サンザイム
Sanzyme Biologics

SPORICH™-total

In-Vitro Studies

SANZYME BIOLOGICS (P) LTD
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CIN : U24110TG2016PTC112002

Factory Unit I: Door No: 7-4-115, Sy. No: 258 & 259, Gaganpahad, Bangalore Road, Hyderabad – 500052.,Telangana, India

Factory Unit II: Plot Nos. 19 to 22, Sy. No's. 321/1,11,12,13 & 276 & 277, Biotech Park, Phase - III, Genome Valley, Karkapatla (V), Markook (Mdl.), Siddipet (Dist.), Pin Code : 502 281,Telangana, India

QUALITY CONTROL DEPERTMENT

Acid Tolerance Studies

Product: SPORICH TOTAL

Strain: *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB

Objective: To determine Acid tolerance assay of SPORICH TOTAL

Initial Potency for Study: 3000 CFU/ml

- **Acid Tolerance & Recovery Assay Protocol:**
  1. Prepare appropriate buffers as glycine HCl (Gly-HCl, pH 2.2 and 3.0) and Phosphate Buffered Saline (PBS, pH 7.0)
  2. Use overnight grown SPORICH TOTAL *(*~12 hour) culture, wash it thrice with PBS
  3. This washed and diluted culture is further diluted at 1:10 dilution in appropriate buffer at pH 2.0, 3.0 and 7. 0
  4. Measure Optical Density at 600 nm in a spectrophotometer at every hour for 6 hours starting from '0' hour
  5. At the same time, for each hour sample subject it for assay by taking 500 µl of sample, pour plate with Nutrient agar medium in triplicates, and incubate at 37^0C for 24 hours.
  6. Keep the culture sample prepared in normal saline without acid/buffer treatment as control.
  7. Below assay protocol can be adopted for pour plating.
  8. Plating:
     A. *Bacillus licheniformis* & *Bacillus subtilis*
        a) Set out sterile petri plates and add 1 ml of control and heat treated final dilution into each petri plate and then pour 15-20 ml molten Nutrient Agar (NA) medium into each of the petri plate and mix thoroughly (temperature around 45 °C)
        b) Incubate the solidified plates in an inverted position at 37 °C for 24 hours.

     B. *Bacillus coagulans*
        c) Set out sterile petri plates and add 1 ml of control and heat shocked (at 75 ^{0}C for 30 minutes) final dilution into each petri plate and then pour 15-20 ml molten

PNY medium into each of the petri plate and mix thoroughly (temperature around 45 °C)

d) Incubate the solidified plates in an inverted position at 37 °C for 48 hours.

C. *Saccharomyces boulardii*

e) Set out five sterile petri plates and add 1 ml of final dilution into each petri plate and then pour 15 ml of Sabouraud Chloramphenicol Agar into each of the petri plate and mix thoroughly.(temperature around 45°C)

f) The solidified plates are incubated in an inverted position at 25°C (± 2)°C for 5 days.

9. Counting:

a) Count the number of CFU in each petri plate and calculate by taking the average number of CFU.

10. Calculation:

$$\text{Viable Count} = \frac{\text{Average No. of CFU x Dilution factor}}{\text{Weight of sample taken x } 10^9} = \text{Billion CFU /g}$$

*Note: Perform all the dilutions in the vicinity of Uni directional Air Flow Unit
*Note: Vortex all the serial dilutions, while performing the assay.

- Media Composition:
  - Nutrient Agar : Hi media Code: M001
  - PNY Medium : Hi media Code: M835
  - Sabouraud Chloramphenicol Agar Composition (SCA) (Himedia code: M1067)
- Preparation of sterile Isotonic Saline Solution
  - Weigh accurately about 9 g of Sodium Chloride in 1000 ml of distilled water. The solution shall be sterilized with steam at 1.5 kg/cm2 pressure at 121°C (or 15 lbs pressure) for 20 min and then cool.
- SB Buffer Preparation and Composition:

Ingredients	Grams / Liter
Potassium Dihydrogen Phosphate (KH_2PO_4)	1.5
Di-sodium hydrogen orthophosphate (Na_2HPO_4)	9
Mycological Peptone	10
Sodium Chloride	5
Dissolve the above ingredients in 1000 ml of distilled water.	
Sterilize by autoclaving at 121°C for 15 minutes. pH: 7.2 (± 0.2)	

Result:

Time (Hours)	pH 2.0 (CFU/ml)	% of Recovery	pH 3.0 (CFU/ml)	% of Recovery	pH 7.0 (CFU/ml)	% of Recovery
0	3000	100.00	3000	100.00	3000	100.00
1	2980	99.33	2977	99.23	2960	98.67
2	2976	99.20	2970	99.00	2950	98.33
3	2969	98.97	2964	98.80	2943	98.10
4	2967	98.90	2963	98.77	2934	97.80
5	2960	98..67	2960	98.67	2931	97.70
6	2938	97.93	2957	98.57	2920	97.33

Conclusion:

- SPORICH TOTAL was recovered 97.93% (2938 CFU/ml) at pH 2 and 6 Hours Incubation
- SPORICH TOTAL was recovered 98.57% (2957 CFU/ml) at pH 3 and 6 Hours Incubation
- SPORICH TOTAL was recovered 97.33% (2920 CFU/ml) at pH 7 and 6 Hours Incubation

QUALITY CONTROL DEPERTMENT

Bile Tolerance Studies

Product: SPORICH TOTAL

Strain: *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB

Objective: To determine Bile tolerance of SPORICH TOTAL

Initial Potency for Study: 3000 CFU/ml

- **Bile Tolerance & Recovery Assay Protocol:**
  1. Prepare appropriate bile solutions at concentrations 0.15%, 0.3% and 0.6%
  2. Use overnight grown SPORICH TOTAL (~12 hour) culture, wash it thrice with PBS
  3. This washed and diluted culture is further diluted at 1:10 dilution in appropriate bile solution at 0.15%, 0.3% and 0.6% concentration
  4. Measure Optical Density at 600 nm in a spectrophotometer at every hour for 6 hours starting from '0' hour
  5. At the same time, for each hour sample subject it for assay by taking 500 µl of sample, pour plate with Nutrient agar medium in triplicates, and incubate at 37 ^{0}C for 24 hours.
  6. Keep the culture sample prepared in normal saline without bile solution treatment as control.
  7. Below assay protocol can be adopted for pour plating.
  8. Plating:
     - *A. Bacillus licheniformis & Bacillus subtilis*
       a) Set out sterile petri plates and add 1 ml of control and heat treated final dilution into each petri plate and then pour 15-20 ml molten Nutrient Agar (NA) medium into each of the petri plate and mix thoroughly (temperature around 45 °C)
       b) Incubate the solidified plates in an inverted position at 37 °C for 24 hours.
     - *B. Bacillus coagulans*
       c) Set out sterile petri plates and add 1 ml of control and heat shocked (at 75 ^{0}C for 30 minutes) final dilution into each petri plate and then pour 15-20 ml molten PNY medium into each of the petri plate and mix thoroughly (temperature around 45 °C)
       d) Incubate the solidified plates in an inverted position at 37 °C for 48 hours.
     - *C. Saccharomyces boulardii*
       e) Set out five sterile petri plates and add 1 ml of final dilution into each petri plate and then pour 15 ml of Sabouraud Chloramphenicol Agar into each of the petri plate and mix thoroughly.(temperature around 45°C)

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f) The solidified plates are incubated in an inverted position at 25°C (± 2)°C for 5 days.

9. Counting:
   - Count the number of CFU in each petri plate and calculate by taking the average number of CFU.

10. Calculation:

$$\text{Viable Count} = \frac{\text{Average No. of CFU x Dilution factor}}{\text{Weight of sample taken x } 10^9} = \text{Billion CFU /g}$$

*Note: Perform all the dilutions in the vicinity of Uni directional Air Flow Unit
*Note: Vortex all the serial dilutions, while performing the assay.

- Media Composition:
  - Nutrient Agar : Hi media Code: M001
  - PNY Medium : Hi media Code: M835
  - Sabouraud Chloramphenicol Agar Composition (SCA) (Himedia code: M1067)
- Preparation of sterile Isotonic Saline Solution
  - Weigh accurately about 9 g of Sodium Chloride in 1000 ml of distilled water. The solution shall be sterilized with steam at 1.5 kg/cm2 pressure at 121°C (or 15 lbs pressure) for 20 min and then cool.
- SB Buffer Preparation and Composition:

Ingredients	Grams / Liter
Potassium Dihydrogen Phosphate (KH_2PO_4)	1.5
Di-sodium hydrogen orthophosphate (Na_2HPO_4)	9
Mycological Peptone	10
Sodium Chloride	5
Dissolve the above ingredients in 1000 ml of distilled water.	
Sterilize by autoclaving at 121°C for 15 minutes. pH: 7.2 (± 0.2)	

Result:

Time (Hours)	Bile 0.15 % (CFU/ml)	% of Recovery	Bile 0.3 % (CFU/ml)	% of Recovery	Bile 0.6 % (CFU/ml)	% of Recovery
0	3000	100.00	3000	100.00	3000	100.00
1	2961	98.70	2961	98.70	2943	98.44
2	2950	98.33	2951	98.37	2937	97.90
3	2945	98.17	2942	98.07	2934	97.80
4	2931	97.70	2929	97.63	2929	97.63
5	2927	97.57	2927	97.57	2922	97.40
6	2922	97.40	2909	96.97	2918	97.27

Conclusion:

- SPORICH TOTAL was recovered 97.40% (2922 CFU/ml) at 0.15% Bile concentration and 6 Hours Incubation
- SPORICH TOTAL was recovered 96.97% (2909 CFU/ml) at 0.3% Bile concentration and 6 Hours Incubation
- SPORICH TOTAL was recovered 97.27% (2918 CFU/ml) at 0.6% Bile concentration and 6 Hours Incubation

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QUALITY CONTROL DEPERTMENT

Antibiotic / AGP Compatibility Studies

Product: SPORICH TOTAL

Strain: *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB

Objective: To determine Antibiotic / AGP Compatibility of SPORICH TOTAL with commonly used Antibiotics / AGPs in Poultry Production Systems

