Research Article

Effect of Dietary Supplementation of Certain Probiotics on Serum Biochemical 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 serum biochemical 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 experimental period significant ($p<0.05$) difference in total protein, albumin and glucose among various experimental diets i.e T2, T3 and T4 compared with control diet (T1) and Serum biochemical parameters namely ALT, AST, creatinine and cholesterol also did not reveal any significant difference among various experimental diets i.e T2, T3 and T4 when compared with control diet (T1)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: Serum Biochemistry; 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 world wide. 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 serum biochemistry 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

During the experimental period of 60 days, blood samples were collected at fortnight intervals for evaluating glucose, total protein, albumin, ALT, AST, creatinine and cholesterol.

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 Vacutainer with clot activator tubes for serum collection. Immediately after collection, the samples were labeled and transported to the laboratory in an ice packed container and Serum vials were stored at -20°C until further analysis.

Blood glucose

BD Vacutainer™ fluoride tubes were used to collect blood samples for glucose determination. Glucose was estimated using diagnostic kits supplied by BioSystems Diagnostics Pvt. Ltd., India. The results were expressed in mg/dl.

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

Glucose (mg/dl)

The effects of supplementation of different probiotics on glucose (mg/dl) concentration at the end of fortnight duration in growing pigs were presented in Table 3..The levels of glucose concentration in growing pigs of treatments T2, T3 and T4 at 0th day were 70.0 ± 0.97, 70.67 ± 1.86 and 71.67 ± 1.23 respectively, whereas the glucose concentration of growing pigs in treatments T2, T3 and T4 at 60th day were 79.00 ± 1.73, 72.50 ± 1.34 and 87.33 ± 1.71 respectively. Among all the treatments, T4 showed highest glucose concentration (87.33 ± 1.71) at the end of the experiment while T2 and T3 showed 79.00 ± 1.73and 72.50 ± 1.34 respectively. The results showed a significant (P<0.01) difference in glucose concentration at end of the experiment among treatments (T2 and T4) compared when compared with control (T1).

Table 3 Effect of supplementation of different probiotics on glucose (mg/dl) levels in growing pigs.

DAYS	Treatments			
	T1	T2	T3	T4
0	71.17^{a}±1.30	70.0^{a}±0.97	70.67^{a}±1.86	71.67^{a}±1.23
15	71.17^{a}±1.40	79.0^{bc}±0.97	74.50^{ab}±1.57	85.50^{c}±1.57
30	69.17^{a}±1.78	79.33^{b}±0.88	76.00^{ab}±1.41	85.50^{c}±1.96
45	70.17^{a}±1.89	79.67^{b}±2.04	75.00^{ab}±1.89	85.00^{c}±1.53
60	69.33^{a}±1.36	79.00^{b}±1.73	72.50^{a}±1.34	87.33^{c}±1.71

^{abc}Means bearing different superscripts in a row differ significantly **(p<0.01), n=6

Serum total protein (g/dl)

The effect of supplementation of different probiotics on serum total protein (g/dl) concentration at the end of fortnight duration in growing pigs were presented in Table 4. It could be observed from the table that, total protein concentration in growing pigs of treatments T2, T3 and T4 at 0th day were 7.42 ± 0.12, 7.45 ± 0.11 and 7.60 ± 0.12 respectively. Whereas the total protein concentration of growing pigs in treatments T2, T3 and T4 at 60th day were 8.00 ± 0.10, 7.78 ± 0.06 and 8.65 ± 0.08 respectively.

Among treated groups, T4 showed highest total protein concentration (8.65 ± 0.08) at the end of the experiment while T2 and T3 showed 8.00 ± 0.10 and 7.78 ± 0.06 respectively. There was significant (P<0.01) difference among total protein concentration at the end of the experiment treatments (T2 and T4) compared to control (T1).

