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CARCASS CHARACTERISTICS, PLASMA BIOCHEMICAL PROFILE AND FAECAL MICROBIAL COUNT OF CROSSBRED PIGS SUPPLEMENTED WITH PROBIOTIC, PREBIOTIC AND SYNBIOTIC

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ABSTRACT

An experiment was carried out for a period of 131 days in forty weaned (twenty castrated male and twenty female) Large White Yorkshire x Desi piglets to study the effect of dietary supplementation of probiotic, prebiotic and synbiotic on carcass characteristics, plasma biochemical parameters and faecal microbial count. The piglets were divided into four groups with five replicates of two piglets in each replicate. The piglets were randomly allotted to the four dietary treatments, T1 (control ration as per NRC, 1998), T2 (control ration + 0.2 per cent yeast as probiotic), T3 (control ration + 0.2 per cent mannan oligosaccharide (MOS) as prebiotic) and T4 (control ration + 0.2 per cent of synbiotic containing 0.1 per cent yeast and 0.1 per cent MOS) using completely randomized design. The results of the study indicated that all the four dietary treatments were similar (P>0.05) in carcass characteristics and plasma biochemical parameters. However, the faecal microbial load was reduced (P<0.05) by synbiotic supplementation showing lowered values for total viable count and coliform count. Results concluded that synbiotic at 0.2 per cent level (0.1 per cent yeast + 0.1 per cent MOS) can be used as beneficial feed additive for maintaining healthy gut microflora in crossbred pigs.

Keywords: Crossbred pigs, Probiotics, Prebiotics, Synbiotics, Carcass characteristics, Plasma biochemical profile

INTRODUCTION

Pork is the most consumed meat worldwide. Intake of pork represents more than 36 per cent of the global consumption of animal protein (UN-FAO, 2019). In the Indian livestock sector, piggery plays an important role in uplifting the socio-economic status of the rural livelihood (Shehnaz *et al*., 2022). Feed additives such as antibiotics, probiotics, prebiotics, organic acids, enzymes and immune modulators have been used widely to improve the gut health in pigs. Among these, antibiotics were most widely used as feed additives in the past decade. However, concerns

associated with antibiotic residues in pork and development of antibiotic-resistant bacteria in human, had led to its ban in European Union and South Korea. This has intensified the need for viable alternatives to antibiotics as growth promoters (Haupenthal *et al*., 2020). Use of probiotics and prebiotics alone, or in combination, thus emerged as an alternative to antibiotic growth promoters in pigs (Joysowal *et al*., 2018). Probiotics are beneficial microbes which stabilize eubiosis by competitive growth against harmful microbes (Park *et al*., 2016); while prebiotics are a class of complex carbohydrates, that are not absorbed or digested in the small intestine of animals, but readily fermented by large intestinal micro flora, acting as substrates for beneficial gut microbes. Synergistic effects of probiotics and prebiotics (synbiotics) can also be considered in stimulating the growth of beneficial bacteria to improve the gut health. To better understand the effects of these feed additives in intensive pig production, it is necessary to study their effect on carcass quality, blood biochemical profile and faecal microbial count. Moreover, systematic studies comparing the effect of probiotic, prebiotic and synbiotic in crossbred pigs are scanty in literature (Rybarczyk *et al*., 2021). Hence, the present experiment was conducted to study the effect of dietary supplementation of probiotic, prebiotic and synbiotic on carcass characteristics, plasma biochemical parameters and faecal microbial count in crossbred pigs.

MATERIALS AND METHODS

Experimental animals

A feeding experiment was conducted for a period of 131 days in forty crossbred (Large White Yorkshire X Desi) pigs (twenty castrated male and twenty females) with an average body weight of 17.66 kg at the Centre for Pig Production and Research, Mannuthy. All the animals were dewormed and vaccinated against Classical Swine Fever (CSF) and Foot and Mouth Disease (FMD) before the commencement of the experiment. The piglets were divided as uniformly as possible with regard to age, sex and body weight into four groups of ten animals each. The animals of each group were randomly allotted to five pens with two animals in each pen, forming five replicates of each treatment. The four groups of animals were randomly allotted to four dietary treatments using completely randomised block design.