Initial Potency for Study: 4 Billion Cells/g

- **Antibiotic Compatibility & Recovery Assay Protocol:**
  1) SPORICH TOTAL formulation was weighed upto100 g in sterile LDPE polythene bags.
  2) Appropriate antibiotic was added as per dosage pattern to the above bags containing SPORICH TOTAL formulation.
  3) The sample was blended thoroughly.
  4) SPORICH TOTAL and Antibiotic blended covers were kept at desired temperature (between 25 °C to 30 °C and RH 60% to 75%) for 15 days.
  5) At periodic time intervals, 1.0 g of samples from above pre-blended formulation bags at 0, 1, 3, 6 hours, were kept for total viable cells assay. Same samples stored at desired conditions were drawn and continued for assay counts after 24 hrs. 7days and 15 days.
  6) The total viable cells count were enumerated by maintaining antibiotic free formulation sample as control.
  7) Tenfold serial dilution technique was adopted in order to enumerate total viable cells.
  8) While conducting enumeration Nutrient agar (*Bacillus subtilis* & *Bacillus licheniformis*), PNY Medium (*Bacillus coagulans*) were used for plating.
  9) After incubation, colony forming units (CFU) were counted and compared against respective controls in order to find the survivability %
  10) Below assay protocol was adopted for pour plating.
  11) Plating:
      A. *Bacillus licheniformis* & *Bacillus subtilis*
         a) Set out sterile petri plates and add 1 ml of control and heat treated final dilution into each petri plate and then pour 15-20 ml molten Nutrient Agar (NA) medium into each of the petri plate and mix thoroughly (temperature around 45 °C)
         b) Incubate the solidified plates in an inverted position at 37 °C for 24 hours.
      B. *Bacillus coagulans*
         c) Set out sterile petri plates and add 1 ml of control and heat shocked (at 75 ^{0}C for 30 minutes) final dilution into each petri plate and then pour 15-20 ml molten

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PNY medium into each of the petri plate and mix thoroughly (temperature around 45 °C)

d) Incubate the solidified plates in an inverted position at 37 °C for 48 hours.

C. *Saccharomyces boulardii*

e) Set out five sterile petri plates and add 1 ml of final dilution into each petri plate and then pour 15 ml of Sabouraud Chloramphenicol Agar into each of the petri plate and mix thoroughly.(temperature around 45°C)

f) The solidified plates are incubated in an inverted position at 25°C (± 2)°C for 5 days.

12) Counting:

a) Count the number of CFU in each petri plate and calculate by taking the average number of CFU.

13) Calculation:

$$\text{Viable Count} = \frac{\text{Average No. of CFU x Dilution factor}}{\text{Weight of sample taken x } 10^9} = \text{Billion CFU /g}$$

*Note: Perform all the dilutions in the vicinity of Uni directional Air Flow Unit

*Note: Vortex all the serial dilutions, while performing the assay.

- Media Composition:
  - Nutrient Agar : Hi media Code: M001
  - PNY Medium : Hi media Code: M835
  - Sabouraud Chloramphenicol Agar Composition (SCA) (Himedia code: M1067)
- Preparation of sterile Isotonic Saline Solution
  - Weigh accurately about 9 g of Sodium Chloride in 1000 ml of distilled water. The solution shall be sterilized with steam at 1.5 kg/cm2 pressure at 121°C (or 15 lbs pressure) for 20 min and then cool.
- SB Buffer Preparation and Composition:

Ingredients	Grams / Liter
Potassium Dihydrogen Phosphate (KH_2PO_4)	1.5
Di-sodium hydrogen orthophosphate (Na_2HPO_4)	9
Mycological Peptone	10
Sodium Chloride	5
Dissolve the above ingredients in 1000 ml of distilled water.	
Sterilize by autoclaving at 121°C for 15 minutes. pH: 7.2 (± 0.2)	

Results:

Time	Control	BMD	CTC	LIN	ZnB	MAD	NEO	TYL
	Potency Recovered (Bn CFU/g)							
0 Hr.	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
1 Hr.	3.99	3.99	3.99	3.99	3.99	3.99	3.99	3.99
3 Hrs.	3.90	3.99	3.99	3.99	3.88	3.94	3.96	3.95
6 Hrs.	3.93	3.99	3.95	3.87	3.81	3.90	3.91	3.93
1 Day	3.84	3.83	3.72	3.76	3.79	3.86	3.84	3.91
7 Days	3.82	3.79	3.68	3.70	3.75	3.83	3.83	3.86
15 Days	3.80	3.78	3.67	3.62	3.72	3.78	3.79	3.80

*BMD ➔ Bacitracin Methyl Disalicylate
*CTC ➔ Chlortetracycline
*LIN ➔ Lincomycin
*ZnB ➔ Zinc Bacitracin
*MAD ➔ Maduramicin
*NEO ➔ Neomycin
*TYL ➔ Tylosin
*Bn ➔ Billion

Conclusions:

- SPORICH TOTAL is compatible with Bacitracin Methylene Disalicylate and 94.50% (3.78 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
- SPORICH TOTAL is compatible with Chlortetracycline and 91.75% (3.67 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
- SPORICH TOTAL is compatible with Lincomycin and 90.50% (3.62 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
- SPORICH TOTAL is compatible with Zinc Bacitracin and 93.00% (3.72 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
- SPORICH TOTAL is compatible with Maduramycin and 94.50% (3.78 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
- SPORICH TOTAL is compatible with Neomycin and 94.75% (3.79 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
- SPORICH TOTAL is compatible with Tylosin and 95.00% (3.80 Billion CFU/g) SPORICH TOTAL was recovered after 15 days
    - SPORICH TOTAL is compatible with with commonly used Antibiotics / AGPs in Poultry Production Systems

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QUALITY CONTROL DEPERTMENT

Antimicrobial Activity Studies

Product: SPORICH TOTAL

Strain: *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB

Objective: To determine Antimicrobial Activity of SPORICH TOTAL

Initial Potency for Study: 4 Billion Cells/g

Standard bacterial Cultures

The below mentioned bacteria were used along with test sample simultaneously to verify the susceptibility zones

S. No	Organism
1	*Salmonella enteritides* ATCC 13076
2	*Clostridium perfringens* ATCC 13124
3	*Escherichia coli* ATCC 8739

Protocol adopted for the experiment:

- One gram of SPORICH TOTAL was inoculated in to nurtient broth and incubated for 24 hours at 37 °C.
- Overnight grown listed standard cultures were taken and spreaded on pre-incubated Nutrient agar and Fluid thioglycolate agar plates
- Wells were made using cork borer, the wells were added with SPORICH TOTAL supernatant, pellet and culture, and plates were incubated for 24 hours at 37 °C.
- Plates were observed for presence/absence of zones around the wells followed by incubation.

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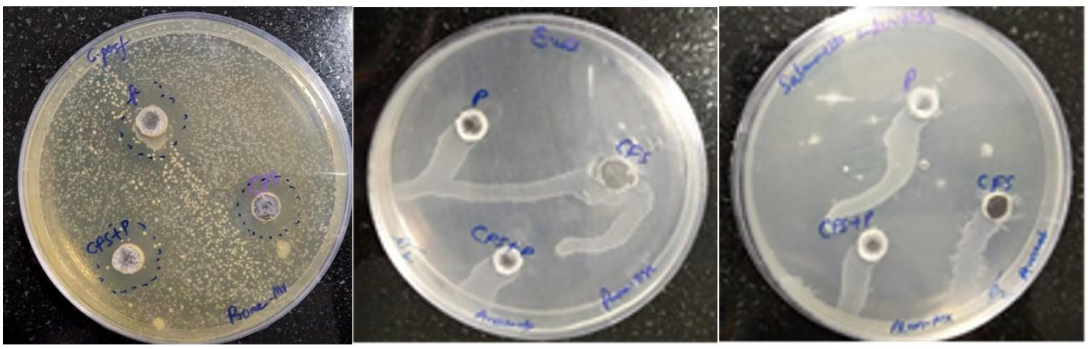
Observations and Results:

Antibacterial activity against	SPORICH TOTAL		
	Pellet	CFS	WB
Salmonella enteridis ATCC 13076	-	+	+
Clostridium perfringens ATCC 13124	+	++	++
Escherichia coli ATCC 8739	+	-	+

*CFS ➔ Cell free supernatant
*WB ➔ Whole broth
*- ➔ Negative
*+ ➔ Positive
*++ ➔ Moderate Zone

Zones of Inhibition against:



Clostridium perfringens *Escherichia coli* *Salmonella enteridis*

Conclusions:

- SPORICH TOTAL demonstrated significant Antimicrobial Activity against *Clostridium perfringens*, *Escherichia coli* & *Salmonella enteridis.*

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QUALITY CONTROL DEPERTMENT

Probiotic Recovery from GIT of Poultry Layer Birds

Product: SPORICH TOTAL

Strain: *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB

Objective: To recover the Probiotic Organisms of SPORICH TOTAL from Different Parts of the Gastro Intestinal Tract of Poultry Layer Birds, fed with SPORICH TOTAL 8 Billion CFU/g @ 100 g/MT of feed from Day 0

Initial Potency for Study: 8 Billion CFU/g

Heat Treatment & Recovery Assay Protocol:

1) Sample Preparation:

   a. Collected the Poultry Layer Bird GIT section cutting in 100 ml sterile normal saline and transferred to QC lab with the help of ice pack within 2 hours from sample collection site.

   b. Added this section cutting part (~10-12 g) to 90 ml of sterile Soyabean Casein Digest Medium and incubated for 24-36 hrs. at 37 °C**.**

2) Heat Shock

   c. Section cutting part with sterile Soyabean Casein Digest Medium post incubation was subjected for heat shock at 75 °C for 30 minutes

   d. Post heat shock, cooled Section cutting part with sterile Soyabean Casein Digest Medium to room temperature followed by tenfold serial dilution taking 9 ml normal saline and 1 ml test solution from post heated Soyabean Casein Digest Medium until we get desired dilution with countable CFU.