Table 4 Effect of supplementation of different probiotics on total protein (g/dl) levels in growing pigs

Days	Treatments			
	T1	T2	T3	T4
0	7.35^{a}±0.14	7.42^{a}±0.12	7.45^{a}±0.11	7.60^{a}±0.12
15	7.57^{a}±0.11	7.98^{ab}±0.06	7.68^{a}±0.06	8.47^{b}±0.04
30	7.32^{a}±0.13	7.98^{b}±0.06	7.70^{ab}±0.05	8.32^{c}±0.09
45	7.60^{a}±0.12	7.95^{a}±0.06	7.75^{ab}±0.04	8.58^{b}±0.06
60	7.53^{a}±0.13	8.00^{b}±0.10	7.78^{ab}±0.06	8.65^{c}±0.08

n=6, ^{ab}Means bearing different superscripts in a row differ significantly **(P<0.01),

Albumin (g/dl)

The effect of supplementation of different probiotics on albumin (g/dl) concentration at the end of fortnight duration in growing pigs were presented in Table 5. It could be observed from the table that, albumin concentration in growing pigs of treatments T2, T3 and T4 at 0th day were 2.78 ± 0.06, 2.82 ± 0.09 and 2.65 ± 0.06 respectively. Whereas the albumin concentration of growing pigs in treatments T2, T3 and T4 at 60th day were 2.98 ± 0.08, 3.34 ± 0.13 and 3.82 ± 0.12 respectively. Among the treatments, T4 has highest albumin concentration (3.82 ± 0.12) at the end of the

experiment while that of T2 and T3 was 2.98±0.08and 3.34 ± 0.13 respectively. There was significant (P<0.01) difference among albumin concentration at end of the experiment in treatments (T3 and T4) compared to treatment T1 (control).

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

Days	Treatments			
	T1	T2	T3	T4
0	2.96^{a}±0.08	2.78^{a}±0.06	2.82^{a}±0.09	2.65^{a}±0.06
15	2.59^{a}±0.06	2.7^{a}±0.07	3.26^{b}±0.12	3.71^{c}±0.11
30	2.92^{a}±0.07	3.06^{a}±0.11	3.32^{ab}±0.11	3.63^{b}±0.13
45	2.86^{a}±0.10	3.1^{a}±0.12	3.56^{b}±0.14	3.85^{b}±0.14
60	2.74^{a}±0.11	2.98^{ab}±0.08	3.34^{b}±0.13	3.82^{c}±0.12

^{ab}Means bearing different superscripts in a row differ significantly **($p<0.01$), n=6

Creatinine (mg/dl)

The effect of supplementation of different probiotics on creatinine (mg/dl) at the end of fortnight duration in growing pigs were presented in Table 6. It was observed from the results that, creatinine concentration in growing pigs of treatments T2, T3 and T4 at 0^{th} day were 1.41 ± 0.04, 1.35 ± 0.04 and 1.32 ± 0.03 respectively, while the creatinine concentration of growing pigs in treatments T2, T3 and T4 at 60^{th} day were 1.34 ± 0.04, 1.36 ± 0.04 and 1.37 ± 0.04 respectively. Creatinine concentration was not significantly different among treatments (T2, T3 and T4) and control (T1).

Table 6 Effect of supplementation of different probiotics on creatinine (mg/dl) levels in growing pigs

Days	Treatments (n=6)			
	T1	T2	T3	T4
0	1.30±0.04	1.41±0.04	1.35±0.04	1.32±0.03
15	1.37±0.03	1.33±0.04	1.34±0.02	1.39±0.05
30	1.35±0.04	1.33±0.04	1.35±0.02	1.39±0.04
45	1.37±0.04	1.36±0.05	1.38±0.04	1.40±0.05
60	1.36±0.04	1.34±0.04	1.36±0.04	1.37±0.04

Alanine aminotransferase (ALT) (IU/L)

The effect of supplementation of different probiotics on ALT (IU/l) activity at the end of fortnight duration in growing pigs were presented in Table 7. It could be observed from the table that, ALT activity in growing pigs of treatments T2, T3 and T4 at 0^{th} day were 61.6 ± 0.43, 60.8 ± 0.30 and 64.2 ± 0.66 respectively, while that of growing pigs in treatments T2, T3 and T4 at 60^{th} day were 62.4 ± 0.56, 61.4 ± 0.70 and 59.8 ± 0.55 respectively. ALT activity was not significantly different among treatments (T2, T3 and T4) and control (T1).