Housing and management

All animals were maintained under identical management conditions, fed twice daily (9.00 am in the morning and 3.00 pm in the evening) and were allowed to consume as much as they could, within a period of one hour. Balance of feed if any,

was collected and weighed before the next feeding. Fresh and clean drinking water was provided *ad libitum*.

Experimental ration and feeding

The experimental ration consisted of grower ration containing 18 per cent crude protein (CP) and 3265 kcal of metabolizable energy (ME) per kg feed, up to 50 kg body weight and finisher ration with 16 per cent CP and 3265 kcal of ME per kg feed from 50 kg onwards (NRC, 1998).

The four experimental rations were formulated as given below.

T1 – Control ration (NRC, 1998)

T2 – Ration containing 0.2 per cent probiotic (yeast)

T3 – Ration containing 0.2 per cent prebiotic (mannan oligosaccharide)

T4 – Ration containing 0.2 per cent synbiotic (combination of 0.1 per cent yeast and 0.1 per cent mannan oligosaccharide)

All the rations were made isocaloric and isonitrogenous. Ingredient composition of grower and finisher rations are furnished in Tables 1 and 2, respectively. Piglets were fed with their respective grower ration until they attained an average body weight of 50 kg and thereafter with finisher ration until the animals attained an average body weight of 70 kg.

Bioyeast (Varsha Group, Bangalore) containing 20 billion cfu per gram of *Saccharomyces cerevisiae*

Mannan oligosaccharide (Varsha Group, Bangalore) containing 50 per cent mannans and glucans

Nicomix AB_2D_3K (Nicholas Piramal

Table 1. Ingredient composition of experimental starter rations, %

Ingredients	Starter rations			
	T_1	T_2	T_3	T_4
Yellow maize, kg	69.80	69.80	69.80	69.80
Soyabean meal, kg	28.00	28.00	28.00	28.00
Dicalcium phosphate, kg	0.90	0.90	0.90	0.90
Calcite, kg	0.80	0.80	0.80	0.80
Salt, kg	0.50	0.50	0.50	0.50
Total	100	100	100	100
To 100 kg of the above mixture were added				
Bioyeast, g	-	200	-	100
Mannan oligosaccharide, g	-	-	200	100
Nicomix AB_2D_3K [1], g	25	25	25	25
Nicomix BE [2], g	25	25	25	25
Zinc Oxide, g	75	75	75	75

Table 2. Ingredient composition of experimental finisher rations, %

Ingredients	Finisher rations			
	T_1	T_2	T_3	T_4
Yellow maize, kg	75.30	75.30	75.30	75.30
Soyabean meal, kg	22.50	22.50	22.50	22.50
Dicalcium phosphate, kg	0.90	0.90	0.90	0.90
Calcite, kg	0.80	0.80	0.80	0.80
Salt, kg	0.50	0.50	0.50	0.50
Total	100	100	100	100
To 100 kg of the above mixture were added				
Bioyeast, g	-	200	-	100
Mannan oligosaccharide, g	-	-	200	100
Nicomix AB_2D_3K, g	25	25	25	25
Nicomix BE, g	25	25	25	25
Zinc Oxide, g	75	75	75	75

India Ltd, Mumbai) containing Vitamin A - 82,500 IU, Vitamin B_2 - 50 mg, Vitamin D_3 - 12,000 IU and Vitamin K - 10 mg, per gram.Nicomix BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B_1 - 4 mg, Vitamin B_6 - 8 mg, Vitamin B_{12} - 40 mg, Niacin - 60 mg, Calcium pantothenate - 40 mg and Vitamin E - 40 mg, per gram.

