacillus reuteri* ZJ625, *Lactobacillus reuteri* VB4, *Lactobacillus salivarius* ZJ614, and *Streptococcus salivarius* NBRC13956) administered as direct-fed microorganisms on growth performance and blood parameters of weaned piglets. Forty-five weaned piglets were divided into five treatments: antibiotic (PC), no antibiotic and no probiotic (NC), probiotic (P1), probiotic (P2), and combination of probiotics (P3). Fecal and ileum samples were collected for microbial count analysis. Blood samples were also collected from the animals at the end of the trial for the hematological and biochemical analysis and the ability of the probiotics to stimulate immunoglobulin G (IgG). Supplementation of probiotics had no effect on feed intake (FI). However, average daily weight gained (ADG) in the P3 treatment was higher than in other treatments and lowered the value of feed conversion ratio (FCR) of weaned piglets. Microbial count of fecal samples did not differ in all the treatments while ileum samples had lower enteric bacteria in P3 treatment when compared to other treatments. Concentration of albumin, globulin, neutrophils and basophils were higher in the NC treatment when compared to other treatment groups. The IgG concentration was highest in P3 compared to other treatments. Results suggested that probiotics have beneficial effects on growth performances, blood parameters, and IgG stimulation of weaned piglets. This advocates that probiotics will offer a significant benefit in pig farming by reducing the risk of post weaning diarrheal syndromes, and therefore enhance pig industry's economy.

Keywords: Blood chemistry, feed conversion ratio, immunoglobulin G, post-weaning diarrheal syndromes, probiotics

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Introduction

Weaning transition is a complicated phase in pig production because piglets are weaned from the ages of three to four weeks old (Moeser *et al.*, 2007). During this phase, the piglets have to cope with unexpected hasty separation from the sows, mixed with other clutters in a strange environment, and their diet changes from easily digestible (milk) to less digestible (solid) food (Lalles, 2007). Weaning is a very critical period in pig production, as it may result in digestive disturbance, which causes growth set-back, and low feed intake in piglets. This can ultimately result in mortality in some cases (Hacin *et al.*, 2008). This transition period is frequently linked with high occurrence of Post-Weaning Diarrheal Syndromes (PWDS), which is triggered by potential enteric pathogens such as *Escherichia coli* (*E. coli*) (Rist *et al.*, 2013). Post-Weaning Diarrheal Syndrome is a multifactorial disorder that arises after weaning, but is characterized by the proliferation of Enterotoxigenic *E. coli* (ETEC) in the gut (Tang *et al.*, 2009). Once PWDS outbreaks occur in farms, the morbidity may be over 50% among the weaned piglets (Laine *et al.*, 2008) and this eventually results in huge economic losses in the pig industry (Kiers *et al.*, 2003).

Antibiotics have been added in the diet of just weaned piglets in order to reduce the occurrence of diarrhoea, while at the same time promoting growth performance (Thu *et al.*, 2011). However, in some European countries, antibiotics have been legislatively and voluntarily banned for use in pigs' diet as growth promoters (Pluske *et al.*, 2002). This is because of the concerns about antibiotic residues in animal food products and the development of bacterial resistance to antibiotics; hence, development for alternative to antibiotics is necessary. Probiotics are potential alternatives (Gu *et al.*, 2006); probiotics are non-toxic live microorganisms that are normally used as animal feed additives. They produce beneficial effect to equilibrate

intestinal flora of an animal (Yirga, 2015). Probiotics compete with harmful gut flora, stimulate the immune system of the animal, and increase its resistance to infectious agents in order to promote growth (Kritas & Morrison, 2005); and have been reported to be able to lower blood cholesterol (Ooi & Liong, 2010). Probiotics execute these health benefits by reducing metabolic reactions that produce toxic substances, produce vitamins and anti-microbial compounds such as bacteriocins (Hemaiswarya *et al.*, 2013).

The most commonly used probiotics microorganisms belong to lactic acid bacteria (LAB), such as *Lactobacillus spp.*, *Bifidobacterium spp.*, and *Enterococcus spp.* (Ljungh & Wadstrom, 2006), and *Streptococcus spp.* These bacteria are normally isolated from intestinal microflora of the intended species and selected based on conditions such as resistance to stomach acids, bile salts, ability to colonize the intestinal harmful microorganisms (Cho *et al.*, 2011). Thus, the objective of this study was to test the efficacy of putative probiotic strains of *Lactobacillus reuteri* ZJ625, *Lactobacillus reuteri* VB4, *Lactobacillus salivarius* ZJ614, and *Streptococcus salivarius* NBRC 13956 on growth performance, hematological and biochemical parameters, and antibody stimulation on weaned piglets.

Materials and Methods

Procedures involved in this study complied with all the relevant legislation regarding the protection of animal welfare and were approved by the Agricultural Research Council (ARC), Animal Production Institute (API) Ethics Committee (APIEC13/008).