Table 7 Effect of supplementation of different probiotics on alanine aminotransferase (IU/L) activity in growing pigs

Days	Treatments (n=6)			
	T1	T2	T3	T4
0	63.3±0.33	61.6±0.43	60.8±0.30	64.2±0.66
15	62.3±0.65	63.2±0.42	63.8±0.79	62.8±0.33
30	64.4±0.65	59.6±0.55	62.6±0.43	60.4±0.56
45	62.8±0.58	64.6±0.54	60.8±0.55	63.2±0.33
60	61.8±0.63	62.4±0.56	61.4±0.70	59.8±0.55

Aspartate aminotransferase (IU/L)

The effect of supplementation of different probiotics on AST (IU/L) at the end of fortnight duration in growing pigs were presented in Table 8. The data presented showed AST activity in growing pigs of treatments T2, T3 and T4 at 0^{th} day were 50.8 ± 0.56, 51.6 ± 0.83 and 50.2 ± 0.66 respectively, while that of growing pigs in treatments T2, T3 and

T4 at 60th day were 54.8 ± 0.61, 52.6 ± 0.64 and 51.4 ± 0.60 respectively.AST activity was not significantly different among treatments (T2, T3 and T4) and control (T1).

Total cholesterol (mg/dl)

The effect of supplementation of different probiotics on total cholesterol (mg/dl) at the end of fortnight duration in growing pigs were presented in Table 9. The levels of total cholesterol in growing pigs of treatments T2, T3 and T4 at 0th day were 88 ± 1.10, 87 ± 1.08 and 86 ± 1.09 respectively, while that of growing pigs in treatments T2, T3 and T4 at 60th day were 82 ± 1.52, 92 ± 0.97 and 91 ± 0.97 respectively. The data presented showed that total cholesterol was not significantly altered among treatments (T2, T3 and T4) and control (T1).

Table 8 Effect of supplementation of different probiotics on aspartate aminotransferase (IU/L) activity in growing pigs

Days	Treatments (n=6)			
	T1	T2	T3	T4
0	49.8±0.65	50.8±0.56	51.6±0.83	50.2±0.66
15	53.6±0.76	53.6±0.48	55.4±0.74	55.6±0.54
30	52.8±0.50	54.2±0.52	53.2±0.68	51.8±0.51
45	52.6±0.44	50.4±0.48	50.8±0.52	53.4±0.48
60	52.3±0.57	54.8±0.61	52.6±0.64	51.4±0.60

Table 9 Effect of supplementation of different probiotics on total cholesterol (mg/dl) concentration in growing pigs.

Days	Treatments (n=6)			
	T1	T2	T3	T4
0	87±1.23	88±1.10	87±1.08	86±1.09
15	95±0.85	92±0.93	94±0.94	91±0.94
30	86±1.41	85±1.61	89±1.26	87±1.12
45	89±0.82	90±0.97	87±1.15	91±0.95
60	88±1.31	82±1.52	92±0.97	91±0.97

Discussion

Serum biochemistry refers to the chemical analysis of blood serum. Testing for these various substances provides information about the organs and tissues in the body as well as the metabolic state of the animal.

Glucose

Various experimental diets i.e., T2, T3 and T4 at the end of the experimental period elicited significant ($p<0.01$) difference in Glucose (mg/dl) concentrations compared with the control diet T1.

Probiotic supplementation effect in consume more average daily feed intake, its helping efficient digestion and absorption of carbohydrates from gastro intestinal tract and probably an improvement in intestinal morphology could be the reason for improved digestion and nutrient utilization resulting in elevated glucose levels in probiotic diets T2, T3 and more in T4 at the end of the experiment period.