3) Plating:

   A. *Bacillus licheniformis* & *Bacillus subtilis*

   e. Set out sterile petri plates and add 1 ml of control and heat treated final dilution into each petri plate and then pour 15-20 ml molten Nutrient Agar (NA) medium into each of the petri plate and mix thoroughly (temperature around 45 °C)

   f. Incubate the solidified plates in an inverted position at 37 °C for 24 hours.

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B. *Bacillus coagulans*

g. Set out sterile petri plates and add 1 ml of control and heat shocked (at 75 °C for 30 minutes) final dilution into each petri plate and then pour 15-20 ml molten PNY medium into each of the petri plate and mix thoroughly (temperature around 45 °C)

h. Incubate the solidified plates in an inverted position at 37 °C for 48 hours.

C. *Saccharomyces boulardii*

i. Set out five sterile petri plates and add 1 ml of final dilution into each petri plate and then pour 15 ml of Sabouraud Chloramphenicol Agar into each of the petri plate and mix thoroughly.(temperature around 45 °C)

j. The solidified plates are incubated in an inverted position at 25 °C (± 2) °C for 5 days.

4) Counting:

k. Count the number of CFU in each petri plate and calculate by taking the average number of CFU.

5) Calculation:

$$\text{Viable Count} = \frac{\text{Average No. of CFU x Dilution factor}}{\text{Weight of sample taken x } 10^9} = \text{Billion CFU /g}$$

*Note: Perform all the dilutions in the vicinity of Uni directional Air Flow Unit
*Note: Vortex all the serial dilutions, while performing the assay.

- Media Composition:
  - Nutrient Agar : Hi media Code: M001
  - PNY Medium : Hi media Code: M835
  - Soyabean Casein Digest Medium, Hi media Code: M011
- Preparation of sterile normal saline
  - Weighed about 9 g of Sodium Chloride dissolved in 100 ml of distilled water and followed by made final volume to 1000 ml with distilled water. The solution was sterilized with steam at 1.5 kg/cm2 pressure at 121°C for 20 min and then cooled.

Results:

Recovery/g of Tissue (Billion CFU)		
S.No.	*GIT-Section*	*Probiotic sp.*
1	Crop	**0.00070**
2	Mid Gut	**0.00112**
3	Hind Gut	**0.00032**
TOTAL		**0.00214**
Extrapolated to Average Total Weight (Billion CFU)		
S.No.	*GIT-Section*	*Probiotic sp.*
1	Crop	0.00434
2	Mid Gut	0.06931
3	Hind Gut	0.00447
TOTAL (Billion CFU)		**0.07812**
Intake - Billion CFU/Bird/Day		*0.0880*
% Recovery		**88.77%**

Conclusions:

- SPORICH TOTAL was recovered from the all segments of the Gastro Intestinal Tract of the Poultry Layer Bird.
- The Percentage Recovery of SPORICH TOTAL from the Gastro Intestinal Tract was upto 89% of the daily intake of the bird.

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Factory Unit II: Plot Nos. 19 to 22, Sy. No's. 321/1,11,12,13 & 276 & 277, Biotech Park, Phase - III, Genome Valley, Karkapatla (V), Markook (Mdl.), Siddipet (Dist.), Pin Code : 502 281,Telangana, India

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เลขประจำตัวผู้เสียภาษี 0994000158378

Room 542, Research Laboratory Unit, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Rd., Siriraj, Bangkoknoi, Bangkok 10700, Thailand

Antimicrobial Resistant Gene Detection Report

Date: 04.02.2020

Name & Address of customer		Sanzyme Biologics Plot No. 13, Sugar Society, Road No.2, Banjara Hills, Hyderabad-500 034	
Sample ID / type		*Bacillus subtilis* San144BS	
Date of sample receipt	22-01-2020	Method of test	Real-time PCR with specific probes
Date of sample testing	23-01-2020 to 03-02-2020	Condition of sample when receive	O.K.

Results

	Antimicrobial classes	Target gene	Results
1	β-lactams	CTX-M1	NF
2	(penicillin,	CTX-M2-M74	NF
3	amoxicillin,	CTX-M8-M25	NF
4	cephalosporin)	CTX-M9	NF
5		PER	NF
6		VEB	NF
7		CMY1-MOX	NF
8		CMY2-LAT	NF
9		DHA	NF
10		FOX	NF
11		ACT-MIR	NF
12		OXA-1	NF
13		OXA-9	NF
14	Carbapenems	KPC	NF
15		GES	NF
16		NDM	NF
17		VIM	NF
18		IMP	NF
19		OXA-48	NF
20	Folate pathway	*sul1*	NF
21	inhibitors	*sul2*	NF
22		*sul3*	NF
23		*dfrA1*	NF
24		*dfrA5-14*	NF
25		*dfrA12*	NF
26		*dfrA17*	NF

	Antimicrobial classes	Target gene	Results
27	Polymyxins	*mcr-1*	NF
28		*mcr-2*	NF
29	Tetracyclines	*tetA*	NF
30		*tetB*	NF
31	Phenicols	*cmlA*	NF
32		*floR*	NF
33		*catA1*	NF
34		*catB3*	NF
35	Aminoglycosides	*aacC1*	NF
36		*aacC2*	NF
37		*aacC4*	NF
38		*aphA1*	NF
39		*aphA6*	NF
40		*aadA1-2-17*	NF
41		*aadB*	NF
42		*armA*	NF
43		*rmtB*	NF
44	Macrolides	*ermB*	NF
45		*mphA*	NF
46	Quinolones	*qnrA*	NF
47		*qnrS*	NF
48		*qnrB1*	NF
49		*qnrB4*	NF
50		*Qep*A	NF

~ 3-8 X10^6 cells was tested. Positive grading criteria: 1+: ≥ 10^1-10^2, 2+: >10^2-10^3 and 3+ = >10^3 positive cells NF = Not found

Tested person: Rattapha Chinli
(Ms. Rattapha Chinli)

Authorized person: Suporn Foongladda
(Assoc.Prof.Dr. Suporn Foongladda)

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Antimicrobial Resistant Gene Detection Report

Date: 04.02.2020

Name & Address of customer		Sanzyme Biologics Plot No. 13, Sugar Society, Road No.2, Banjara Hills, Hyderabad-500 034	
Sample ID / type		*Bacillus coagulans* San135BC	
Date of sample receipt	22-01-2020	Method of test	Real-time PCR with specific probes
Date of sample testing	23-01-2020 to 03-02-2020	Condition of sample when receive	O.K.

Results

	Antimicrobial classes	Target gene	Results
1	β-lactams	CTX-M1	NF
2	(penicillin,	CTX-M2-M74	NF
3	amoxicillin,	CTX-M8-M25	NF
4	cephalosporin)	CTX-M9	NF
5		PER	NF
6		VEB	NF
7		CMY1-MOX	NF
8		CMY2-LAT	NF
9		DHA	NF
10		FOX	NF
11		ACT-MIR	NF
12		OXA-1	NF
13		OXA-9	NF
14	Carbapenems	KPC	NF
15		GES	NF
16		NDM	NF
17		VIM	NF
18		IMP	NF
19		OXA-48	NF
20	Folate pathway	*sul1*	NF
21	inhibitors	*sul2*	NF
22		*sul3*	NF
23		*dfrA1*	NF
24		*dfrA5-14*	NF
25		*dfrA12*	NF
26		*dfrA17*	NF

	Antimicrobial classes	Target gene	Results
27	Polymyxins	*mcr-1*	NF
28		*mcr-2*	NF
29	Tetracyclines	*tetA*	NF
30		*tetB*	NF
31	Phenicols	*cmlA*	NF
32		*floR*	NF
33		*catA1*	NF
34		*catB3*	NF
35	Aminoglycosides	*aacC1*	NF
36		*aacC2*	NF
37		*aacC4*	NF
38		*aphA1*	NF
39		*aphA6*	NF
40		*aadA1-2-17*	NF
41		*aadB*	NF
42		*armA*	NF
43		*rmtB*	NF
44	Macrolides	*ermB*	NF
45		*mphA*	NF
46	Quinolones	*qnrA*	NF
47		*qnrS*	NF
48		*qnrB1*	NF
49		*qnrB4*	NF
50		*Qep*A	NF

~ 3-8 X10^6 cells was tested. Positive grading criteria: 1+: ≥ 10^1-10^2, 2+: >10^2-10^3 and 3+ = >10^3 positive cells NF = Not found

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Antimicrobial Resistant Gene Detection Report

Date: 11.11.2021

Name & Address of customer		SANZYME BIOLOGICS (P) LTD Plot No.13. Sagar Society, Road No.2, Banjara Hills, Hyderabad-500 034., Telangana, India	
Sample ID / type		*Bacillus licheniformis* - SAN 136BL	PTA337
Date of sample receipt	01-11-2021	Method of test	Real-time PCR with specific probes
Date of sample testing	05-11-2021 to 10-11-2021	Condition of sample when receive	Pure colonies on slant agar

Results

	Antimicrobial classes	Target gene	Results
1	β-lactams	CTX-M1	NF
2	(penicillin,	CTX-M2-M74	NF
3	amoxicillin,	CTX-M8-M25	NF
4	cephalosporin)	CTX-M9	NF
5		PER	NF
6		VEB	NF
7		CMY1-MOX	NF
8		CMY2-LAT	NF
9		DHA	NF
10		FOX	NF
11		ACT-MIR	NF
12		OXA-1	NF
13		OXA-9	NF
14		GES	NF
15	Carbapenems	KPC	NF
16		NDM	NF
17		VIM	NF
18		IMP	NF
19		OXA-48	NF
20	Folate pathway	*sul1*	NF
21	inhibitors	*sul2*	NF
22		*sul3*	NF
23		*dfrA1*	NF
24		*dfrA5-14*	NF
25		*dfrA12*	NF
26		*dfrA17*	NF

