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## Effect of feeding probiotics with and without milk powder on incidence and severity of piglet diarrhoea

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### Abstract

A study was conducted to evaluate the effect of feeding Probiotics on the incidence and severity of diarrhoea in piglets under different feeding treatments viz., T<sub>0</sub> (fed conventional creep feed), T<sub>1</sub> (fed 5% milk powder supplemented creep feed), T<sub>2</sub> (fed probiotics supplemented creep feed added at manufacturer's recommended rate) and T<sub>3</sub> (fed creep feed supplemented with 5% milk powder and probiotics as per manufacturer's recommendation). The overall incidence rate (%) of piglet diarrhoea was highest in T<sub>0</sub> group and then gradually decreased in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> group. The average values of diarrhoea scores indicated that the severity of diarrhoea was promptly by the supplementation of Probiotics in T<sub>2</sub> and T<sub>3</sub> groups and the piglets fully recovered by 14<sup>th</sup> day followed by T<sub>1</sub> on 29<sup>th</sup> day while the control T<sub>0</sub> group suffered up to 45<sup>th</sup> day and then recovered. The occurrence and severity of diarrhoea was reduced in the experiment indicating the effectiveness of the used Probiotics.

**Keywords:** Probiotics, lactose, probios, prebiotics, piglets, diarrhoea

### 1. Introduction

Pig rearing forms a very important component of animal husbandry in India, holding the potential to meet the food and nutrition demand of the fast-growing population in the country as pig is the most potential livestock species for meat production and efficient feed converter after the broilers. Efficient piglet rearing right from their birth undoubtedly is an important aspect of overall successful pig farming.

The post-weaning growth of pigs is closely related to their pre-weaning health status. While piglets are exposed to many stressors immediately after birth, gastrointestinal problems are among the most severe. When the basal liquid milk diet is reduced and the stage where-in the piglets are offered with solid creep feed from the 10<sup>th</sup> day after birth, the digestive physiology changes where in the intervention of different feed additives is needed to have the maximum nutrient utilization [1]. For decades, sub-therapeutic levels of antibiotics have been used as growth promoters to combat the problems caused by GI infections [2]. However, the indiscriminate use of antimicrobials has been associated to the development of antimicrobial resistance and the ban of growth-promoting antibiotics throughout the EU since 1<sup>st</sup> January 2006 have triggered increased interest in alternative ways to promote the health of production animals, including pigs [3, 4]. Probiotics, sometimes used interchangeably with the term direct fed microbials (DFM), are gaining acceptance as potential alternatives to antibiotics to improve production efficiency [5]. A definition adopted by FAO/WHO (2001) [6] states that "Probiotics are mono or mixed cultures of live organisms which when administered in adequate amounts confer a health benefit to the host." Probiotics may contain one or more strains of microorganisms and may be given either alone or in combination with other additives in feed or water [7]. Probiotics have "GRAS" (generally regarded as safe) status [8]. The species commonly used in probiotics products for pigs include *Lactobacillus acidophilus*, *L. reuteri*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. brevis*, *L. delbreuckii* subsp. *bulgaricus*, *Lactococcus lactis*, *Streptococcus salivarius* subsp. *thermophilus*, *Enterococcus faecalis*, *E. faecium*, *Bacillus licheniformis*, *B. subtilis*, *Bifidobacterium bifidum*, *B. pseudolongum*, *B.*

*thermophilus*, *Clostridium butyricum*, and yeasts like *Saccharomyces* spp. [9]. Probiotics help establish a microenvironment in the gut that favours beneficial microorganisms and reduces the colonization of pathogenic bacteria by: (1) creating a hostile environment for harmful bacteria species (through production of lactic acid, SCFA, and reduction in pH); (2) competing for nutrients with undesired bacteria; (3) production and secretion of antibacterial substances (e.g. bacteriocins by *Lactobacillus*, *Bacillus* spp.); and (4) inhibition of bacterial adherence and translocation [10-15].

Probiotics are most effective in animals during microflora development or when microflora stability is impaired [16]. Therefore, it is suggested that the effects of probiotics appear to be more consistent and positive in piglets rather than in growing finishing pigs [17]. On farms with a high incidence of diarrhoeal disease, it may be appropriate to introduce a probiotics strain as early as possible to colonize the digestive tract with probiotic strains that inhibit pathogens, reduce piglet diarrhoea and reduce pre-weaning mortality [18]. In the suckling piglets, acid secretion is low and the principal source of acidity is bacterial fermentation of lactose from sows' milk to lactic acid. A number of possible solutions have been tested with varying degrees of success such as addition of lactose in dry feeds or, in water [19]. The milk sugar lactose has been proposed to have prebiotic properties in certain situations [20, 21], and many gastrointestinal *Lactobacillus* spp. and *Streptococcus* spp. are known to utilize lactose by readily converting it to lactic acid [22]. Prebiotics are non-digestive food / feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the digestive tract and thus attempt to improve host health [23, 24]. Nonfat dried skim milk (DSM) contains 50% lactose [25].

