Research Article

Effect of Dietary Supplementation of Certain Probiotics on Hematological Parameters in Growing Pigs

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Abstract

The present study was undertaken to evaluate the effect of probiotics (*Lactobacillus acidophilus* and *Saccharomyces* cerevisiae) individually and in combination on hematological parameters in growing pigs. Twenty four pigs (75% LWY x 25% DESI), 170 days of age, weighing about 36.1±0.6 kg body weight (BW) were selected and randomly divided into 4 groups of 6 pigs per pen. They were fed with different diets: Basal diet (T1), Basal diet + *Lactobacillus acidophilus* (T2), Basal diet + *Saccharomyces cerevisa*e (T3) and Basal diet + *Lactobacillus acidophilus* + *Saccharomyces cerevis*ae (T4), In this experiment blood samples were collected at fortnight intervals for a period of 60 days. At the end of the experiment various experimental diets in the present study i.e., T2, T3 and T4 elicited non-significant ($p<0.05$) difference in total leucocyte count ($x10^3$/µl), total erythrocyte count ($x10^6$/µl), hemoglobin (g/dl), hematocrit (%) and lymphocyte count (%) compared with the control diet T1 indicating that the feed and feed supplementation with probiotics has no toxic effect. The current study demonstrates that the mixture of bacteria and yeast viz., *L. acidophilus* and *S. cerevisiae* has the potential to be used as a probiotic dietary supplement in growing pigs.

Keywords: Hematology; Lactobacillus; Saccharomyces; Probiotics; Pigs.

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Introduction

Pork is a very important source of animal protein. It is the most widely consumed meat in the world accounting for 38% of meat production worldwide. Pork is nutritionally rich and palatable human food containing 17% protein and 24% fat. The antibiotics were earlier used as efficient growth promoters in pork production but their use is now condemned due to the adverse effects of antibiotic residues in food products in the development of resistant bacteria. Probiotics, which are live microbial feed supplements, have received attention as suitable alternatives to antibiotics to promote growth in the pig industry. They selectively stimulate the composition, growth and activity of gut microflora thus ultimately improving the growth performance of the animal.

Materials and Methods

The Experimental protocols describing the management and care of animals were reviewed and approved by the animal ethical committee of Sri Venkateswara Veterinary University, Tirupati.

Source of probiotic

The probiotics used in T2, T3 and T4 diets Viz., *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were procured from AVA BIOTECH, Hyderabad as gratis.

Experimental design and diets

Animals of haematological study were categorized into four groups with three dietary treatments consisting of six pigs in each group. The first group is the control group (T1) fed on a basal diet. In the remaining three groups basal diet was supplemented with probiotic organisms through water as described below during the experimental period of 60 days.

Table 1 Experimental design

Groups	Diets	Number of Animals
Group-I (T1)	Basal diet	6
Group-II (T2)	Basal diet +*Lactobacillus acidophilus*, (1x 10^9 CFU/g) at 0.1%	6
Group-III (T3)	Basal diet + *Saccharomyces cerevisiae*,(1x 10^9CFU/g) at 0.1%	6
Group- IV (T4)	Basal diet + *Lactobacillus acidophilus* (1x 10^9 CFU/g) at 0.1% + *Saccharomyces cerevisiae*, (1x 10^9 CFU/g) at 0.1%	6

Table 2 Ingredients and chemical composition of basal diet

Ingredients	Kg/100 Kg
Maize	60.0
Soybean meal	24.0
DORB	14.0
Mineral mixture (Agrimin)	1.4*
Salt	0.5
Lysine	0.1
Total	100
Chemical composition	%
Dry matter	92.5
Organic matter	91.2
Crude protein	18.2
Crude fibre	7.9
Ether extract	2.6
Nitrogen free extract	62.5
Total carbohydrates	70.3
Total Ash	8.9
Acid insoluble ash	5.4
Calcium	0.74
Phosphorous	1.35
DE kcal/kg	3100

*composition of mineral mixture for kg: cobolt-150 mg, copper-1200 mg, iodine-325 mg, iron-5000 mg, magnesium-6000 mg, manganese-1500 mg, potassium-100 mg, sodium-5.9 mg, sulphur-0.922%, zinc-9600 mg, DL-methionine-1920 mg, L-lysine-4400 mg, calcium-24% and phosphorous- 12%.

Methods

Blood sample collection, transport and storage

Blood samples were collected at fortnight intervals during the experimental period from ear vein of pigs in 4 ml K3E (EDTA) BD Vacutainer tubes for whole blood. Immediately after collection, the samples were labeled and transported to the laboratory in an ice packed container and hematological parameters were assayed immediately.