Probiotic bacteria were isolated and characterized from the gastro-intestinal tracts (GIT) of eight piglets of South African Windsnyer pig breed. These pigs have proven records of tolerance to diseases, high weaning rates and low incidences of post weaning diarrhoea (PWD). Combinations of both the molecular sequencing (16S ribosomal RNA) and analytical profile index (API® Biomerieux, SA) methods were used to identify the probiotics. The probiotics were prepared in MRS agar slants and stored in ultra-low-freezer. *Lactobacillus reuteri* ZJ625, *Lactobacillus reuteri* VB4, *Lactobacillus salivarius* ZJ614 and *Streptococcus salivarius* NBRC 13956 probiotic strains were revived by inoculation in Man, Rogosa and Sharpe broth (MRS; Oxoid, England). The probiotics were then grown in anaerobic jars with CO₂-generating kits (Anaerogen; Thermofisher, UK) at 37 °C for 24 hr. The revived probiotic strains were inoculated with the appropriate culture at 1% (v/v) and grown anaerobically in MRS broth overnight following the procedure described by Casey *et al.* (2007).

The study was carried out at the Agricultural Research Council (ARC) Gastrointestinal Microbiology and Biotechnology Unit and Pig Nutrition Unit in Gauteng, South Africa. Weaned piglets (n = 45), of which 23 were commercial (large white x landrace) breed piglets and 22 were South African indigenous Windsnyer breed piglets, entered the study at four weeks old, at a weight range of 7.5 to 9.5 kg and were in a trial for a period of 30 days. Pigs were kept individually in a temperature-controlled building in flat deck type cages (1.5 x 1.0 m) with perforated metal floor, equipped with self-feeders and automatic water nipple; and piglets had unrestricted access to water for the whole trial period.

The piglets were randomly assigned to five treatments (9 per treatment). Treatment 1: diet containing no antibiotic and no probiotic (NC); Treatment 2: diet containing antibiotic – no probiotic (PC); Treatment 3: diet supplemented with probiotic (*L. reuteri* ZJ625) (P1); Treatment 4: diet supplemented with probiotic (*S. salivarius* NBRC13956) (P2); and Treatment 5: diet supplemented with combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC 13956) (P3). *L. reuteri* VB4 and *L. salivarius* ZJ614 have been tested and confirmed in our previous studies (forthcoming) to have probiotics properties; hence, in this study they are combined with newly tested probiotics to confirm synergy when all are combined together. The diet used in these experiments was in the form of pellets and was formulated to provide the nutrient requirements regardless of treatments, although for positive control group, in-feed antibiotic lincospectin was added. The ingredients and composition of the experimental diets are presented in Table 1 and viable cell counts of probiotics used in the study are presented in Table 2.

The piglets were supplied with sufficient feed and water through self-feeders. Feed intake (FI) and body weight were monitored and measured weekly to determine the average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR). The FI of pigs was recorded once a week by offering a weighed quantity of feed and weighing their residues, while the ADG was calculated by subtracting the initial body weight from the final body weight and thereafter divided by the number of feeding days on trial. The FCR for each piglet was calculated by dividing feed intake by the body weight gain. Piglets in a probiotic treatment were administered with 10 mL of 24 hour old culture once a week, orally using a syringe. Mortalities and morbidities were monitored, noted, and subjected to post mortem examination.

Table 1 Nutritional levels of experimental diets (Meadow weaner feeds South Africa)

Nutrients	g/kg
Protein	180
Fat	25
Fibre	50
Dry matter	880
Calcium	10
Total lysine	11

Table 2 Average viable cell counts of probiotics administered to piglets

Probiotic	Concentration (cfu/mL)
<i>L. reuteri</i> ZJ625	6.8×10^9
<i>L. reuteri</i> VB4	5.5×10^{10}
<i>L. salivarius</i> ZJ614	2.6×10^{10}
<i>S. salivarius</i> NBRC13956	2.9×10^{10}

A day after oral administration of probiotics, 10 g of fresh fecal samples were collected weekly from each piglet, for bacterial enumeration and stored at $-80\text{ }^{\circ}\text{C}$. At the end of the trial (30 days), pigs were slaughtered and a segment of ileum was removed and placed in a sterile tube containing 9% (v/v) normal saline for bacterial enumeration. Collected fecal and ileum samples were serially diluted from 10^{-1} to 10^{-8} . Dilutions were subsequently plated in triplicate on selective agar media for enumeration of target bacterial strains. Total bacteria, enteric and lactic acid bacteria (LAB) were enumerated using nutrient agar (Oxoid, England), violet red bile agar (VRB; Oxoid, England) and Man, Rogosa and Sharpe agar (MRS; Oxoid, England) respectively, following the traditional method of An *et al.* (2008). Each plate was incubated at $37\text{ }^{\circ}\text{C}$ for 24 hour, anaerobically for LAB and aerobically for enteric and total bacteria. Colonies were counted using automated colony counter (Ben-art product, USA). Results were presented as the average of the three dilutions at base 10- logarithm colony forming unit per mL (cfu/mL).

At the end of the trial, approximately 20 mL of blood were collected from sacrificed piglets, for the analysis of hematological, biochemical and IgG stimulation. Blood samples collected into EDTA (BD Diagnostics, pre-analytical systems, Midrand, USA) were transported on ice to the Diagnostic Laboratory, for the determination of selected hematological parameter analysis. The hematological parameters analyzed included red blood cells (RBC) (hemoglobin and Hematocrit), white blood cells (WBC) (neutrophils, monocytes, lymphocytes, eosinophils, basophils) and platelets. In addition, biochemical parameters analyzed include total serum protein, albumin, globulin, cholesterol and glucose.