Pollmann *et al.* (1980) [26] suggested that pigs receiving lactose in combination with the *Lactobacillus* inoculums had the best average daily gain in comparison with other groups. Considering the above facts and views, the present research work of feeding probiotics with or without milk powder supplemented creep feed in pre-weaned piglets was undertaken to study its effect in diarrhoea incidence and severity.

## 2. Materials and methods

The feeding trial of the piglets was carried out at the 30-sow Teaching Unit of the Department of Livestock Production and Management while laboratory analysis of feed and blood samples were done respectively in the Department of Animal Nutrition and Department of Animal Physiology as well as T.V.C.C., College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, India. The animal experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India.

### 2.1 Selection of animal and treatment allocation

Four Hampshire litters from sows of similar parity were selected for the study. Seven healthy piglets of uniform size and body weight from each litter were finally selected for feeding trial and recording of experimental data. The seven piglets of particular litter constituted one group and thus four treatment groups were formed. Each group of the experimental piglets was randomly assigned to one of the four treatments as in the following treatment schedule.

**Table 1:** Group wise feeding treatment of piglets

Treatment group	No. of piglet	Average initial body weight (kg)	Experimental feed for the treatment groups
T <sub>0</sub> (Control)	7	2.280	Conventional farm feed
T <sub>1</sub> (Treatment-1)	7	2.314	Conventional farm feed + 5% supplementation of milk powder (SAGAR BRAND)
T <sub>2</sub> (Treatment-2)	7	2.315	Conventional farm feed+ probiotics (PROBIOS <sup>R</sup> from VETS PLUS, Inc and CHR. Hansen) as per manufacturer's recommendation
T <sub>3</sub> (Treatment-3)	7	2.300	Conventional farm feed+ 5% milk powder+ probiotics as per manufacturer's recommendation

### 2.2 Housing

The piglets together with their mothers were sheltered in conventional farrowing pens till weaning at 8 weeks of age. The floor of the pens was cement concrete and floor area was of 9 sq. meters with an equal floor area in the outdoor run.

### 2.3 Feeding and management

All the experimental piglets were raised entirely on their dams' milk from birth to 7 days of age. From day-8 onward, the piglets of T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups were offered respective experimental feed twice daily as per the feeding schedule up to weaning at 56 days of age. The piglets were separated from

their sows daily in the morning and evening for a period of 1-2 hours following suckling and offered feed in the outdoor run and fed to appetite. The piglets were provided clean wholesome water ad libitum round the clock. Other day to day routine managerial tasks like cleaning of pens, disinfection of floors, washing of feeding and water troughs etc. were strictly followed. The compositions of the ration, milk powder and probiotics used for the piglets are as shown below in the Tables 2. The proximate analysis of the feed samples was done as per methods described in AOAC (Association of Official Analytical Chemists, 1990) [27].

**Table 2:** Composition of farm ration, milk powder (Sagar brand) and Probiotics (Probios).

Farm ration (%)		Milk powder(Per 100gm)		Probiotics(Viable lactic acid bacteria/ gm)	
DM (basal feed)	95	Energy(Kcal)	366	<i>Enterococcus faecium</i> , <i>Lactobacillus acidophilus</i> , <i>L. casei</i> and <i>L. plantarum</i>	10 million CFU
DM(left over feed)	92	Total fat(gm)	1.5		
CP	22.1	Total carbohydrate (gm)	52		
EE	5.06	Protein(gm)	35		
CF	4.75	Sodium(mg)	549		
Total ash	7.75	Calcium(mg)	1200		
NFE	60.34				

## 2.4 Recording the incidence rate of diarrhoea.

The weekly percent incidence rates of diarrhoea in each treatment group were calculated as:

$$\text{Incidence rate} = \frac{\text{Nos. of new cases}}{\text{Piglets-days at risk}} \times 100$$

Where, piglets – days at risk = [(nos. of piglets at risk at the beginning of the time interval+ nos. of piglets at risk at the end of the time interval)/2] × (nos. of time unit in the time intervals).