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Slaughter studies

On attaining the slaughter weight of 70 kg, five animals from each group were slaughtered at Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy to study the carcass characteristics and dressing percentage. The head was removed at the atlanto-occipital joint and weight of the carcass without head was recorded, to determine the dressing percentage of the hot carcass (carcass weight / live weight). Carcass length was measured from the anterior edge of aitch bone (os-sacrum) to the anterior edge of first rib. The back fat

thickness was estimated as the measurement of subcutaneous fat with skin at the level of tenth rib, at a perpendicular point 3/4th the length of longissimus dorsi muscle. The loin eye area or area of longissimus dorsi muscle at the tenth intercostal space was cut and traced on a transparent paper and the area was measured. Weight of internal organs such as lungs, liver, spleen, kidney, heart, stomach and intestine was recorded and was expressed as percentage of body weight.

Plasma biochemical studies

Blood samples were collected from five animals from each group during the time of slaughter using sodium citrate as anticoagulant. Plasma was separated immediately after centrifugation at 3000 rpm for 10 minutes and analyzed for Phosphorus (Bernhart and Wreath, 1955) (phosphomolybdate method), total cholesterol (Lie *et al*., 1976) (CHOD-PAP Method, Agappe Diagnostics), HDL cholesterol (Haar *et al.*, 1978) (DGKC, SCE, Kinetic Method, Agappe Diagnostics), using standard kits. Plasma minerals such as Ca, P and Mg were also estimated using Atomic Absorption Spectrophotometer (Perkin – Elmer Model Pinnacle 900H).

Faecal microbial population

Fresh faecal samples were collected randomly towards the end of feeding trial from animals belonging to the four dietary treatment groups and subjected to microbiological analysis on the same day of collection. Nine grams of samples were homogenized in 90 milliliter of phosphate buffered saline (PBS) and this form the initial test sample. Total Viable Count (TVC) of all samples was estimated by pour plate technique, as described by Morton (2001). From the selected tenfold dilution of each sample, one milliliter of inoculum was transferred into duplicate petridishes of uniform size. To each of the inoculated plates about 10-15 milliliter sterile molten standard plate count agar (HiMedia) maintained at 45^{0}C was poured and mixed with inoculum by gentle rotary movement. The inoculated plates were left at room temperature and allowed to solidify and were incubated at 37^{0}C for 24h. At the end of incubation, plates showing colonies between 30 and 300 were selected and counts were taken with the help of a colony counter. The number of colony forming units (cfu) per mg/ml of sample was calculated by multiplying the mean colony count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu/g or ml. Coliform count per milliliter of samples was estimated according to the procedure described by Kornacki and Johnson (2001). From the selected tenfold dilution, 0.1 milliliter of inoculum was inoculated onto duplicate plates of Violet

Red Bile Agar (VRBA) (HiMedia) and was uniformly distributed with a sterile 'L' shaped glass rod. The inoculated plate was incubated at 37^0C for 24h. At the end of incubation, purplish red colonies with a diameter of at least 0.5 mm, surrounded by a reddish precipitation zone were counted as coliforms. The number of organisms was estimated by multiplying the mean count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu/g or ml

Statistical analysis

Data collected on various parameters were statistically analyzed by ONE WAY ANOVA method as described by Snedecor and Cochran (1994). Means were compared by Duncan Multiple Range Test (DMRT) using Statistical Package for Social Studies (SPSS. 17.0.1v, 2008) software.

RESULTS AND DISCUSSION

Carcass characteristics

Data on carcass weight, carcass length, loin eye area, back fat thickness and dressing percentage in pigs reared on the four dietary treatments are shown in Table 3.

Sekar (2003) observed an average carcass length of 23.66, 24.09 and 24.37inch respectively for control, 0.25 and 0.5 per cent probiotic (Baker's yeast) supplemented group of crossbred pigs and concluded that the carcass length was not affected by dietary supplementation of probiotic as observed in the present study. Similar results were also reported by Czech *et al*. (2009) in prebiotic (8 g per pig per day) supplemented growing pigs. The carcass weight of pigs maintained on four dietary treatments was also similar. Sreeparvathy (2011) reported that when pigs were fed diet supplemented with spent brewer's yeast at 0, 2.5 and 5 per cent levels, the carcass weight were similar (P>0.05) with values 52.17, 53.00 and 52.17 kg respectively, and the reported values were closer to those obtained in the present study. In agreement with the results of present