The preparation of serum was carried out using the method of Szabo *et al.* (2009). Aliquots (2 mL) of blood samples collected into serum bottles were transferred into sterile 5 mL microcentrifuge tubes and centrifuged at 1200 rpm for 15 min using Sorvall MC-12V microcentrifuge (American Laboratory trading, Inc., USA). The fraction sera were later aspirated using Pasteur pipettes (Sigma-Aldrich, Johannesburg, South Africa) into sterile 5 mL centrifuge tubes and used within 12 hour of preparation. Swine serum samples were diluted (1:35, 000) and concentrations of IgG were quantified using Pig IgG enzyme-linked immunosorbent assay (ELISA) kits (Bethyl laboratories, Inc.; USA), following manufacturer's instructions. A Multiskan GO Microplate Spectrophotometer (Thermofisher Scientific, USA) was then used to measure the substrate absorbance at a wavelength of 450 nm. The results obtained (ng/mL) were multiplied by 10^{-5} dilution factor and expressed as mg/mL. In each experiment serial dilution of standard samples were tested in order to obtain calibration curve, which was then computer-adjusted (with Fin graph software program). From this calibration curve, values of unknown IgG concentration were calculated.

The data was submitted to one-way analysis of variance (ANOVA), comparing five treatments as main effects, using SAS statistical software (SAS 9.3) (2003). Means were separated using Tukey's Test and differences were considered significant at $P \leq 0.05$.

Results and Discussion

In all treatment groups, no significant difference was observed ($P > 0.05$) for FI (Table 3). The ADG on P3 treatment group was higher ($P \leq 0.05$) as compared to other treatment groups and FCR was better in P3 treatment group.

Table 3 Growth performance of piglets based on different treatments group

Parameters	Treatment					P-value
	NC	PC	P1	P2	P3	
FI (kg/d)	0.83	0.81	0.82	0.86	0.80	0.57
ADG (kg/d)	0.40 ^c	0.41 ^c	0.40 ^c	0.47 ^b	0.57 ^a	0.04
FCR	2.10 ^a	2.00 ^a	2.10 ^a	1.90 ^a	1.40 ^b	0.03

^{a-c}Mean values within a row with different superscripts are significantly different ($P \leq 0.05$)

ADG = Average daily gain, FI = Feed Intake, FCR = Feed conversion ratio, NC = Negative control, PC = Positive Control P1 = *L. reuteri* ZJ625, P2 = *S. salivarius* NBRC13956, P3 = Combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC13956).

Table 4 shows effect of probiotic bacteria on biochemical parameters. No significant difference ($P > 0.05$) was observed in all treatments when total serum protein, cholesterol and glucose were measured in the experimental animals. The concentration of albumin and globulin showed higher significant difference ($P \leq 0.05$) in NC treatment group as compared to other treatment groups.

Table 4 Biochemical parameters of piglets based on different treatments groups

Parameters	Treatments					P-value
	NC	PC	P1	P2	P3	
TSP (g/L)	54.1	55.1	58.9	54.2	60.5	0.69
ALB (g/L)	47.1 ^a	45.6 ^b	44.9 ^b	43.0 ^b	38.5 ^c	0.03
GLO (g/L)	65.5 ^a	63.3 ^b	60.9 ^b	59.2 ^c	58.4 ^c	0.05
CHO (mmol/L)	2.10	2.03	2.27	2.13	2.08	0.99
GLU (mmol/L)	7.13	7.33	7.11	7.33	7.13	0.94

^{a-c}Mean values within a row with different superscripts are significantly different ($P \leq 0.05$)

NC = Negative control, PC = Positive Control P1 = *L. reuteri* ZJ625, P2 = *S. salivarius* NBRC13956, P3 = Combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC13956).

TSP = Total serum protein, ALB = Albumin, GLO = Globulin, CHO = Cholesterol, GLU = Glucose

Table 5 shows effect of probiotic bacteria on hematological parameters. No significant difference ($P > 0.05$) observed in all treatments except for segmented neutrophils and basophils. Segmented neutrophils and basophils had higher concentration ($P \leq 0.05$) in NC treatment group as compared to other treatment groups.

Table 5 Hematological analysis of piglets based on different treatment groups

Parameters	Treatments					P-value
	NC	PC	P1	P2	P3	
Hb (g/L)	140	145	137	136	131	0.15
Cells/L (10E9)						
Ht	0.44	0.45	0.44	0.43	0.40	0.09
Wcc	19.4	21.1	15.2	14.7	21.1	0.13
Sn	14.4 ^a	9.5 ^b	7.33 ^b	4.92 ^c	4.81 ^c	0.01
Bn	0.00	0.38	0.58	0.00	1.71	0.34
Lym	8.58	9.30	6.41	8.87	7.65	0.75
Mon	1.14	0.66	0.74	0.68	0.71	0.39
Eos	0.48	0.20	0.66	0.11	0.20	0.16
Bas	0.16 ^a	0.10 ^a	0.07 ^b	0.14 ^a	0.04 ^c	0.05
Plt	311	430	380	430	420	0.71

^{a-c}Mean values within a row with different superscripts are significantly different ($P \leq 0.05$)

NC = Negative control, PC = Positive Control P1 = *L. reuteri* ZJ625, P2 = *S. salivarius* NBRC13956, P3 = Combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC13956).