## 2.5 Recording the severity of diarrhoea by scoring.

The number of piglets affected by diarrhoea in each group was recorded daily. Diarrhoea was assessed visually and scores were given from 0-3 based on the consistency of the faeces, according to the score: 0-pellet faeces; 1-semi pellet faeces; 2- soft faeces; and 3-watery faeces. This observation was followed using the method described by Loh *et al.* (2002) [28] and Dowarah *et al.* (2016) [29].

## 3. Results and Discussion

The data for diarrhoea incidence rate (%) and diarrhoea score were depicted in Table 3 and 4 and further illustrated in Fig 1. and 2.

The average values of incidence rate (%) were 4.49, 3.87, 2.97 and 1.17 percent in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> group respectively. The highest incidence rate in different treatment groups were as 12.24 % at 6<sup>th</sup> week in T<sub>0</sub>, 9.52% at 2<sup>nd</sup> week in T<sub>1</sub>, 14.00% at 2<sup>nd</sup> week in T<sub>2</sub> and 3.57% at 3<sup>rd</sup> week in T<sub>3</sub> groups. Likewise, the lowest percentage of 0.00% which indicated nil or no diarrhoea in a particular group was found at 5<sup>th</sup> week in T<sub>3</sub>, 6<sup>th</sup> week in T<sub>2</sub> and 8<sup>th</sup> week both in T<sub>0</sub> and T<sub>1</sub> groups.

The average values of diarrhoea incidence (%) indicated that the incidence rate was lowest in probiotics cum milk powder supplemented group (T<sub>3</sub>) followed by probiotics (T<sub>2</sub>), milk powder (T<sub>1</sub>) and control (T<sub>0</sub>) without any supplement. The findings indicated the beneficial effect of probiotics which helped to stabilize the helpful intestinal microbiota, shedding of *E. coli* organisms in faeces resulting in efficient digestion and utilization of feed nutrients. Addition of milk powder containing lactose might further helped the existing

*Lactobacillus* population or *Lactobacillus* contained in the probiotics to multiply and work in a better way (Wells *et al.*, 2004) [30]. Higher incidence of diarrhoea rate in the beginning of the experiment in the treatment groups might be due to adaptive change to the supplements for a few days. However, it is found that probiotics helped to subside diarrhoea in the T<sub>3</sub> and T<sub>2</sub> groups early (5<sup>th</sup> and 6<sup>th</sup> week respectively) which lasted up to 8<sup>th</sup> week in T<sub>1</sub> and T<sub>0</sub> groups receiving no probiotics.

The data of table 4 depicted that diarrhoeal scores were 0.29±0.29, 0.29±0.29, 2.29±0.18 and 0.86±0.40 at day-0 and 1.00±0.00, 0.24±0.24, 1.22±0.46 and 0.86±0.34 at day-3 in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. The highest score were as 1.71±0.42 at 7<sup>th</sup> day in T<sub>0</sub>, 2.14±0.04 at 7<sup>th</sup> day in T<sub>1</sub>, 1.22±0.46 at 3<sup>rd</sup> day in T<sub>2</sub> and 1.14±0.26 at 7<sup>th</sup> day in T<sub>3</sub> groups. On the other hand, 0.00±0.00 score indicating nil or no diarrhoea were found at 14<sup>th</sup> day both in T<sub>2</sub> and T<sub>3</sub> groups, at 29<sup>th</sup> day in T<sub>1</sub> group and 45<sup>th</sup> day in T<sub>0</sub> group. Analysis of variance of the data (Table 5) revealed significant effect of treatment on diarrhoeal score at 7<sup>th</sup> and 29<sup>th</sup> day. The findings were further illustrated in fig 1. And 2.

From the findings it was evident that probiotics promptly dropped diarrhoea scores in the high incidence T<sub>2</sub> group (from 2.29±0.18 in day-0 to 1.22±0.46 in day 3), followed by T<sub>1</sub> and T<sub>3</sub> groups. Probiotics helped to arrest diarrhoea as early as 14<sup>th</sup> day in T<sub>2</sub> and T<sub>3</sub>, followed by milk powder at 29<sup>th</sup> day in T<sub>1</sub> while control group piglets suffered from diarrhoea upto 45 days. This might be due anti-diarrhoeal property of probiotics by stabilizing intestinal micro biota, competitive expulsion of diarrhoea causing bacteria like *E. coli* etc. The lactose of milk powder might have helped the existing helpful bacterial micro flora by acting as a substrate resulting in slight arrest of diarrhoea in the respective groups. The present findings are in agreement with the findings of Wells *et al.* (2004) [30], Venkatachalapathy *et al.* (2013) [31] and Dowarah *et al.* (2016) [29] who found beneficial effect of different probiotics preparations in controlling diarrhoea in piglets. However, Lahtinen *et al.* (2015) [32] observed mild diarrhoea in the adaptation period in some piglets in his feeding trial of multispecies *Lactobacillus* in piglets. Pustal *et al.* (2015) [33] from his work reported no significant difference ( $P>0.05$ ) in diarrhoea occurrence in milk supplemented piglets from the control.