Hematological parameters

Different hematological parameters such as Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Hemoglobin (Hb) content, Packed Cell Volume (PCV) and Lymphocyte Count (LC) were estimated by using Unitron Bio-medical Auto Hematology Analyzer Fx-19T at Department of Veterinary Physiology, College of Veterinary Science, Tirupati.

Statistical analysis

The data were analyzed using General Linear Model procedure of statistical package for social sciences (SPSS) 15th version and comparison of means was tested using Duncan's multiple range test [1] and significance was considered at 1% and 5% level of significance ($P<0.01$ and $P<0.05$).

Results

Total leucocyte count (x 10^3/µl)

The effect of supplementation of different probiotics on total leucocyte count (x 10^3/µl) at the end of fortnight duration in growing pigs were presented in Table 3.

It could be observed from the table 11 that, total leucocyte count in growing pigs of treatments T2, T3 and T4 at 0^{th}day were 20.50 ± 0.61, 19.17 ± 0.76 and 20.50 ± 0.69 respectively. Whereas the total leucocyte count of growing pigs in treatments T2, T3 and T4 at 60^{th} day were 19.52 ± 0.91, 19.78 ± 0.94 and 19.73 ± 0.96 respectively. Total leucocyte count was not altered significantly among different treatments (T2, T3 and T4) when compared with control (T1).

Table 3 Effect of supplementation of different probiotics on WBC (x10^3/µl) in growing pigs

DAYS	Treatments (n=6)			
	T1	T2	T3	T4
0	20.13±0.49	20.50±0.61	19.17±0.76	20.50±0.69
15	19.55±0.67	19.75±0.80	20.58±0.76	19.58±0.69
30	19.45±0.65	19.45±1.04	19.35±0.69	19.02±0.80
45	19.43±0.73	19.62±0.83	20.08±0.95	19.58±1.20
60	19.83±0.96	19.52±0.91	19.78±0.94	19.73±0.96

Total erythrocyte count(x10^6/µl)

The effect of supplementation of different probiotics on total erythrocyte count (x10^6/µl) at the end of fortnight duration in growing pigs were presented in Table 4.

It could be observed from the Table 12 that, total erythrocyte count in growing pigs of treatments T2, T3 and T4 at 0^{th} day were 6.67 ± 0.18, 6.76 ± 0.17 and 6.91 ± 0.10 respectively. Whereas the total erythrocyte count of growing pigs in treatments T2, T3 and T4 at 60^{th} day were 6.93 ± 0.09, 6.85 ± 0.13and 6.90 ± 0.10 respectively. Total erythrocyte count was not significantly altered among different treatments (T2, T3 and T4) when compared with control (T1).

Table 4 Effect of supplementation of different probiotics on RBC(x10^6/µl) in growing pigs

DAYS	Treatments (n=6)			
	T1	T2	T3	T4
0	6.72±0.18	6.67±0.18	6.76±0.17	6.91±0.10
15	6.93±0.15	6.90±0.17	6.83±0.16	7.09±0.13
30	6.74±0.06	6.81±0.08	6.88±0.13	6.76±0.09
45	6.70±0.10	6.77±0.11	6.89±0.11	6.85±0.07
60	6.78±0.09	6.93±0.09	6.85±0.13	6.90±0.10

Hemoglobin (g/dl)

The effects of supplementation of different probiotics on hemoglobin (g/dl) at the end of fortnight duration in growing pigs were presented in Table 5.It could be observed from the data that, hemoglobin concentration in growing pigs of treatments T2, T3 and T4 at 0^{th} day were 11.70 ± 0.72, 11.93 ± 0.37 and 11.87 ± 0.48 respectively. Whereas the hemoglobin concentration of growing pigs in treatments T2, T3 and T4 at 60^{th} day were 11.37 ± 0.42, 11.62 ± 0.53 and 11.63 ± 0.49 respectively. There was no significant difference in the levels of hemoglobin among different treated groups (T2, T3 and T4) when compared with control (T1).