Wcc = White cell count, Sn = Segmented neutrophils, Bn = Band neutrophils, Lym = Lymphocytes, Mon = Monocytes, Eos = Eosinophil, Bas = Basophil, Plt = Platelet

Several studies on probiotics have been published in recent years, suggesting that probiotics offer beneficial nutrition and protection against pathogens for animals and humans, particularly in the pig industry (Chaucheyras-Durand and Durand, 2010; Ezema, 2013; Devi and Kim, 2014). In the present study, no significant difference was observed for FI between the treatment groups (Table 3). However, ADG on the P3 treatment group (combination of probiotics) was higher ($P \leq 0.05$) as compared to other treatment groups and resulted in a drastic improvement in FCR in the P3 treatment group. The FCR of this P3 group improved from 2 kg of feed consumed for 1 kg of weight gain to 1.4 kg of feed consumed for 1 kg of weight gain, which is an improvement of 30% in efficiency. The extrapolation from these results is that the producer would have invested less money per kilo of weight when the pig reaches slaughter weight. Lojanica *et al.* (2010) reported similar results when evaluated effects of probiotic *Enterococcus faecium* DSM 7134 in the weaned piglets nutrition. They found that piglets fed *E. faecium* had greater daily weight gain and improved feed conversion ratio. Vrotniakiene & Jatkauskas (2013) also reported improvement in both daily weight gain and feed conversion ratio, when pig diet is supplemented with Enterococcus-based probiotics. These results demonstrate that supplementation of a combination of probiotics has potential to be used as a dietary supplementation in weaned piglets.

A properly balanced gut microbiota has the ability to positively affect the integrity of the intestinal barrier against colonization by pathogens (Jandhyala *et al.*, 2015), through its protective and metabolic function and can stimulate the immune system in an anti-inflammatory manner (Wu and Wu, 2012). However, physiological and/or psychological stresses such as weaning lead to dysfunctional of gut microbial composition (McLamb *et al.*, 2013). By supplementing probiotics to the piglets feeding during post weaning, there is a potential to modulate intestinal microbial diversity by improving the establishment of beneficial bacterial populations for health and reducing pathogenic bacteria such as enterotoxigenic *E. coli* (Brousseau, *et al.*, 2015). In this study, the supplementation of probiotics did not reduce enteric bacterial counts nor increased lactic acid bacterial (LAB) counts in fecal samples (Figure 1). These results are similar to those reported by Giang *et al.* (2011), where how probiotics supplementation affects performance, nutrient digestibility and fecal microflora in growing-finishing pigs was evaluated. The authors reported that inclusion of *Bacillus* in pig diet did not affect fecal microflora. However, Figueiredo-Silva *et al.* (2010) found different results, while studying probiotics and antibiotics as additives for sows and piglets during nursery phase. They found that the prevalence of *E. coli* associated with pathogenic groups was lower with the use of probiotics. Hu *et al.* (2014) also reported that probiotic supplementation on diet of weaned piglets reduced *E. coli* counts in fecal samples.

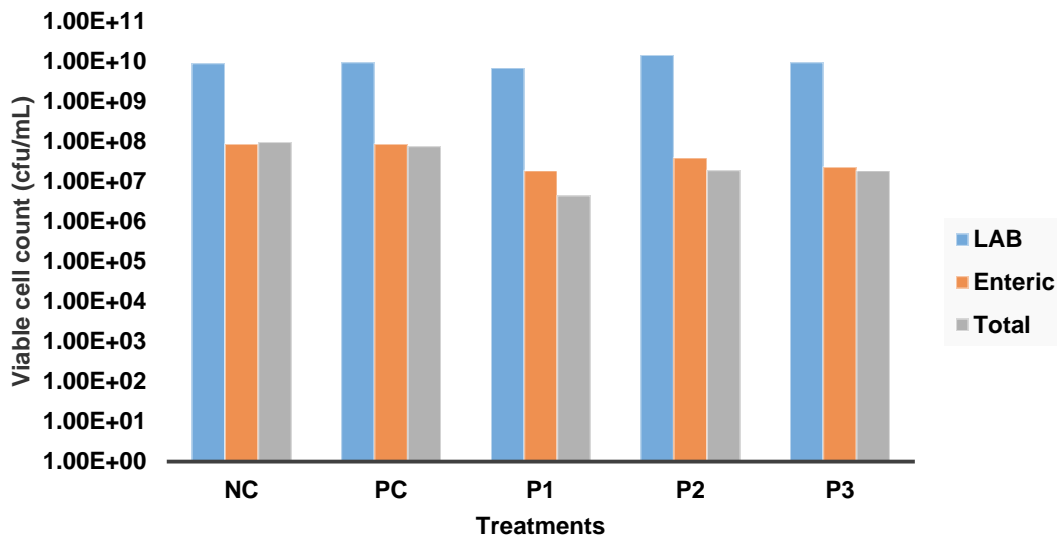


Figure 1 Microbial enumeration of lactic acid, enteric and total bacteria from piglets fecal samples

NC = Negative control, PC = Positive Control P1 = *L. reuteri* ZJ625, P2 = *S. salivarius* NBRC13956, P3 = Combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC13956).