	Antimicrobial classes	Target gene	Results
27	Polymyxins	*mcr-1*	NF
28		*mcr-2*	NF
29	Tetracyclines	*tetA*	NF
30		*tetB*	NF
31	Phenicols	*cmlA*	NF
32		*floR*	NF
33		*catA1*	NF
34		*catB3*	NF
35	Aminoglycosides	*aacC1*	NF
36		*aacC2*	NF
37		*aacC4*	NF
38		*aphA1*	NF
39		*aadA4-5*	NF
40		*aphA6*	NF
41		*aadA1-2-17*	NF
42		*aadB*	NF
43		*armA*	NF
44		*rmtB*	NF
45	Macrolides	*ermB*	NF
46		*mphA*	NF
47	Quinolones	*qnrA*	NF
48		*qnrS*	NF
49		*qnrB1*	NF
50		*qnrB4*	NF
51		*Qep*A	NF

~ 3-8 X10^6 cells was tested. Positive grading criteria: 1+: ≥ 10^1-10^2, 2+: >10^2-10^3 and 3+ = >10^3 positive cells NF = Not found

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Antimicrobial Resistant Gene Detection Report

Date: 11.11.2021

Name & Address of customer		SANZYME BIOLOGICS (P) LTD Plot No.13. Sagar Society, Road No.2, Banjara Hills, Hyderabad-500 034., Telangana, India	
Sample ID / type		*Saccharomyces boulardii* - SAN 158SB	PTA338
Date of sample receipt	01-11-2021	Method of test	Real-time PCR with specific probes
Date of sample testing	05-11-2021 to 10-11-2021	Condition of sample when receive	Pure colonies on slant agar

Results

	Antimicrobial classes	Target gene	Results
1	β-lactams	CTX-M1	NF
2	(penicillin,	CTX-M2-M74	NF
3	amoxicillin,	CTX-M8-M25	NF
4	cephalosporin)	CTX-M9	NF
5		PER	NF
6		VEB	NF
7		CMY1-MOX	NF
8		CMY2-LAT	NF
9		DHA	NF
10		FOX	NF
11		ACT-MIR	NF
12		OXA-1	NF
13		OXA-9	NF
14		GES	NF
15	Carbapenems	KPC	NF
16		NDM	NF
17		VIM	NF
18		IMP	NF
19		OXA-48	NF
20	Folate pathway	*sul1*	NF
21	inhibitors	*sul2*	NF
22		*sul3*	NF
23		*dfrA1*	NF
24		*dfrA5-14*	NF
25		*dfrA12*	NF
26		*dfrA17*	NF

	Antimicrobial classes	Target gene	Results
27	Polymyxins	*mcr-1*	NF
28		*mcr-2*	NF
29	Tetracyclines	*tetA*	NF
30		*tetB*	NF
31	Phenicols	*cmlA*	NF
32		*floR*	NF
33		*catA1*	NF
34		*catB3*	NF
35	Aminoglycosides	*aacC1*	NF
36		*aacC2*	NF
37		*aacC4*	NF
38		*aphA1*	NF
39		*aadA4-5*	NF
40		*aphA6*	NF
41		*aadA1-2-17*	NF
42		*aadB*	NF
43		*armA*	NF
44		*rmtB*	NF
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48		*qnrS*	NF
49		*qnrB1*	NF
50		*qnrB4*	NF
51		*QepA*	NF

~ 3-8 X10^6 cells was tested. Positive grading criteria: 1+: ≥ 10^1-10^2, 2+: >10^2-10^3 and 3+ = >10^3 positive cells NF = Not found

Tested person: Rattapha Chinli
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"EFFECT OF PROBIOTIC FEED SUPPLEMENT IN COMMERCIAL LAYER CHICKENS"

Conducted by Dr. Daida Krishna, Associate Professor & Head, College Of Veterinary Science, Korutla, Jagtial Dist., Telangana State, India

ABSTRACT

The study was conducted to assess the three types of Competitor-BS 1 Billion CFU/g, Sporich-Total 4 Billion CFU/g (100 & 200 g/ton of feed), Prome-Max 4 Billion CFU/g (100 & 200 g/ton of feed) (SANZYME BIOLOGICS PVT. LTD.) and Bacitracin Methylene Disalicylate (50 g/ton of feed) supplemented in diet on *isocaloric* and *isonitrogenous* basis fed to a total of 288 White Leghorn layers (BV300), allocated to each of the eight treatments in a replicated manner, six replicates/treatment from 16 weeks of age (six birds/replicate) and evaluated production performance from 22 to 37 week of age.

The trial results revealed that, the per cent hen day egg production for four laying periods in White Leghorn layers from 22 to 37 weeks of age was significantly ($P<0.05$) influenced by supplementation of Competitor-BS, Sporich-Total and Prome-Max and Bacitracin Methylene Disalicylate (BMD). The higher Percent Egg Production was noticed in Sporich-Total @ 200 g/ton of feed (89.37%) compared with control and remaining treatments.

The mean Feed Intake and Feed Conversion Ratio per Dozen Eggs were significantly ($P<0.05$) influenced by supplementation of Competitor-BS, Sporich-Total & Prome-Max and BMD. The feed intake was significantly ($P<0.05$) lower in Control group (112.16g) and highest in Prome-Max @ 200 g/ton of feed (118.79g). The better Feed Conversion Ratio per Dozen Eggs was noticed in Sporich-Total @ 100 g/ton of feed (1.563).

Egg weight was significantly difference ($P<0.05$) was observed. The higher egg weight in Sporich-Total @ 100g/ton of feed (58.85g). The internal and external egg quality parameters of Haugh Unit, Yolk Index, Shell Thickness, Shell Percentage and Shell Strength were significantly ($P<0.05$) influenced by supplementation of Probiotic and BMD at different graded

levels. Whereas Albumen Index, Shell Weight and Density were not influenced by all the dietary supplementation groups. The mortality was within the limitation during 22-37 weeks of age.

The Competitor-BS, Sporich-Total and Prome-Max supplemented in diet influenced Net Profit per Dozen Eggs is highest in Sporich-Total @ 100 g/ton of feed (Rs.9.43) compared to other treatment groups during the 22-37 weeks of age.

It can be concluded that, supplementation of Multi Strain Probiotic Sporich-Total @ 100 g/ton of feed and Prome-Max @ 100 g/ton of feed as an alternative to antibiotic (BMD) and has better performance compared to the single strain Competitor-BS @ 100 g/ton of feed and Control.

Sporich-Total @ 100g/ton of feed has exhibited better production performance of commercial White Leghorn layer birds during the 22-37 weeks of age.

INTRODUCTION

Probiotics are defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance and there mode of action is by "competitive exclusion of harmful pathogens" in the gut. The use of sub-therapeutic levels of antibiotics as routine feed additive has been banned in many countries because of public concern, over possible antibiotic residual effects and the development of drug resistant bacteria. This has led to the development and application of many non-antibiotic substances performance enhancers. Initially Probiotics were introduced as an alternative to antibiotics and subsequently became an area of great research interest. Several studies in layers indicated that supplementation of Probiotics can serve as alternatives to antibiotics for increasing performance and disease resistance in poultry (Patterson and Burkholder, 2003). The addition of Probiotics could improve egg production, egg weight, and egg quality (Mohan *et al*., 1995; Ramasamy *et al.,* 2009) in laying hens.

MATERIALS AND METHODS

HOUSING AND MANAGEMENT

The experiment was conducted during August to December 2018. A total of 288 White Leghorn pullets (BV300) at the age of 16 weeks were procured from private agency then, leg banded and weighed individually & housed in California type cages (16"Hx 12"Wx18"D) having provision of feeders and nipple watering system. The birds housed in cages are given feed and water *ad lib* and the birds are raised under identical management conditions.

Before the commencement of the actual experiment, the cages, feeders and nipple drinking system were thoroughly cleaned, disinfected, and sprayed against external parasites. Other health precautions and sanitary measures were also taken throughout the study period. Diets were offered in separate feeder for different treatments with clear demarcation between replicates. Fluorescent lamp was placed for the lighting system to increase the lighting period to 16 h per day in order to increase feed intake and laying (Yasmeen *et al.,* 2008). Birds were adapted to respective treatment diet for a week before the commencement of the actual data collection.

EXPERIMENTAL DESIGN

The White Leghorn pullets (16weeks of age) were distributed randomly in to 8 different treatments with 6 replicates having 6 birds/replicate. Prior to experiment, the pre layer ration was fed to the birds until 21 weeks and layer ration from 22 weeks onwards.

EXPERIMENTAL DIETS

Three types of Probiotic supplementation at graded levels of 100 & 200 g/ton and Bacitracin Methylene Disalicylate (BMD) at 50 g/ton feeding fed to commercial layer chickens.

An experimental trial was conducted in layer chickens by feeding diets incorporated with various levels of Single Strain Probiotic - **COMPETITOR-BS 1 Billion CFU/g** and Multi Strain Probiotics of **SANZYME BIOLOGICS: SPORICH -TOTAL 4 Billion CFU/g and PROME-MAX 4 Billion CFU/g** in mash feed and to evaluate production performance in four laying periods (each period consist of 28 days).

The experimental layer diets were formulated (*isonitrogenous* and *isocaloric).*

- (T1 / Control) – The Basal Diet consisted of corn and soybean meal.
- (T2) – Basal Diet + 1×10^{9} CFU/g of Competitor-BS contain (*Bacillus subtilis*) @ 100 g/ton of feed
- (T3) – Basal Diet + 1×10^{9} CFU/g of Competitor-BS (*Bacillus subtilis*) @ 200 g/ton of feed;
- (T4) – Basal Diet + 4×10^{9} CFU/g of Sporich-Total contain (*Bacillus subtilis, Bacillus coagulans, Bacillus licheniformis & Saccharomyces boulardii*) @ 100 g/ton of feed
- (T5) – Basal Diet + 4×10^{9} CFU/g of Sporich-Total (*Bacillus subtilis, Bacillus coagulans, Bacillus licheniformis & Saccharomyces boulardii*) @ 200 g/ton of feed
- (T6) – Basal Diet + 4×10^{9} CFU/g of Prome-Max containing (*Bacillus subtilis, Bacillus coagulans & Bacillus licheniformis*) @ 100 g/ton of feed
- (T7) – Basal Diet + 4×10^{9} CFU/g of Prome-Max (*Bacillus subtilis, Bacillus coagulans & Bacillus licheniformis*) @ 200 g/ton of feed
- (T8) – Basal Diet + of AGP Bacitracin Methylene Disalicylate (BMD) 50 g/ton of feed
    - Production Performance of Hen Day Egg Production, Feed Intake, Feed Conversion Ratio per Dozen Eggs, Egg Weight, Egg Quality Parameters, Livability and Relative Economics were evaluated & studied for a total of four laying periods of 28 days each from 22 to 37 week of age.