Table 5 Effect of supplementation of different probiotics on Hb (g/dl) in growing pigs

DAYS	Treatments (n=6)			
	T1	T2	T3	T4
0	11.65±0.44	11.70±0.72	11.93±0.37	11.87±0.48
15	11.95±0.33	11.63±0.38	11.68±0.48	11.68±0.50
30	11.48±0.61	11.47±0.28	11.60±0.46	11.59±0.49
45	11.52±0.42	11.39±0.63	11.58±0.52	11.47±0.64
60	11.65±0.44	11.37±0.42	11.62±0.53	11.63±0.49

Hematocrit (PCV) (%)

The effect of supplementation of different probiotics on hematocrit value (%) at the end of fortnight duration in growing pigs were presented in Table 6. It could be observed from the results that, hematocrit values in growing pigs of treated groups T2, T3 and T4 at 0^{th} day were 38.38 ± 0.64, 37.86 ± 0.55 and 36.32 ± 0.59 respectively. Whereas the hematocrit values of growing pigs in treatments T2, T3 and T4 at 60^{th} day were 37.08 ± 0.60, 38.62 ± 0.72 and 41.26 ± 0.49 respectively. Hematocrit values were not significantly different among treatments (T2, T3 and T4) when compared to control (T1).

Table 6 Effect of supplementation of different probiotics on hematocrit value (%) in growing pigs

DAYS	Treatments (n=6)			
	T1	T2	T3	T4
0	36.74±0.48	38.38±0.64	37.86±0.55	36.32±0.59
15	39.25±0.80	40.32±0.83	38.36±0.73	36.86±0.57
30	40.2±0.96	36.82±0.51	39.32±0.69	39.02±0.75
45	41.08±0.64	42.72±0.59	38.93±0.74	40.08±0.56
60	42.24±0.56	37.08±0.60	38.62±0.72	41.26±0.49

Lymphocyte count (%)

The effect of supplementation of different probiotics on lymphocyte count (%) at the end of fortnight duration in growing pigs were presented in Table 7. The data showed lymphocyte count in growing pigs of treatments T2, T3 and T4 at 0^{th} day were 45.98 ± 0.64, 48.4 ± 0.71 and 46.92 ± 0.57 respectively. Whereas the lymphocyte count of growing pigs in treated groups T2, T3 and T4 at 60^{th} day were 43.76 ± 0.59, 45.86 ± 0.65 and 44.08 ± 0.61 respectively. There was no significant difference in lymphocyte count among different treatments (T2, T3 and T4) when compared with control (T1).

Table 7 Effect of supplementation of different probiotics on lymphocyte (%) in growing pigs

DAYS	Treatments (n=6)			
	T1	T2	T3	T4
0	43.28±0.58	45.98±0.64	48.4±0.71	46.92±0.57
15	45.32±0.83	43.88±0.72	44.96±0.79	44.03±0.45
30	46.28±0.81	46.48±0.71	43.86±0.67	45.26±0.47
45	47.52±0.61	46.26±0.67	45.48±0.70	46.84±0.55
60	46.42±0.94	43.76±0.59	45.86±0.65	44.08±0.61

Discussion

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease [2]. Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment [3]. Haematological parameters are good indicators of the physiological status of animals [4]. As reported by [5] animals with good blood composition are likely to show good performance. Haematological components, which consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals [6].

Various experimental diets in the present study i.e., T2, T3 and T4 at the end of the experimental period elicited non-significant difference in total leucocyte count ($x10^3$/µl), total erythrocyte count ($x10^6$/µl), hemoglobin (g/dl), hematocrit (%) and lymphocyte count (%) compared with the control diet T1 indicating that the feed and feed supplementation with probiotics has no toxic effect.

These results are in agreement with [7] who proved that *Bacillus* based probiotic supplementation in finishing pigs had no influence on WBC count. [8] also revealed no significant differences ($P > 0.05$) in red blood cell (RBC) count and hemoglobin among treatment groups supplemented with *Lactobacillus* based probiotics in the diet of nursery pigs.

The findings of [9] showed non- significant ($P > 0.05$) variation in WBC, RBC and hemoglobin values with probiotic-supplemented diet in growing finishing pigs

[10] and [11] investigated the effect of dietary treatment with probiotics on RBC, WBC and Lymphocyte count revealed no significant difference ($P > 0.05$) among the treatment groups.

The results of [12] and [13] showed that Haematocrit (PVC), Haemoglobin (Hb) and Total number of erythrocyte count (RBC) values did not differ significantly with probiotic treatment and the variations observed were between the physiological limits depending on species and age.

It implies that the probiotics supplements used at the given dose as mono-strain or multi-strain combination in the present study have no adverse effect on the health of growing pigs as reflected by the non-significant variation in haematological parameters which are commonly used as indices of health status in livestock species.

Conclusion

Blood samples were collected at fortnight intervals and the hematological parameters namely WBC, RBC, Hb, PCV and Lymphocyte count showed no significance difference among groups supplemented with different experimental diets T2, T3 and T4 compared with control diet (T1).

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