From ileum samples counts, no significant difference was observed in all treatment groups, with an exception of P3 treatment group regarding cfu/mL of lactic acid bacteria, enteric bacteria and total bacteria. P3 treatment group had lower cfu/mL of enteric bacteria as compare to lactic acid bacteria and total bacteria (Figure 2). It has been reported that probiotic bacteria maintain normal gut microflora in two ways: competitive exclusion and antagonism. Once probiotic bacteria reach the gut and established themselves, they may produce bactericidal compounds (bacteriocins) such as organic acids, hydrogen peroxide, and lactoferrin. These substances reduce the pH in the gut and this creates an environment where pathogenic bacteria cannot grow (Kamada, 2013).

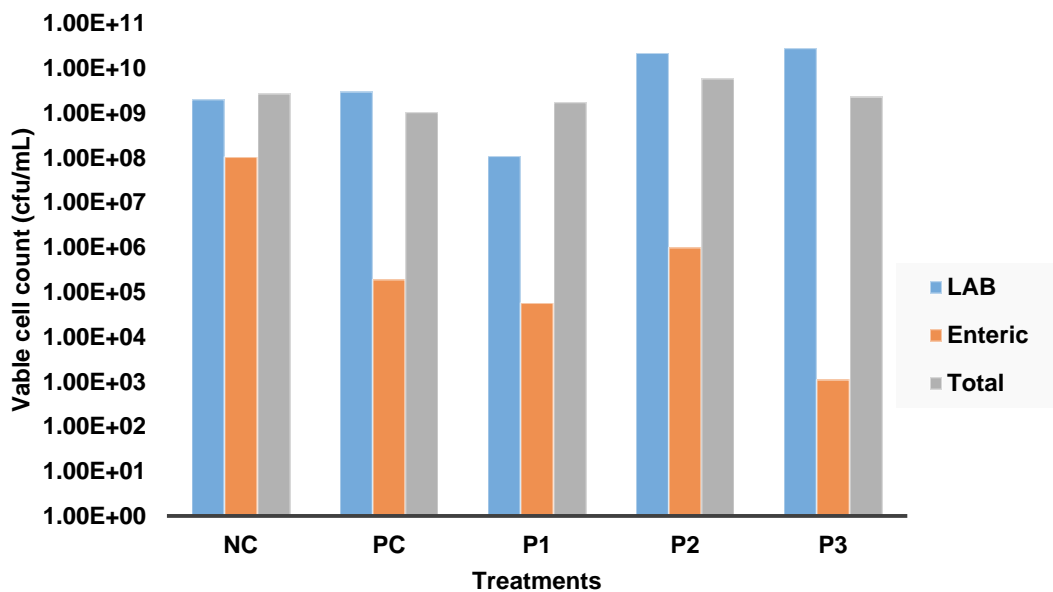


Figure 2 Microbial enumeration of lactic acid, enteric and total bacteria from piglets ileum samples.

NC = Negative control, PC = Positive Control P1 = *L. reuteri* ZJ625, P2 = *S. salivarius* NBRC13956, P3 = Combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC13956).

These results are consistent with results of Mallo *et al.* (2010), who found that the addition of *Enterococcus faecium* to diet improves the intestinal microbiota of the piglets. In their study, they observed that piglets receiving the probiotic bacteria in the diet had significantly higher cfu/mL of lactic acid bacteria in the ileum while the cfu/mL of *E. coli* was significantly lower. Enterobacteria are potential intestinal pathogens when an imbalance of the intestinal microflora occurs. It has been reported that a decrease of *E. coli* in probiotic-fed piglets led to improved LAB/*E. coli* ratio, a suggested indicator of gut health (Dong *et al.*, 2013). The decrease in enteric bacteria in ileum samples of piglets fed a combination of probiotics (P3) indicates improved gut health.

A well-balanced gut microflora is able to affect the integrity of the intestinal barrier against colonization through its protective and metabolic function and can stimulate the immune system in an anti-inflammatory manner. However, physiological or psychological stresses caused by weaning process compromise gut microbiota and results to intestinal dysfunction. Probiotics are mostly applied on this kind of situation, in order to re-establish the gut microbial balance (Ahmed *et al.*, 2014). The results from this study indicates that supplementation of probiotics in weaned piglets feed enhance the immune system.

Results on Table 4 show the probiotics effect on blood biochemistry parameters of the slaughtered piglets. No significant difference was observed for parameters such as total serum protein, cholesterol and glucose among all treatment groups. It was only observed for globulin and albumin; negative control group had high concentration of albumin and globulin. These results are similar to those observed by Busanello *et al.* (2015), who concluded that high concentration of albumin and globulin indicate the presence of infection and dehydration. This suggest that piglets in the control treatment group were more exposed to infections as compared to piglets in the probiotic treatment groups. On the contrary, Kumar *et al.* (2012) reported no significant difference observed in the concentration of albumin and globulin in all treatment groups.