Table 3. Carcass characteristics of pigs maintained on four dietary treatments (Mean±SE)

Parameter	Treatments				
	T1	T2	T3	T4	P value
Live weight, kg	75.60 ± 2.38	73.30 ± 3.28	76.70 ± 2.41	75.50 ± 1.39	0.80
Carcass weight, kg	54.44 ± 2.24	50.42 ± 2.84	54.03 ± 1.71	51.91 ± 1.85	0.55
Dressing percentage	71.87 ± 1.12	68.76 ± 2.00	70.49 ± 2.93	68.82 ± 5.61	0.55
Carcass length, inch	25.11 ± 1.42	24.11 ± 0.99	25.39 ± 0.79	24.67 ± 0.35	0.13
Loin eye area, cm^2	26.75 ± 2.52	25.80 ± 1.27	25.60 ± 3.20	27.80 ± 1.70	0.42
Back fat thickness, cm	4.70 ± 0.22	4.62 ± 0.24	4.62 ± 0.25	3.92 ± 0.14	0.07

study, Rekiel *et al*. (2007) also observed no difference (P>0.05) in the carcass weight of pigs fed diet supplemented with prebiotic (0.1 per cent MOS) and those fed basal diet. The values of back fat thickness of pigs maintained on four dietary treatments were also similar (p>0.05). Similar results were earlier reported (Rekiel *et al.*, 2005) in fatteners supplemented probiotic (*Pediococcus acidilactici*) at 0.1 per cent level. Prebiotic supplementation also did not affect the back fat thickness in this study. Similar observations were noted earlier (Rekiel *et al.*, 2007) in pigs fed diet supplemented with prebiotic and those fed control diet (2.26 vs 2.28 cm). However, Czech *et al.* (2009) reported a reduction in back fat thickness (2.26 vs 2.10 cm) on supplementation of prebiotic at 0.25 per cent level in growing pigs. The loin eye area of the pigs maintained on four dietary treatments were similar (P>0.05) in accordance with the earlier observations (Grela *et al*., 2001; Rekiel *et al.*, 2005; Sreeparvathy, 2011) after supplementation of probiotic. Rekiel *et al.* (2007) reported statistically similar values of loin eye area (49.2 vs 52.5 cm^2) for pigs fed diet supplemented with prebiotic (0.2 per cent MOS) compared to those fed control diet in agreement with the results obtained in the current study. From the data on carcass characteristics it could be seen that the dressing percentage were statistically similar in all the groups as observed in earlier studies in pigs supplemented with probiotics (Grela *et al*., 2001; Rekiel *et al.* 2005; Sreeparvathy, 2011). Rekiel *et al.* (2007) reported no improvement in dressing percentage of pigs fed diet supplemented prebiotic (0.1 per cent MOS) over those fed basal diet (76.1 vs 75.9 per cent). However, Datt *et al.* (2011) reported higher dressing percentage in pigs fed diet supplemented with 0.075 per cent of complex probiotic containing *Saccharomyces cerevisiae* (1.5 x 10^8 cfu per gram) and *Lactobaccilus sporogens* (5 x 10^7 cfu per gram)

The data on weight of internal organs such as heart, lungs, liver, kidney, spleen, stomach and intestine are expressed as percentage of live weight (Table 4). Statistical analysis revealed that there was no difference (P>0.05) among the animals of the four dietary treatments with regard to any of the organ weight studied. Sreeparvathy (2011) also reported similar (P>0.05) weight of internal organs in pigs fed diet supplemented with probiotic on comparison with those fed basal diet. Similar results were also reported by Krause *et al*. (2010), who found similar weights of internal organs such as liver, stomach and spleen in weanling pigs supplemented with prebiotics. The supplementation of synbiotic also had no effect on weights of liver, spleen and stomach in pigs as reported earlier (Krause *et al*., 2010).