**Table 3:** Incidence rate of diarrhoea in the piglets

Groups	Incidence rate (%)							
	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week	7 <sup>th</sup> week	8 <sup>th</sup> week	Average
T <sub>0</sub>	2.60	3.57	2.04	7.79	12.24	3.17	0.00	4.49
T <sub>1</sub>	9.52	4.76	2.38	2.85	4.76	2.86	0.00	3.87
T <sub>2</sub>	14.00	2.60	2.04	2.20	0.00	0.00	0.00	2.97
T <sub>3</sub>	2.60	3.57	2.04	0.00	0.00	0.00	0.00	1.17

**Table 4:** Average diarrhoea scores of piglets of different treatment group

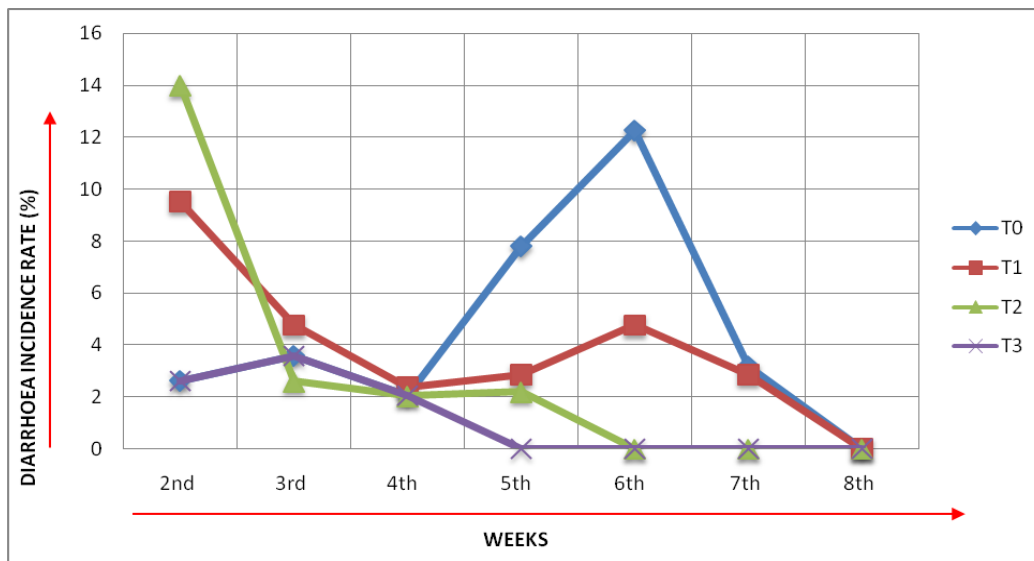
Period (day)	Treatment				P value
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
0	0.29 <sup>a</sup> ±0.29	0.29 <sup>a</sup> ±0.29	2.29 <sup>b</sup> ±0.18	0.86 <sup>a</sup> ±0.40	<0.001
3	1.00±0.00	0.24±0.24	1.22±0.46	0.86±0.34	0.272
7	1.71 <sup>b</sup> ±0.42	2.14 <sup>b</sup> ±0.40	0.57 <sup>a</sup> ±0.29	1.14 <sup>ab</sup> ±0.26	0.024
14	0.00±0.00	0.57±0.37	0.00±0.00	0.00±0.00	0.043
21	0.00±0.00	0.24±0.18	0.00±0.00	0.00±0.00	0.093
29	1.43 <sup>b</sup> ±0.37	0.00 <sup>a</sup> ±0.00	0.00 <sup>a</sup> ±0.00	0.00 <sup>a</sup> ±0.00	<0.001
37	0.43±0.20	0.00±0.00	0.00±0.00	0.00±0.00	0.157
45	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-

( $P<0.05$ )