Findings of probiotic effect on hematology of slaughtered piglets' blood are shown on Table 5. No significant difference was observed amongst treatment groups regarding hematology parameters, except for segment neutrophils and basophils. Segment neutrophils and basophils had higher (concentration in the control treatment group than in probiotic groups. These findings are similar to those of Nurliyani and Marsetyawan (2011) who reported that rat fed probiotic fermented goat milk had lower levels of neutrophils and basophils than rats in control treatment. Moreover, high levels of neutrophils and basophils indicates occurrence of infection. These findings clearly indicate that piglets in probiotic groups were in a healthier state as compared to those in the control treatment group. However, Al-Saiady *et al.* (2010) did not find significant improvement on hematology parameters from the use of probiotics.

The Figure 3 shows the effect of probiotics on IgG stimulation in piglets. Piglets fed probiotics had higher concentration of IgG than piglets fed positive and negative control feed. This significant increase of IgG in the probiotic group could be due to the persistent of probiotic bacteria in the intestinal tract and acting as immune adjuvant to the immune system and therefore stimulating IgG production (Naqid *et al.*, 2015).

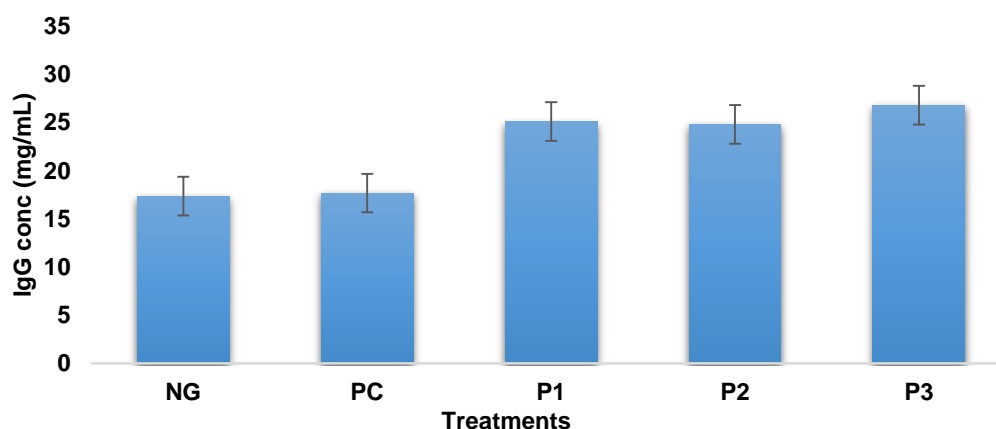


Figure 3 Effect of different treatments on Immunoglobulin G (IgG) stimulation of piglets

NC = Negative control, PC = Positive Control P1 = *L. reuteri* ZJ625, P2 = *S. salivarius* NBRC13956, P3 = Combination of probiotics (*L. reuteri* ZJ625, *L. reuteri* VB4, *L. salivarius* ZJ614 and *S. salivarius* NBRC13956).

These results are in accordance with results of Yu *et al.* (2004). Yu and his co-authors evaluated the effect of probiotics and selenium combination on the immune and blood cholesterol concentration of pig. In their findings, they reported that piglets fed probiotics showed higher concentration of IgG than piglets fed treatment without probiotics. On contrary, Chen *et al.* (2005) reported different result when studied the effect of dietary probiotics on growth performances, nutrient digestibility, blood characteristics and fecal noxious gas content in growing pigs. In their findings, they reported IgG concentration was not affected by dietary treatment of complex probiotics. These results suggest that piglets administered with probiotics were healthier than those getting antibiotics. The findings correlates with challenges piglets face during weaning phase; such as low feed intake, stressful environment and weight loss. Thus, piglets in NC presented poor immune system.

Conclusions and Recommendations

From this study, the application of a probiotics blend in piglets clearly revealed that probiotics have great potential as alternatives to antibiotics. Although different probiotics strains, even of the same species may have different metabolic effects; which in turn affect growth performances, microbial count and blood parameters of piglets differently. Moreover, probiotics may be used to control post weaning diarrheal syndromes during weaning period. However, the mechanism of actions of probiotics is largely unknown. Hence, it is of utmost importance for more research to be carried out in order to elucidate the mechanism of probiotic actions, to provide more rigid recommendations about probiotics strains and their optimal performance levels. Additionally, studies of shelf life, supplementation stages, alternatives to oral administration, dose size, effect on different pig breeds and gender are also required.

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Authors' Contributions

ZCD conducted the research for her MSc studies and drafted the manuscript, RLSL helped with animal trials and laboratory work, OAA and AIO conceived, designed and supervised the research. Finally, all authors commented on early and final versions of the manuscript.

Conflict of Interest Declaration

The authors have declared that there is no conflict of interests.