Table 4. Weight of internal organs as percentage of live weight of pigs maintained on four dietary treatments of feed additives (Mean±SE)

Parameter	Treatments				
	T_1	T_2	T_3	T_4	P value
Heart	0.25 ± 0.03	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.55
Lungs	0.54 ± 0.60	0.61 ± 0.09	0.55 ± 0.03	0.68 ± 0.07	0.44
Liver	1.62 ± 0.08	1.65 ± 0.07	1.52 ± 0.03	1.49 ± 0.03	0.17
Kidney	0.27 ± 0.03	0.29 ± 0.02	0.24 ± 0.01	0.26 ± 0.01	0.35
Spleen	0.19 ± 0.01	0.21 ± 0.02	0.36 ± 0.19	0.24 ± 0.01	0.61
Stomach and intestine	12.90 ± 2.13	7.78 ± 0.30	11.36 ± 2.43	8.61 ± 0.34	0.44

Table 5. Concentration of minerals, protein and lipids in plasma of cross bred pigs maintained on four dietary treatments of feed additives (Mean ± S.E)

Parameter	Treatments				
	T_1	T_2	T_3	T_4	P value
Calcium, mg/dl	10.27 ± 0.20	10.26 ± 0.13	9.87 ± 0.36	10.34 ± 0.25	0.46
Phosphorus, mg/dl	5.57 ± 0.83	6.74 ± 0.68	7.00 ± 0.71	6.37 ± 0.28	0.55
Magnesium, mg/dl	2.34 ± 0.05	2.36 ± 0.05	2.33 ± 0.03	2.34 ± 0.05	0.97
Total Protein, g/dl	7.55 ± 0.16	8.07 ± 0.20	7.67 ± 0.23	6.42 ± 0.08	0.41
Total cholesterol, mg/dl	106.53 ± 0.96	113.88 ± 0.25	116.04 ± 0.83	122.60 ± 0.47	0.08
HDL cholesterol, mg/dl	43.70 ± 1.38	44.38 ± 0.70	45.49 ±0.80	48.30 ±.19	0.06
LDL cholesterol, mg/dl	62.83 ±0.87	69.50 ± 0.62	70.55 ± 1.13	74.30 ± 0.29	0.06
Triglycerides, mg/dl	29.21 ± 0.29	32.36 ± 1.59	33.32 ± 0.99	34.28 ± 2.27	0.13

Plasma biochemical parameters

Average values of minerals (Ca, P and Mg), total protein and lipids in plasma of pigs during slaughter were similar in all the groups and are given in Table 5.

Similar values were also reported by Rossi *et al*. (2009) who found no significant difference in plasma Ca (2.29 vs 2.29 m mol/l) and P (2.65 vs 2.98 m mol/l) concentrations in pigs fed diet supplemented with probiotic (0.15 per cent *S. cerevisiae*) and control group. Piva *et al*. (2005) also reported similar (P>0.05) concentration of plasma Ca (2.42 vs 2.64 m mol/l) and total protein (61.2 vs 63.2 g/l) in pigs fed diet supplemented with synbiotic (Lactitol + Lactic acid bacteria) on comparison with those fed basal diet. It has been reported (Holdsworth *et al*., 1991) that yeast supplementation inhibited liver cholesterol synthesis and lowered the plasma total cholesterol level in rats. This effect is attributed to compounds such as pantethine and nicotinamide riboside, which inhibit cholesterol synthesis by inhibiting incorporation of acetyl groups into non-saponifiable lipid. However, in the current study, no difference in plasma cholesterol concentrations were noted between the pigs

fed diet supplemented with probiotic and those fed basal diet. Similar to the present result, Rekiel *et al*. (2007), reported no significant difference in total cholesterol (2.26 vs 2.08 m mol/l) and triglyceride (0.42 vs 0.41 m mol/l) concentrations when prebiotic was supplemented at 0.1 per cent level to growing and finishing pigs. Similar results were also obtained by Czech *et al*. (2009) in pregnant sows, where no difference (P>0.05) in plasma cholesterol (1.39 vs 1.43 m mol/l) and HDL cholesterol (0.68 vs 0.68 m mol/l) concentrations were reported when pigs were fed diet supplemented with prebiotic. Piva *et al.* (2005) also noted statistically similar values for plasma total cholesterol in pigs fed diet supplemented with synbiotic (Lactitol + Lactic acid bacteria) and those fed basal diet. However, Liong *et al.* (2007) observed that supplementation of synbiotic (*L. acidophilus* ATCC 4962 1 g + fructose oligosaccharide 1.25 g + mannitol 1.56 g and inulin) at a level of 2.20 g per day in hypercholesterolaemic pigs reduced plasma total cholesterol, which is in contrast with the findings of current study. The variations in the results could be due to factors such as the breed of animals selected, feed offered, the quality and quantity of probiotic/ prebiotic/synbiotic used and genetic make-up of the animals (Chen *et al*., 2009).