Table 1. Ingredient Composition of Basal Diets (in Kg.) fed to the commercial White Leghorn layer birds from 22 to 37 week of age

Ingredients	Control / T1	T2	T3	T4	T5	T6	T7	T8
Maize	56	56	56	56	56	56	56	56
Soya bean meal	23	23	23	23	23	23	23	23
DORB	8.63	8.62	8.61	8.62	8.61	8.62	8.61	8.58
Shell grit	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2
DCP	1.1	1.1	1.1	1.1	1.1	1.1	1.1	2
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vit. mix*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Trace Mineral Mixture**	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline chloride (50%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium bicarbonate	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Probiotic	0.0	0.01	0.02	0.01	0.02	0.01	0.02	0.05
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

* Vitamin premix provided per Kg diet: Vitamin A 200000 IU, Vitamin B2 25 mg, Vitamin D3 3000IU, Vitamin K 2mg.

Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg and Niacin 15mg.

** Trace mineral provided per Kg diet: Manganese 120mg, Zinc 80mg, Iron 25mg, Copper 10mg, Iodine 1mg and Selenium 0.1mg.

Nutrient Composition								
CP (%)	16.97	16.97	16.97	16.97	16.97	16.97	16.97	16.97
ME(kcal/Kg)	2620	2620	2620	2620	2620	2620	2620	2620
Calcium (%)	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45
Available (%) Phosphorus	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Lysine (%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Methionine (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38

RESULTS

1. PRODUCTION PERFORMANCE IN WHITE LEGHORN LAYERS DURING 22-37 WEEKS OF AGE

1.1 Percent Hen- Day Egg Production

The data on Percent Hen Day Egg Production as influenced by two types of Competitor-BS, Sporich-Total and Prome-Max at graded levels as shown in the Table 2. The Per Cent Hen Day Egg Production in White Leghorn layers were significantly (P<0.05) influenced during period-P1 (22-25wks.), period-P2 (26-29wks.), period-P3 (30-33wks.), period-P4 (34-37wks.) and over all period (22-37 wks.) of age.

It is observed that in overall period (22-37wks.) was significant (P<0.05) difference in Percent Hen Day Egg Production among different levels of Probiotic fed groups. Highest Percent Hen Day Egg Production in Sporich-Total @ 200 g/ton of feed (89.37%) followed by Sporich-Total @ 100 g/ton of feed (89.02%), Prome-Max @ 200 g/ton of feed (88.90%), Competitor-BS @ 100 g/ton of feed (88.40%), Competitor-BS @ 200 g/ton of feed (87.95%), Prome-Max @ 100 g/ton of feed (86.64%), Control (86.09%) and BMD @ 50 g/ton of feed (85.33%)

Table 2. Effect of dietary supplementation of Probiotic at graded levels on Percent Hen Day Egg Production in White Leghorn Layers during 22-37 weeks of age

Treatment		g /ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	73.61^{d}	87.34^{f}	92.20^{b}	91.20^{cd}	**86.09^{d}**
T2	Competitor-BS	100	74.26^{cd}	93.10^{ab}	94.11^{ab}	92.11^{cd}	**88.40^{c}**
T3	Competitor-BS	200	72.50^{cd}	93.85^{ab}	92.87^{b}	92.56^{c}	**87.95^{c}**
T4	Sporich-Total	100	80.56^{a}	91.01^{a}	91.45^{a}	93.04^{a}	**89.02^{a}**
T5	Sporich-Total	200	80.27^{c}	92.09^{e}	91.09^{c}	94.04^{d}	**89.37^{d}**
T6	Prome-Max	100	72.40^{cd}	91.26^{cd}	91.60^{a}	91.28^{b}	**86.64^{bc}**
T7	Prome-Max	200	76.65^{c}	92.05^{bc}	93.53^{a}	93.35^{b}	**88.90^{b}**
T8	BMD	50	70.78^{b}	89.38^{de}	90.97^{ab}	90.18^{b}	**85.33^{bc}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate

1.2 Feed intake

The present study shows that the data on Feed Intake in commercial White Leghorn layers as influenced by different dietary treatments is presented in Table 3. The feed consumption was significant (P<0.05) difference during in first (22-25wks.), second (26-29 wks.), third (30-33 wks.) and fourth (34-37 wks.) periods and overall period (22-37 wks.) was significantly (P<0.05) influenced by supplementation with Competitor-BS, Sporich-Total & Prome-Max and BMD.

It is observed that in overall period (22-37wks.) was significant (P<0.05) difference in Feed Intake among different levels of Probiotic fed groups. Highest feed intake was with Prome-Max @ 200 g/ton of feed (118.79 g) followed by Sporich-Total @ 200 g/ton of feed (117.45 g), Sporich-Total @ 100 g/ton of feed (116.43 g), BMD @ 50 g/ton of feed (116.14 g), Prome-Max @ 100 g/ton of feed (115.55 g), Competitor-BS @ 100 g/ton of feed (115.36 g), Competitor-BS @ 200 g/ton of feed (114.98 g) and least feed intake in Control (112.16 g)

Table3. Effect of dietary supplementation of Probiotic at graded levels on Feed Intake (g/hen/day) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	110.89^{bc}	111.15^{d}	109.87^{c}	116.83^{h}	**112.16^{e}**
T2	Competitor-BS	100	108.54^{de}	116.86^{ab}	116.32^{a}	119.67^{g}	**115.36^{cd}**
T3	Competitor-BS	200	107.24^{e}	116.50^{ab}	115.03^{ab}	121.17^{f}	**114.98^{d}**
T4	Sporich-Total	100	109.48^{c}	115.44^{bc}	116.15^{a}	124.50^{e}	**116.43^{c}**
T5	Sporich-Total	200	111.85^{ab}	117.74^{a}	113.93^{ab}	126.17^{d}	**117.45^{b}**
T6	Prome-Max	100	110.32^{c}	114.24^{c}	110.20^{c}	127.33^{c}	**115.55^{cd}**
T7	Prome-Max	200	112.92^{a}	117.50^{ab}	116.27^{a}	128.33^{b}	**118.79^{a}**
T8	BMD	50	107.11^{e}	114.15c	113.37^{b}	129.83^{a}	**116.14^{c}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05),
BMD= Bacitracin methylene disalicylate

1.3 Feed conversion ratio per dozen eggs

The data on Feed Conversion Ratio per Dozen Eggs (Kg. of Feed consumed for every Dozen Eggs Produced) is presented in the Table 4. The results revealed that, there was significantly (P<0.05) difference in Feed Conversion Ratio per Dozen Eggs during period-P1 (22-25 wks.), period-P2 (26-29 wks.), period-P3 (30-33 wks.), period-P4 (34-37 wks.) and over all period (22-37 wks.) of age.

During the overall period (22-37 wks..) the best Feed Conversion Ratio per Dozen Eggs in commercial White Leghorn layers was observed when fed with supplementation of Sporich-Total @ 100 g/ton of feed (1.563) followed by Sporich-Total @ 200 g/ton of feed (1.566), Prome-Max @ 100 & 200 g/ton of feed (1.569), Competitor-BS @ 100 & 200 g/ton of feed (1.577 & 1.600), BMD @ 50 gton of feed (1.603) and poor in and Control (1.633).

Table 4. Effect of dietary supplementation of Probiotic at graded levels on Feed Conversion Ratio (Kg Feed Consumed for Dozen Eggs) in White Leghorn Layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	1.773^{ab}	1.569^{a}	1.530^{a}	1.661^{a}	**1.633^{a}**
T2	Competitor-BS	100	1.631^{d}	1.558^{c}	1.555^{bc}	1.564^{c}	**1.577^{d}**
T3	Competitor-BS	200	1.790^{a}	1.515^{b}	1.461^{bc}	1.634^{b}	**1.600^{b}**
T4	Sporich-Total	100	1.794^{a}	1.512^{b}	1.428^{c}	1.519^{d}	**1.563^{c}**
T5	Sporich-Total	200	1.661^{d}	1.499^{b}	1.448^{bc}	1.656^{a}	**1.566^{c}**
T6	Prome-Max	100	1.739^{c}	1.496^{b}	1.482^{b}	1.560^{c}	**1.569^{c}**
T7	Prome-Max	200	1.728^{bc}	1.491^{bc}	1.486^{b}	1.571^{c}	**1.569^{c}**
T8	BMD	50	1.776^{ab}	1.525^{bc}	1.487^{d}	1.624^{b}	**1.603^{c}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate

2. EGG WEIGHT

The data on Egg Weight (g) is presented in the Table 5. The Egg Weight was significant (P<0.05) influenced by Competitor-BS, Sporich-Total, Prome-Max and BMD supplementation.

The overall Egg Weight was significantly (P<0.05) higher in Sporich-Total @ 100 g/ton of feed (58.85 g) followed by Competitor-BS @ 200 g/ton of feed (58.18 g), Prome-Max @ 200 g/ton of feed (58.12 g), Sporich-Total @ 200 g/ton of feed (58.09 g), Competitor-BS @ 100 g/ton of feed (57.90 g), BMD @ 50 g/ton of feed (57.74 g), Prome-Max @ 100 g/ton of feed (57.39 g) and Control (57.00 g).