References

- Ahmed, S.N., Hoon, J., Mun, H.S. & Yang, C.J., 2014. Evaluation of Lactobacillus and Bacillus-based probiotics as alternatives to antibiotics in enteric microbial challenged weaned piglets. *Afr. J. Microbiol. Res.* 8, 96-104.
- Al-Saiady, M.Y., 2010. Effect of probiotic bacteria on immunoglobulin G concentration and other blood components of new calves. *J. Anim. Vet. Adv.* 9, 604-609.
- An, B.K., Cho, B.L., You, S.J., Paik, H.D., Chang, H.I., Kim, S.W., Yun, S.W., Yun, C.W. & Kang, C.W., 2008. Growth performance and antibody response of broiler chicks fed yeast derived β -glucan and single-strain probiotics. *Asian-Aust. J. Anim. Sci.* 21, 1027-1032.
- Balasubramanian, B., Li, T. & Kim, I.H., 2016. Effect of supplementing growing-finishing pig diets with bacillus spp. Probiotic on growth performance and meat-carcass grade quality traits. *R. Bras. Zootec.* 45, 93-100.
- Brousseau, J.P., Talbot, G., Beaudoin, F., Lauzon, K., Roy, D. & Lessard, M., 2015. Effect of probiotics *Pediococcus acidilactici* strain MA18/5M and *Saccharomyces cerevisiae* subsp. *boulardii* strain SB-CNM I-1079. On faecal and intestinal microbiota of nursing and weaning piglets. *J. Anim. Sci.* 93, 5313-5326.
- Busanello, M., Pozza, M.S.S., Pozza, P.C., Nunes, R.V., Chambo, A.P.S. & Eckstein, I.I., 2015. Probiotics: viable and inactivated cells on the performance, microflora and blood parameters of piglets. *Rev. Bras. Saúde. Prod. Anim.* 16, 387-396.
- Casey, P.G., Gardner, G.E., Casey, G., Bradshaw, B., Lawler, P.G., Lynch, P.B., Leonard, F.C., Stanton, C., Ross, P.R., Fitzgerald, G.F. & Hill, C., 2007. A five-strain probiotic combination reduces pathogens shedding and alleviates disease signs in pigs challenged with *Salmonella enteric* Serova Typhimurium. *Appl. Environ. Microbiol.* 73, 1858-1863.
- Chaucheyras-Durand, E. & Durand, H., 2010. Probiotics in animal nutrition and health. *J. Benef. Microbes* 1, 3-9.
- Chen, Y.J., Son, K.S., Min, B.J., Cho, J.H., Kwon, O.S. & Kim, I.H., 2005. Effect of dietary probiotic on growth performance, nutrients digestibility, blood characteristics and fecal noxious gas content in finishing pigs. *Asian-Australas. J. Anim. Sci.* 19, 587-592.
- Cho, J.H., Zhao, P.Y. & Kim, I.H., 2011. Probiotics as dietary additives for pigs: A review. *J. Anim. Vet. Adv.* 10, 2127-2134.