The results are in agreement with [2] who obtained significantly higher ($P \leq 0.001$, $P \leq 0.05$) glucose levels in growing-finishing pigs with *Bacillus* spp. supplemented diets at 6 and 16 weeks.

Total Protein and Albumin

Various experimental diets i.e., T2, T3 and T4 at the end of the experimental period elicited significant ($P < 0.01$) increase in total protein (g/dl) and albumin (g/dl) concentrations compared with the control diet

Elevated total protein and albumin concentrations indicate more absorption of amino acids and peptides from gastro intestinal tract because of improved digestion by probiotics. It also indicates more protein synthesis in liver and its concentration may reflect the hepatic protein metabolic status in animals. The increased levels of total protein in the present study indicate improved protein status of the pigs due to probiotic supplementation.

The results are in agreement with [3]. who found that addition of yeast (*Saccharomyces cerevisiae*) at 1.5, 2 and 2.5% levels respectively were responsible for a significant ($P < 0.05$) increase of total serum protein levels. [4] explained that lactic acid and proteolytic enzymes produced by *L. plantarum* and various proteases produced by *B.*

subtilis interact to enhance the digestion and assimilation of dietary protein. Supplementation of a mixture of *L. plantarum* and *B. subtilis* resulted in elevated serum total protein concentration in piglets compared with any other treatments [5].

Hence the elevated total protein concentration with selected probiotic supplementation in the present study elucidated the positive nitrogen balance ultimately resulting in enhanced growth in growing pigs.

Creatinine

Creatinine is a by-product of muscle metabolism and it is excreted entirely by the kidney. Increased levels of creatinine indicate decreased kidney function.

Various experimental diets i.e., T2, T3 and T4 at the end of the experimental period elicited non-significant difference in creatinine (mg/dl) concentrations compared with the control diet T1 indicating that the probiotic treatment individually and in combination has no effect on the kidney function. Our findings are in agreement with that of the findings of [6] who reported that dietary supplementation of *L. acidophilus* had no significant impact on creatinine concentration.

ALT, AST and Cholesterol

There are many different liver enzymes, but the two that appear in most profiles are alanine aminotransferase (ALT), and aspartate aminotransferase (AST). They are typically found when the cells of the liver are stressed or damaged. Cholesterol is produced in the liver as part of fat metabolism. Increases in cholesterol are associated with hormonal and metabolic diseases, liver disease, and serious kidney disease.

Various experimental diets i.e., T2, T3 and T4 at the end of the experimental period elicited non-significant difference in ALT (IU/L), AST (IU/L) and cholesterol (mg/dl) concentrations compared with the control diet T1.

[7] demonstrated that the activities of ALT and AST were normal and near to control in probiotic-treated animals, thus indicating that probiotics had no side effects on the animal health. Any abnormal increase in serum levels of AST may imply liver damage. Therefore, relatively stable levels of AST may be associated with hepato-protective effects of the yeast probiotic.

The supplementation of *Lactobacillus brevis* ZLB004 increased serum total protein concentrations ($P = 0.044$) compared with control diet and no difference in the levels of ALT, AST, total cholesterol [8]. Similarly, [9] showed that serum chemistry parameters such as total Cholesterol, AST and ALT were not affected by dietary treatments with probiotics ($P > 0.05$).

Conclusion

Serum biochemical parameters namely ALT, AST, creatinine and cholesterol also did not reveal any significant difference among various experimental diets i.e T2, T3 and T4 when compared with control diet (T1) indicating that the feed and feed supplementation with probiotics has no toxic effect. There were significant ($p<0.05$) differences in total protein, albumin and glucose among various experimental diets i.e T2, T3 and T4 compared with control diet (T1) which could be due to the increased absorption from gastro intestinal tract because of improved digestion and nutrient utilization by probiotics. Present study revealed supplementation of probiotics in growing pigs elicited beneficial effects as revealed by improved growth performance in growing pigs with a positive economic effect on pig industry.

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