Table 5. Effect of dietary supplementation of Probiotic at graded levels on Egg Weight (g) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	55.17^{bc}	55.65^{c}	57.12^{c}	60.07^{c}	**57.00^{d}**
T2	Competitor-BS	100	55.77^{b}	57.72^{ab}	57.83^{c}	60.26^{bc}	**57.90^{bc}**
T3	Competitor-BS	200	55.41^{b}	57.61^{ab}	58.34^{bc}	61.35^{ab}	**58.18^{b}**
T4	Sporich-Total	100	57.00^{a}	58.44^{a}	59.83^{a}	60.12^{c}	**58.85^{a}**
T5	Sporich-Total	200	56.32^{ab}	57.06^{b}	58.01^{bc}	60.97^{abc}	**58.09^{b}**
T6	Prome-Max	100	54.14^{cd}	57.78^{ab}	59.21^{ab}	58.43^{d}	**57.39^{cd}**
T7	Prome-Max	200	55.29^{bc}	55.71^{c}	59.91^{a}	61.58^{a}	**58.12^{b}**
T8	BMD	50	53.75^{d}	57.61^{ab}	58.17^{bc}	61.42^{ab}	**57.74^{bc}**
n			6	6	6	6	**6**
P-Value			0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate

3. INTERNAL EGG QUALITY PARAMETERS

3.1 Haugh Unit

Haugh Unit (HU) was significantly (P<0.05) influenced during first (22-25wks.), second (26-29 wks.), third (30-33 wks.) and fourth (34-37 wks.) and overall period (22-37 wks.) by supplemented with Competitor-BS, Sporich-Total & Prome-Max and BMD at graded levels.

The higher Haugh Unit value was observed in Prome Max @ 200 g/ton of feed (87.68) indicating the quality of albumen was better and lower Haugh Unit value observed in BMD @ 50 g/ton of feed (84.95) as shown in the (Table 6).

Table 6. Effect of dietary supplementation of Probiotic at graded levels on Haugh Unit in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	87.61^{a}	78.50^{d}	88.78^{a}	86.95^{cd}	**85.46^{cd}**
T2	Competitor-BS	100	87.77^{a}	80.98^{c}	86.84^{b}	84.43^{c}	**85.01^{d}**
T3	Competitor-BS	200	87.53^{a}	81.72^{c}	88.67^{a}	84.22^{e}	**85.53^{cd}**
T4	Sporich-Total	100	84.89^{b}	84.92^{a}	89.72^{a}	87.67^{bcd}	**86.80^{b}**
T5	Sporich-Total	200	86.41^{a}	85.20^{a}	86.56^{b}	86.34^{d}	**86.12^{bc}**
T6	Prome-Max	100	86.29^{a}	83.17^{b}	86.97^{b}	88.81^{ab}	**86.31^{b}**
T7	Prome-Max	200	87.56^{a}	84.65^{a}	90.28^{a}	88.26^{abc}	**87.68^{a}**
T8	BMD	50	83.97^{b}	79.28^{d}	87.05^{b}	89.51^{a}	**84.95^{d}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05),

BMD= Bacitracin methylene disalicylate

3.2 Albumen index

The present study shows that the data on Albumen Index as influenced by different dietary treatments is presented in Table 7. Albumen Index was significantly (P<0.05) influenced by supplementation of Probiotic during fourth period (34-37 wks.) of age.

The higher Albumen Index value for overall period (22-37 wks.) was noticed in Sporich-Total @ 100 g/ton of feed (0.085) followed by Competitor BS @ 100 g/ton of feed, Sporich-Total @ 200 g/ton of feed and Prome-Max @ 100 & 200 g/ton of feed (0.082), Control, Competitor BS @ 200 g/ton of feed and BMD @ 50 g/ton of feed (0.080) respectively.

Table7. Effect of dietary supplementation of Probiotic at graded levels on Albumen Index in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	0.084	0.084	0.084	0.083^{b}	**0.080**
T2	Competitor-BS	100	0.084	0.084	0.084	0.084^{b}	**0.082**
T3	Competitor-BS	200	0.085	0.083	0.083	0.083^{b}	**0.080**
T4	Sporich-Total	100	0.085	0.085	0.083	0.087^{a}	**0.085**
T5	Sporich-Total	200	0.083	0.084	0.085	0.083^{b}	**0.082**
T6	Prome-Max	100	0.085	0.083	0.083	0.084^{b}	**0.082**
T7	Prome-Max	200	0.086	0.085	0.085	0.084^{b}	**0.082**
T8	BMD	50	0.086	0.084	0.083	0.083^{b}	**0.080**
	n		6	6	6	6	**6**
	P-Value		0.078	0.078	0.122	0.016	**0.251**

Means with different superscripts in a column differ significantly (P<0.05),
BMD= Bacitracin methylene disalicylate

3.3 Yolk Index

It is observed that in period-P1 (22-25wks.), period-P2 (26-29wks.), period-P4 (34-37wks.) and over all period (22-37 wks.) was significant (P<0.05) difference in Yolk Index among different levels of Probiotic fed groups.

The overall period Yolk Index was better in the Sporich-Total @ 100 g/ton of feed, Prome-Max @ 100 & 200 g/ton of feed (0.473), followed by Competitor-BS @ 200 g/ton of feed (0.467), Competitor-BS @ 100 g/ton of feed & Sporich-Total @ 200 g/ton of feed (0.465), BMD @ 50 g/ton of feed (0.463) and Control (0.462).

Table 8. Effect of dietary supplementation of Probiotic at graded levels on Yolk Index in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	0.453^{d}	0.455^{c}	0.472	0.458^{a}	**0.462^{a}**
T2	Competitor-BS	100	0.493^{a}	0.467^{bc}	0.463	0.447^{ab}	**0.465^{ab}**
T3	Competitor-BS	200	0.487^{ab}	0.467^{bc}	0.465	0.452^{ab}	**0.467^{ab}**
T4	Sporich-Total	100	0.490^{ab}	0.492^{a}	0.463	0.453^{ab}	**0.473^{a}**
T5	Sporich-Total	200	0.463^{cd}	0.468^{bc}	0.470	0.460^{a}	**0.465^{ab}**
T6	Prome-Max	100	0.475^{abc}	0.485^{ab}	0.462	0.462^{a}	**0.473^{a}**
T7	Prome-Max	200	0.487^{ab}	0.492^{a}	0.462	0.440^{b}	**0.473^{a}**
T8	BMD	50	0.472^{bcd}	0.475^{abc}	0.458	0.447^{ab}	**0.463^{ab}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.002	0.669	0.036	**0.043**

Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate

4. EXTERNAL EEGG QUALITY PARAMETERS

4.1 Egg Shell Weight

The present experiment revealed that there is no significant difference was observed statically between control and various experimental groups of Egg Shell Weight. However, the values ranged between 5.581 to 5.712 g (Table 9).

Table 9. Effect of dietary supplementation of Probiotic at graded levels on Egg Shell Weight (g) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	5.438	5.473	5.778	6.161	**5.712**
T2	Competitor-BS	100	5.333	5.622	5.841	5.969	**5.692**
T3	Competitor-BS	200	5.21	5.433	5.624	6.091	**5.589**
T4	Sporich-Total	100	5.257	5.472	5.743	6.053	**5.631**
T5	Sporich-Total	200	5.163	5.411	5.807	6.122	**5.626**
T6	Prome-Max	100	5.121	5.534	5.635	6.032	**5.581**
T7	Prome-Max	200	5.415	5.336	5.682	6.172	**5.652**
T8	BMD	50	5.114	5.608	5.834	6.116	**5.668**
	n		6	6	6	6	**6**
	P-Value		0.227	0.496	0.887	0.846	**0.804**

Means with different superscripts in a column differ significantly (P<0.05),
BMD= Bacitracin methylene disalicylate

4.2 Shell Thickness

The Shell Thickens was significant (P<0.05) difference in first and second period between 22-25 and 26-29 weeks of age where as third and fourth period between 30-33 and 34-37 weeks of age there was non-significant. However, during the overall period there was significant (P<0.05) difference among the treatments (Table 10).

Table 10. Effect of dietary supplementation of Probiotic at graded levels on Egg Shell Thickness (mm) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	0.406^{bc}	0.397^{b}	0.41	0.407	**0.404^{bc}**
T2	Competitor-BS	100	0.396^{e}	0.396^{b}	0.397	0.4	**0.397^{d}**
T3	Competitor-BS	200	0.401^{cde}	0.396^{b}	0.402	0.406	**0.401^{bc}**
T4	Sporich-Total	100	0.412^{ab}	0.387^{c}	0.407	0.406	**0.404^{bc}**
T5	Sporich-Total	200	0.394^{e}	0.386^{c}	0.405	0.41	**0.399^{cd}**
T6	Prome-Max	100	0.403^{cd}	0.407^{a}	0.404	0.404	**0.405^{a}**
T7	Prome-Max	200	0.415^{a}	0.398^{b}	0.405	0.407	**0.406^{a}**
T8	BMD	50	0.402^{cde}	0.399^{b}	0.404	0.411	**0.404^{bc}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.001	0.061	0.425	**0.001**

Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate

4.3. Shell Percentage

The data on Shell Percentage was evaluated in layer chicken as influenced by different dietary treatments with Probiotic fed diets are presented in Table 11. During the first period between 22-25 wks. of age the Shell Percentage was significant (P<0.05) difference reported. However, the remaining periods were no significant difference among the treatments. During over all period (22-37wks.) the supplementation of Probiotic significantly (P<0.05) improve the Shell Percentage. The higher Shell Percentage was in the Sporich Total @ 100 g/tom (10.02) when compared with treatment groups.

Table 11. Effect of dietary supplementation of Probiotic at graded levels on Egg Shell Percentage in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	9.248^{cd}	9.542	9.681	10.02	**9.623^{b}**
T2	Competitor-BS	100	9.805^{ab}	9.332	9.736	10.05	**9.732^{b}**
T3	Competitor-BS	200	9.362^{bcd}	9.568	9.777	9.933	**9.658^{b}**
T4	Sporich-Total	100	9.869^{a}	9.838	10.09	10.27	**10.02^{a}**
T5	Sporich-Total	200	9.712^{abc}	9.473	9.728	10.2	**9.775^{ab}**
T6	Prome-Max	100	9.343^{bcd}	9.723	9.686	9.983	**9.680^{b}**
T7	Prome-Max	200	9.536^{abcd}	9.738	10	9.983	**9.818^{ab}**
T8	BMD	50	9.187^{d}	9.593	9.763	10.03	**9.638^{b}**
	n		6	6	6	6	**6**
	P-Value		0.01	0.299	0.55	0.526	**0.019**

Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate

4.4. Shell strength

The Shell Strength was significant (P<0.05) in supplementation with Competitor-BS, Sporich-Total & Prome-Max and BMD at different graded levels during the period and overall period. The Shell Strength was significantly (P<0.05) higher value in Sporich Total @ 100 g/tom (21.15 N) compared with other treatment groups (Table 12).