- Devi, S.M. & Kim, I.H., 2014. Effect of medium chain fatty acids (MCFA) and probiotic (*Enterococcus faecium*) supplementation on the growth performances, digestibility and blood profile in weaned pigs. *Vet. Med.* 59, 572-535.
- Dong, X., Zhang, N., Zhou, M., Tu, Y., Deng, K. & Diao, Q., 2013. Effect of dietary probiotics on growth performances, fecal microbiota and serum profiles in weaned piglets. *Anim. Prod. Sci.* 54, 1-6.
- Dowarah, R., Verma, A.K., Agarwal, N., Patel, B.H.M. & Singh, P., 2017. Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. *Livest. Sci.* 195, 74-79.
- Ezema, C., 2013. Probiotics in animal production: A review. *J. Vet. Med. Anim. Health* 5, 308-316.
- Figueiredo-Silva, M.L.F., de Freitas-Lima, J.A., de Souza-Cantarelli, V., de Oliveira-Amaral, N., Zangeronimo, M.G. & Fialho, E.T., 2010. Probiotics and antibiotics as additives for sows and piglets during nursery phase. *R. Bras. Zootec.* 39, 2453-2459.
- Giang H.H., Viet, T.Q., Ogle, B. & Lindberg, J.E., 2011. Effect of supplementation of probiotics on the performance, nutrient digestibility and fecal microflora in growing-finishing pigs. *Asian-Australas. J. Anim. Sci.* 24, 655-661.
- Gu, X., Li, D., Lu, W., Piao, X. & Chen, X., 2006. Screening of *Bacillus* strains as potential probiotics and subsequent confirmation of the in vitro effectiveness of *Bacillus subtilis* MA139 in pigs. *Antonie. Van. Leeuwenhoek.* 90, 139-146.
- Hacin, B., Rogelj, T. & Matijasic, B.B., 2008. Lactobacillus isolates from weaned piglets mucosa with inhibitory activity against common porcine pathogens. *Folia Microbiol* 53, 569-579.
- Hemaiswarya, S., Raja, R., Ravikumar, R. & Carvalho, I.S., 2013. Mechanism of action of probiotics. *Braz. Arch. Biol. Technol.* 56, 113-119.
- Hu, Y., Dun, Y., Li, S., Zhao, S., Peng, N. & Liang, Y., 2014. Effects of *Bacillus subtilis* KN-42 on growth performance, diarrhea and faecal bacterial flora of weaned piglets. *Asian-Aust. J. Anim. Sci.* 27, 1131-1140.
- Jandhyala, S.M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M. & Reddy, D.N., 2015. Role of the normal gut microbiota. *World. J. Gastroenterol* 21:8787-8803.
- Kamada, N., 2013. Control of pathogens and pathobionts by the gut microbiota. *Nat. Immunol.* 14, 685-690.
- Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. & Van der Meulen, J., 2003. Effect of fermented soybeans on diarrhea and feed efficiency in weaned piglets. *J. Appl. Microbiol.* 95, 545-552.
- Kritas, S.K. & Morrison, R.B., 2005. Evaluation of probiotics as a substitute for antibiotics in a large pig nursery. *Vet. Rec.* 156, 447-448.
- Kumar, S., Verma, A.K., Mondal, S.K., Gupta, M., Patil, A.K. & Lal Jangir, B., 2012. Effect of *Saccharomyces cerevisiae* feeding on serum biochemistry in early-weaned crossbred piglets. *Vet. World* 5, 663-666.
- Laine, T.M., Lyttikäinen, T., Yliaho, M. & Anntila, M., 2008. Risk factors for post-weaning diarrhea on piglets producing farms in Finland. *Acta Vet. Scand.* 50, 1-11.
- Lalles, J.P., Bosi, P., Smidt, H. & Stokes, C.R., 2007. Nutritional management of gut health in pigs around weaning. *Proc. Nutr. Soc.* 66, 260-268.
- Ljungh, A. & Wadstrom, T., 2006. Lactic acid bacteria as probiotics. *Curr. Issues Intestinal Microbiol.* 7, 73-90.
- Lojanica, M., Manojlovic, M. & Jeremic, D. & Petronijevic, S., 2010. The effects of probiotic *Enterococcus faecium* DSM 7134 in the weaned pigs nutrition. *Biotechnol. Anim. Husb.* 26, 57-64.
- Mallo, J.J., Rioperez, J. & Honrubia, P., 2010. The addition of *Enterococcus faecium* to diet improves piglet's intestinal microbiota and performances. *J. Livest. Sci.* 133, 176-178.
- McLamb, B.L., Gibson, A.L., Overman, E.L., Stahl, C. & Moeser, A.J., 2013. Early weaning stress in pigs impairs innate mucosal immune responses to Enterotoxigenic *E. coli* challenge and exacerbates intestinal injury and clinical disease. *Plos. One.* 18, 1-12.
- Moeser, A.J., Ryan, K.A., Nighot, P.K. & Blikslager, A.T., 2007. Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 293, 413-421.
- Naqid, I.A., Owen, J.P., Maddison, B.C., Gardner, D.S., Foster, N., Tchórzewska, M.A. & La Ragione, R.M., 2015. Prebiotic and probiotic agents enhance antibody-based immune responses to *Salmonella Typhimurium* infection in pigs. *Anim. Feed. Sci. Technol.* 201, 57-65.
- Nurliyani, E.H. & Marsetyawan, H.S., 2011. Leukocytes count and lymphocytes proliferation of dinitrochlorobenzene sensitized rat supplemented with fermented goat milk. *Int. J. Biol. Biomolec. Agric. Food Biotechnol. Eng.* 5:357-361.
- Ooi, L.G. & Liong, M.T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings. *Int. J. Mol. Sci.* 11, 2499-2522.
- Pluske, J.R., Pethick, D.W., Hopwood, D.C. & Hampson, D.J., 2002. Nutritional influences on some major enteric bacterial diseases of pigs. *Nutr. Res.* 15, 333-371.
- Rist, V. T.S., Weiss, E., Eklund, M. & Mosenthin, R., 2013. Impact and activity on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: *Anim.* 7, 1067-1078.
- SAS/STAT User's Guide, Version 9, 3rd printing, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.
- Szabo, I., Wieler, L.H., Teden, K., Scharek-Teden, L., Taras, D., Hensel, A., Appel, B. & Nockler, K., 2009. Influence of a probiotic strain of *Enterococcus faecium* on *Salmonella enterica* Serovar Typhimurium DT104 infection in a porcine animal infection model. *Appl. and Environ. Microbiol.* 75, 2621-2628.
- Tang, Z., Yin, Y., Zhang, Y., Huang, R., Sun, Z., Li, T., Chu, W., King, X., Li, L., Geng, M. & Tu, Q., 2009. Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin-lactoferrampin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21d. *Br. J. Nutr.* 101, 998-1005.

- Thu, T.V., Loh, T.C., Foo, H.L., Yaakub, H. & Bejo, M.H., 2011. Effect of liquid metabolite combinations produced by *Lactobacillus plantarum* on growth performance, feces characteristics, intestinal morphology and diarrhea incidence in post-weaning piglets. Trop. Anim. Health. Prod. 43, 69-75.
- Vrotniakiene, V. & Jatkauskas, J., 2013. Effect of probiotics dietary supplementation on diarrhea incidence, fecal shedding of *Escherichia coli* and growth performances in post-weaned piglets. Vet. Med. Zoot. 63, 81-88.
- Wu, H.J. & Wu, E., 2012. The role of gut microbiota in immune homeostasis and autoimmunity. J. Gut. Microbes. 3, 4-14.
- Yirga, H., 2015. The use of probiotics in animal nutrition. J. Prob. Health. 3, 1-10.
- Yu, L.T., Ju, C.C., Ju, J., Wu, H.L. & Yen, H.T., 2004. Effect of probiotics and selenium combination on the immune and blood cholesterol concentration of pigs. J. Anim. Feed. Sci. 13, 625-634.