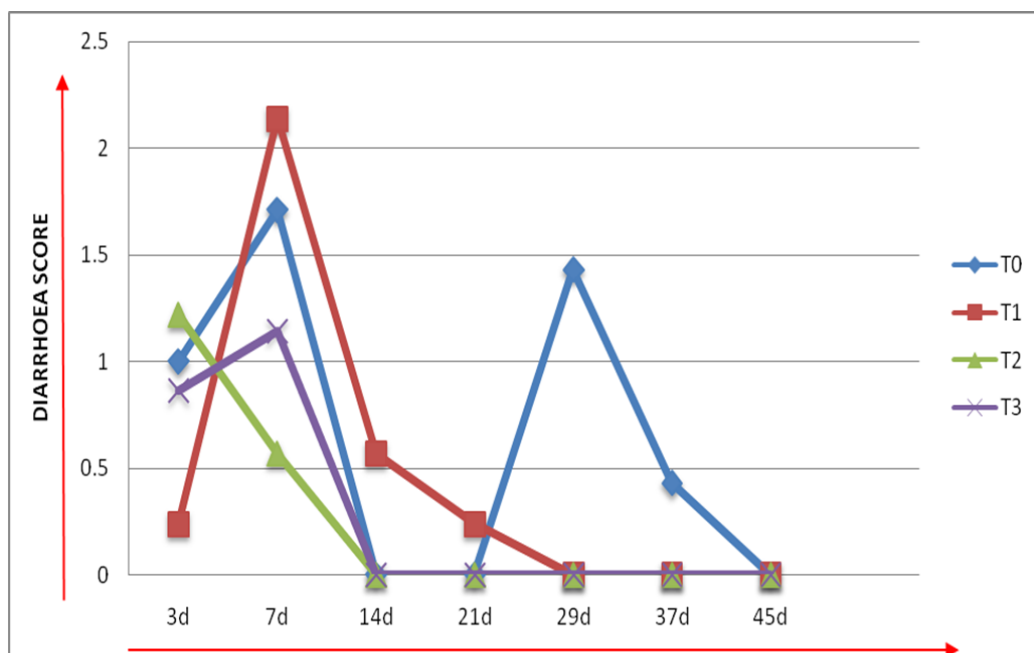
Treatment means having atleast one common superscript in a row do not differ significantly.

**Table 5:** Analysis of variance of diarrhea scores of piglets of different treatment groups

Period (day)	Source of variation	SS	d.f.	MS	F Value	P Value
0-d	Between Groups	18.714	3	6.238	9.887	0.000
	Within Groups	15.143	24	0.631		
3-d	Between Groups	2.964	3	0.988	1.383	0.272
	Within Groups	17.143	24	0.714		
7-d	Between Groups	9.821	3	3.274	3.767	0.024
	Within Groups	20.857	24	0.869		
14-d	Between Groups	1.714	3	0.571	2.400	0.093
	Within Groups	5.714	24	0.238		
21-d	Between Groups	0.429	3	0.143	2.400	0.093
	Within Groups	1.429	24	0.060		
29-d	Between Groups	10.714	3	3.571	15.000	0.000
	Within Groups	5.714	24	0.238		
37-d	Between Groups	1.286	3	0.429	1.895	0.157
	Within Groups	5.429	24	0.226		
45-d	Between Groups	0.000	3	0.000		
	Within Groups	0.000	24	0.000		



**Fig 1:** Incidence rate of diarrhoea (%) of piglets of different treatment groups



**Fig 2:** Diarrhoea score of piglets of different treatment groups

**4. Conclusion**

The Probiotics used in the study has shown its effect in suppressing the occurrence of diarrhoea and the effect was

enhanced when combined with milk powder. Also the diarrhoea score showed that the severity was also reduced in piglets' groups' supplemented probiotics both with and

without milk powder. The overall incidence rate of piglet diarrhoea was highest in T<sub>0</sub> group and then gradually decreased in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> group with corresponding values of 4.49, 3.87, 2.97 and 1.17 percent in the respective group. On the other hand, the average values of diarrhoea scores indicated that though there were incidence of diarrhoea in all the groups initially at 0-day, severity of diarrhoea was promptly reduced by the supplementation of probiotics in T<sub>2</sub> and T<sub>3</sub> groups and the piglets fully recovered by 14<sup>th</sup> day followed by T<sub>1</sub> on 29<sup>th</sup> day while the control T<sub>0</sub> group suffered up to 45<sup>th</sup> day and then recovered. The present findings indicated that feeding of probiotics helps to control the occurrence and severity of diarrhoea in piglets. However more comprehensive study with more no. of litter in a group is needed for proper sampling and assessing more accurate results.

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