Table 12. Effect of dietary supplementation of Probiotic at graded levels on Egg Shell Strength (N*) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	21.02^{b}	17.95^{e}	18.92^{b}	19.33^{d}	**19.30^{de}**
T2	Competitor-BS	100	20.02^{bc}	20.50^{ab}	17.69^{c}	21.01^{c}	**19.81^{cd}**
T3	Competitor-BS	200	24.54^{a}	19.67^{abcd}	18.72^{bc}	19.57^{d}	**20.63^{ab}**
T4	Sporich-Total	100	19.24^{c}	20.10^{abc}	19.55^{b}	25.71^{a}	**21.15^{a}**
T5	Sporich-Total	200	19.27^{c}	19.39^{bcd}	18.76^{bc}	22.84^{b}	**20.06^{bc}**
T6	Prome-Max	100	19.50^{c}	19.05^{cde}	21.35^{a}	23.75^{b}	**20.91^{a}**
T7	Prome-Max	200	19.81^{bc}	20.83^{a}	21.68^{a}	21.33^{c}	**20.92^{a}**
T8	BMD	50	20.22^{bc}	18.53^{de}	17.73^{c}	18.34^{d}	**18.71^{e}**
	n		6	6	6	6	**6**
	P-Value		0.001	0.001	0.001	0.001	**0.001**

.Means with different superscripts in a column differ significantly (P<0.05)
BMD= Bacitracin methylene disalicylate
*Newton's

4.5 Density

The density (g/cm^3) of egg was non-significant in all the treatment groups during the period wise and overall period (Table 13).

Table 13. Effect of dietary supplementation of Probiotic at graded levels on Density (g/cm3) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	1.09	1.09	1.09	1.09	**1.09**
T2	Competitor-BS	100	1.09	1.09	1.09	1.09	**1.087**
T3	Competitor-BS	200	1.09	1.09	1.09	1.09	**1.087**
T4	Sporich-Total	100	1.09	1.09	1.09	1.09	**1.086**
T5	Sporich-Total	200	1.08	1.08	1.09	1.09	**1.085**
T6	Prome-Max	100	1.08	1.08	1.09	1.09	**1.085**
T7	Prome-Max	200	1.09	1.08	1.09	1.09	**1.086**
T8	BMD	50	1.08	1.09	1.09	1.09	**1.087**
	n		6	6	6	6	**6**
	P-Value		0.07	0.06	0.73	0.94	**0.18**

Means with different superscripts in a column differ significantly (P<0.05),
BMD= Bacitracin methylene disalicylate

5. LIVEABILITY

Between the 22-37 weeks of age, the mortality was within the limitation. During first (22-25wks.) second (26-29wks.) and fourth (34-37wks.) period there was no mortality of birds but whereas in third period (30-33 wks.) there was only one bird mortality was recorded in Competitor-BS @ 100 g/ton of feed (Table 14).

Table 14. Effect of dietary supplementation of Probiotic at graded levels on Liveability (%) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	100	100	100	100	**100**
T2	Competitor-BS	100	100	100	97	100	**99.25**
T3	Competitor-BS	200	100	100	100	100	**100**
T4	Sporich-Total	100	100	100	100	100	**100**
T5	Sporich-Total	200	100	100	100	100	**100**
T6	Prome-Max	100	100	100	100	100	**100**
T7	Prome-Max	200	100	100	100	100	**100**
T8	BMD	50	100	100	100	100	**100**

6. RELATIVE ECONOMICS IN WHITE LEGHORN LAYERS DURING 22-37 WEEKS OF AGE

The supplementation of Probiotic at different graded levels influenced Net Profit per Dozen Eggs is highest in Sporich-Total @ 100 g/ton of feed (Rs.9.43) compared with control (Rs.8.67) and also with Competitor-BS and Prome-Max (100 & 200 g/ton) and BMD (50 g/ton) of feed during the 22-37 weeks of age (Table 15).

Table 15. Effect of dietary supplementation of Probiotic at graded levels on Relative Economics in White Leghorn layers during 22-37 weeks of age

S.No	Details	Cntrl	Comp.BS	Comp.BS	SPT	SPT	PMX	PMX	BMD
Supplemented @		T1	T2	T3	T4	T5	T6	T7	T8
			100 g/ton	200 g/ton	100 g/ton	200 g/ton	100 g/ton	200 g/ton	50 g/ton
1	Cost of feed per Kg (Rs.)	23.25	23.3	23.3	23.3	23.3	23.3	23.3	23.3
2	Feed consumption/dozen eggs (Kg)	1.563	1.569	1.569	1.527	1.633	1.568	1.6	1.566
3	Feed cost/dozen eggs (Rs.)	36.33	36.55	36.55	35.57	38.97	36.53	37.28	36.48
4	Selling price of dozen eggs (Rs.)	45	45	45	45	45	45	45	45
5	Net profit per dozen eggs (Rs.)	8.67	8.45	8.45	9.43	6.03	8.47	7.72	8.52
6	Net profit over control (Rs.)		-0.22	-0.22	0.76	-2.64	-0.2	-0.95	-0.15

Selling price of egg Rs: 3.75, Avg. Probiotic cost Rs: 500/Kg
Cntrl = Control
Comp.BS = Competitor BS
SPT = Sporich Total
PMX = Prome Max
BMD = Bacitracin methylene disalicylate

7. BROKEN EGGS AND SOILED EGGS:

Broken Eggs

Broken Eggs was low in Sporich-Total @ 100 g per ton of feed and Prome-Max @ 100g per ton of feed followed by Competitor-BS @ 100 & 200 g per ton of feed per ton compared to Control and BMD treatments.

Table 14. Effect of dietary supplementation of Probiotics at graded levels on Broken Eggs (No.) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	2	2	1	2	7
T2	Competitor-BS	100	1	1	1	2	5
T3	Competitor-BS	200	2	1	1	1	5
T4	Sporich-Total	100	2	1	1	0	4
T5	Sporich-Total	200	1	2	2	2	7
T6	Prome-Max	100	1	2	0	1	4
T7	Prome-Max	200	2	2	1	2	7
T8	BMD	200	2	1	2	2	7

BMD= Bacitracin methylene

Soiled Eggs

Soiled Eggs were low in Sporich-Total @ 100g and Competitor-BS @ 100g per ton of feed compared to control and BMD.

Table 15. Effect of dietary supplementation of Probiotics at graded levels on Soiled Eggs (No.) in White Leghorn layers during 22-37 weeks of age

Treatment		g / ton	Age (weeks)				Mean
			22-25 (P1)	26-29 (P2)	30-33 (P3)	34-37 (P4)	22-37 wks.
T1	Control	0	1	1	1	1	4
T2	Competitor-BS	100	1	1	1	0	3
T3	Competitor-BS	200	0	2	1	1	4
T4	Sporich-Total	100	1	0	1	1	3
T5	Sporich-Total	200	1	2	1	1	5
T6	Prome-Max	100	1	2	2	0	5
T7	Prome-Max	200	2	1	0	2	5
T8	BMD	200	1	0	2	1	4

BMD= Bacitracin methylene

DISCUSSION

1 PRODUCTION PERFORMANCE OF COMMERCIAL LAYERS

1.1 Hen-Day Egg Production

In this experiment the dietary supplementation of graded levels of Probiotic in White Leghorn (BV 300) layers had shown significant effect ($P<0.05$) on Percent Hen Day Egg Production. The Percent Hen Day Egg Production was significantly ($P<0.05$) higher in Sporich Total (200 g/ton) when compared with control and this trend has continued from 22-37 weeks.

The above findings are in agreement with (Tortuero and Fernandez, 1995; Nahashon *et al*., 1996; Zhang *et al*., 2012; Yalcin *et al*., 2010; Panda *et al*., 2006; Abdelqader *et al*., 2013) reported that Percent Hen Day Egg Production was increased and improved in commercial layer birds and also Berrin (2011) & Wei Fen Li *et al*. (2011) reported in Japanese quail and Shaoxing Ducks.

Contrary to the findings of the present study, reported by Davis and Anderson 2002, Mahdavi *et al*., 2005,Yousefi and Karkoodi 2007, Balevi*et al*., 2009, Kalavathy Ramasamy *et al*., 2009, Mikulski *et al*., 2012, Shalaei *et al*., 2014, Sobczak*et et al*., (2015), Forte *et al*., 2016, Upadhaya *et al*., 2016, Sheoran *et al*., 2018, Fathi *et al*., 2018 observed did not show any significant variation of percent hen day egg production in commercial layer birds .

Different opinions were expressed by Sattar Bageri Dizaji and Rasoul Pirmohammadi 2009, Moorthy *et al*., 2010 observed that significantly ($P<0.05$) decrease the per cent hen day egg production by supplemented with Probiotic in White Leghorn layers.

Variability in response to the use of Probiotic giving good results in terms of Percent Hen Day Egg Production in White Leghorn layers with statistically significant, which may be reasonably due to the bacterial sensitivity, health and hygiene of birds used in the trials as well as the environmental factors.

1.2 Feed intake

In the experiment it is observed that the commercial layer birds fed with Probiotic supplemented diet throughout the experimental period had consumed significantly ($P<0.05$) more in Prome Max (200 g/ton) compared with supplemented Probiotic groups & control.

The results were in accordance with the earlier reports of Nahashon *et al*. (1996), Falaki *et al*. (2010) and Sheoran *et al.* (2018) found that significantly ($P<0.05$) increase in feed consumption in White Leg horn layers.