Faecal microflora population

From the data on faecal microbial count presented in Table 6, it could be seen that total viable count and coliform count was lower (P<0.05) for pigs fed diet supplemented with synbiotic than that of other treatment groups. Moreover, the faecal microbial count was not affected by supplementation of probiotic or prebiotic alone.

Li *et al*. (2006) observed no change in gut microbial population upon supplementation of yeast at 0.2 per cent level; whereas, van Heugten *et al*. (2003) reported a reduction in total bacterial count in pigs fed diet supplemented with yeast (1.6×10^7 cfu of *Saccharomyces cerevisiae* SC47 per gram of feed). The proposed mode of action for yeast is to alter gut micro flora by selectively stimulating growth of

Table 6. Faecal microbial count of pigs maintained on four dietary treatments of feed additives (Mean±SE)

Parameter	Treatments				
	T1	T2	T3	T4	P value
Total viable count	9.72^{b}± 1.55	9.53^{b}± 1.65	9.62^{b} ± 1.75	8.87^{a} ± 1.77	0.03*
Coliforms	6.45^{b}± 1.47	6.21^{b}± 1.04	6.30^{b}± 0.70	5.93^{a} ± 1.32	0.01*

Bacterial numbers are expressed as $\log_{10}$ colony forming units per gram

a, b- Means with different superscripts within the same row differ significantly *(p<0.05)

beneficial bacteria while suppressing the growth of pathogenic bacteria (Rekiel *et al*., 2007). But, lack of consistent response to yeast supplementation may be due to several factors such as type of yeast product, dosage level and age of animals (Lessard *et al*., 2009). It was also suggested that the degree of response to yeast supplementation was inversely related to the general well- being of the experimental animals and the response to yeast supplementation under farm conditions may be greater than those occurring in a research station environment, where environment is less stressful (Bontempo *et al*., 2006). This principle might also apply to observations of the present study. The result of the present study confirmed the earlier observation of Suryanarayanan *et al*. (2013), who found no significant change in total count and coliform count after supplementation of prebiotic (1.0 per cent fructo-oligosaccharide). On the contrary, Rekiel *et al*. (2007) reported lowered gut population of *Enterobacteriaceae* family in prebiotic (0.1 per cent MOS) supplemented group as compared to control. Synbiotic supplementation in the current study had significantly reduced the faecal total viable count and coliform count. Similarly, Suryanarayanan *et al*. (2013) also noted a reduction in total viable count and coliform count in pigs fed diet supplemented with synbiotic (0.1 per cent *Saccharomyces cerevisiae* + 1 per cent fructose oligosaccharide). On the other hand, Lee *et al*. (2009) observed no significant difference in faecal bacterial count upon synbiotic (yeast + MOS, lactose, sodium acetate and ammonium citrate) supplementation. Synbiotics helps to decrease the number of harmful bacteria in the intestine and it also aid in the adhesion of beneficial bacteria to the lumen by decreasing the intestinal pH (Underdahl *et al*., 1982).

CONCLUSION

The results of the study indicated that all the four dietary treatments were similar (P>0.05) with regard to plasma biochemical profile and carcass characteristics. However, the animals fed diet supplemented with synbiotic could alter the faecal micro flora as indicated by reduction in total viable count and coliform count. Results concluded that synbiotic at 0.2 per cent level (0.1 per cent yeast + 0.1 per cent MOS) can be used as beneficial feed additive for maintaining healthy gut microflora in crossbred pigs.

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