Contrary to the above results Tortuero and Fernandez 1995, Panda *et al*., 2003, Yoruk *et al*., 2004, Mahdavi *et al*., 2005, Panda *et al*., 2006, Yousefi and Karkoodi 2007, Panda *et al*., 2008 , Kalavathy Ramasamy *et al*., 2009, Moorthy *et al*., 2010, Yalcin *et al*., 2010, Mikulski *et*

al., 2012, Zhang *et al*., 2012, Abdelqader *et al*., 2013,Sobczak *et al*., 2015 and Fathi *et al*.,2018 were reported that no significant difference of feed intake in commercial White Leghorn birds supplemented with Probiotic and yeast culture.

The treatment groups feed consumption increased compare with control it may be Probiotic increasing the occurrence of naturally occurring beneficial bacteria in the intestinal tract of birds, which may result in the improvement of feed consumed.

1.3 Feed Conversion Ratio per Dozen Eggs

The Feed Conversion Ratio per Dozen Eggs was significantly ($P<0.05$) reduced observed when supplemented with Sporich-Total (100 g/ton) of feed in White Leghorn between 22-37 weeks of age.

Better feed efficiency observed in this study with supplementation of Probiotic is in accordance with the earlier findings of Nahashon *et al*., 1996, Yoruk *et al*., 2004, Panda *et al*., 2006 & 2008, Balevi *et al*., 2009, Yalcin *et al*., 2010, Shivani Katoch *et al*., 2011, Mikulski *et al*., 2012, Zhang *et al*., 2012 Abdelqader *et al*., 2013, Shalaei *et al*., 2014 and Sheoran *et al*., 2018 observed that significant ($P<0.05$) improvement in feed conversion ratio in commercial layer birds fed with Probiotic supplementation at different levels.

In contrast to the results of the present study reported by Tortuero and Fernandez 1995, Panda *et al*., 2003, Yoruk *et al*., 2004, Mahdavi *et al*., 2005, Yousefi and Karkoodi 2007, Yalcin *et al*., 2008, Moorthy *et al*., 2010,, Sobczak *et al*., 2015, Forte *et al*., 2016 and Fathi *et al*., 2018) were reported no significant difference in feed conversion ratio per dozen eggs in White Leghorn supplementation with Probiotic.

The effect of Probiotic might be attributable to the probable production of natural antibiotic like acidophil in which is active against pathogenic microbes like *E.coli* and *Salmonella*. Further, the Probiotic not only check the growth of pathogenic microorganisms but also could improve the feed utilization with neutralization of toxins, apparently increased the absorption of nutrients and alteration of microbial metabolism.

2. EGG WEIGHT

The supplementation with Probiotic and BMD in White Leghorn showed significantly ($P<0.05$) increase Egg Weight. Similar to these findings with the Tortuero and Fernandez 1995 & Davis and Anderson 2002, Balevi *et al*., 2009, Kalavathy Ramasamy *et al*., 2009; Yalcin *et al*., 2010, Mikulski *et al*., 2012, Zhang *et al*., 2012, Abdelqader *et al*., 2013 and Shalaei *et al*., 2014.

Contrary to the above results (Nahashon *et al*., 1996, Mahdavi *et al*., 2005, Panda*et al*., 2006 &2008, Yousefi and Karkoodi 2007, Yalcin *et al*., 2008, Sattar Bageri Dizaji and Rasoul Pirmohammadi 2009, Balevi *et al*., 2009, Sobczak *et al*., 2015, Forte *et al*., 2016 and Sheoran *et al*., 2018 found that the White Leghorn layer birds egg weight was non-significant in all treatment groups.

The improved egg weight observed in the present and earlier studies is probably due to the ability of Probiotic cultures to perform well under stressful conditions as egg weight increments in egg-type hens have been reported to be largely affected by environmental factors, although egg weight is a highly heritable trait in chickens. Furthermore, supplementation of Probiotic in animal feed may improve digestion and absorption of nutrients in the host.

The improvement in egg size was associated with the higher calcium and nitrogen retentions in the Probiotic fed hens. Davis reported similar result and Anderson 2002, in which hens supplemented with PrimaLac exhibited a higher percentage of extra-large eggs than control.

3 INTERNAL EGG QUALITY

3.1 Haugh Unit

Albumen quality evaluated as Haugh Unit Score was significantly ($P<0.05$) influenced supplemented Probiotic and BMD during 22-37 weeks of age. Similar to the above findings agreement with , Tortuero and Fernandez 1995, Zhang *et al*., 2012 and Sobczak *et al*., 2015.

Contrary to the observations several others, Nahashon *et al*.,1996, Mahdavi *et al*., 2005, Panda *et al*., 2008, Yalcin *et al*., 2008, Berrin 2011, Mikulski *et al*.,2012 and Fathi *et al*.,2018 results revealed no significant difference among the treatment groups.

3.2 Albumin Index

The effect of different periods was not significant of albumen index. However, there was significant differences of albumen index during third period without following any specific trend. This might be due to biological variation. These findings were in accordance with Yoruk *et al*., 2004, Valavan *et al*., 2006, Yalcin *et al*., 2008 and Berrin 2011.

3.3 Yolk Index

The effect of Yolk Index was significantly ($P<0.05$) influenced during the first (22-25wks.) second (25-29wks.) and fourth period (33-37wks.) and overall period (22-37 wks.) However, during the third period (30-33wks.) non-significant difference was recorded.

Different opinions were expressed by various workers Yoruk *et al*., 2004 Valavan *et al*., 2006, Yalcin *et al*., 2008, Berrin2011 and Mikulski *et al*., 2012 found that no significant difference in yolk index .

4. EXTERNAL EGG QUALITY

4.1 Shell Weight

The effect of different periods was non-significant on Egg Shell Weight. Similar to these findings, Forte *et al*., 2016.

Contrary to the above results, Yousefi and Karkoodi 2007, Panda *et al*., 2008 and Fathi*et al*.,2018 reported that there was significant (P<0.05) difference in Shell Weight among the treatment groups.

4.2 Shell Thickness

The effect of first, second and overall periods were significantly effect on Shell Thickness. However, the Shell Thickness was not affected during third and fourth periods.

These results are in agreement with Panda *et al*., 2006, Yousefi and Karkoodi 2007, Panda *et al*., 2008, Mikulski *et al*., 2012, Zhang *et al*., 2012 Abdelqader *et al*.,2013, Shalaei *et al*., 2014, Sobczak *et al*.,2015 and Fathi *et al*.,2018.

Contrary to the findings of the present study Mahdavi *et al*., 2005 and Yalcin *et al*., 2008 showed that shell thickness was no significant difference among the treatment groups in layers birds.

This beneficial effect may be attributed to a favourable environment in the gastrointestinal tract resulting from the administration of Probiotic to birds (Mohan *et al*., 1995; Panda *et al*., 2008; Mikulski *et al*., 2012). Probiotic bacteria increase the rate of fermentation and the production of short-chain fatty acids (SCFAs), which reduces the luminal pH (Scholz-Ahrens *et al*., 2007). Low luminal pH increases calcium solubility and absorption (Van den Heuvel *et al*., 1999). SCFAs stimulate intestinal epithelial cell proliferation and villus height (Garcia *et al*., 2007), which increases absorption efficiency (Scholz-Ahrens *et al*., 2007). As a result, more nutrients, including calcium, can be assimilated, thus improving eggshell quality

4.3. Shell Percentage

The mean Shell Percentage was significantly (P<0.05) influenced by three types of Probiotic and BMD supplementation. However, differences in Shell Percentage was recorded during the first period.

The findings of the present study are in agreement with those of Mikulski *et al*., 2012 and Sobczak *et al*., 2015.

In contrast to the results of the present study, various workers Yousefi and Karkoodi (2007), Forte *et al*. (2016) Shalaei *et al*. (2014).

4.4. Shell Strength

The Shell Strength was significant differences in White Leghorn eggs during 22-37 weeks of age without following any specific trend.

. The above results are in agreement with Panda *et al*. 2008, Sobczak*et al*., 2015, Upadhaya *et al*., 2016 and Fathi *et al*., 2018 resulted in a significantly (P<0.05) improved Shell Strength in White Leghorn layers.

Shell Breaking Strength was significantly higher in the Probiotic-fed groups. This could be attributed to the higher Shell Thickness, which might have created greater resistance resulting in higher breaking strength

Concomitant to the findings of the present study, Mahdavi *et al*., 2005 and Shalaei *et al*., 2014 found that did not observe any difference in the Shell Strength.

4.5 Density

The Density (g/cm^3) of egg was non-significant in all the treatment groups during the period wise and overall period.

5. Livability

There was no mortality in layer birds. Compared to the findings of the present study, Panda *et al.,* 2006 and Balevi *et al.,* 2009 observed mortality was not effected in White Leghorn chicks supplemented with Probiotic. However, Yoruk *et al*., 2004 and Vicente *et al*., 2007 reported a significant (P<0.01) reduction in mortality.

6. RELATIVE ECONOMICS

The supplementation of Probiotic at different graded levels influenced Net Profit per Dozen Eggs is highest in Sporich-Total @ 100 g/ton (Rs. 9.43) compared with Control (Rs 8.67) and also with Competitor-BS and Prome-Max (100 & 200 g/ton) and BMD (50g/ton), during the 22-37 weeks of age.

Davis and Anderson (2002) noticed that feeding of direct fed microbial, Probiotic supplement “Primalac” to Single Comb White leghorn, Hy-Line W-36 and DeKalb XL laying hens significantly (P<0.002) reduced the feed cost.

Sabiha *et al*. (2005) observed that the cost of production in broilers was lower in 0.025 and 0.05% Probiotic (Yea-sacc1026, *Lactobacillus acidophilus, Streptococcus faecium*etc.) supplemented groups at six and eight weeks of age, respectively.

Moorthy *et al*. (2010) reported that diets supplemented with Probiotic and prebiotic supplements did not affect the return over feed cost per bird in White Leghorn layers aged 21-52 weeks of age.

7. Broken Eggs

Multi strain Probiotics such as Sporich-Total & Prome-Max @ 100g per ton of feed has lower number of Broken Eggs compared to control, BMD and Competitor-BS.

8. Soiled Eggs

Soiled Eggs are lower in Sporich-Total & Competitor-BS @100g per ton of feed.

CONCLUSION

Based on the findings of this study the following conclusions were drawn:

- The supplementation of Sporich-Total @ 100 g per ton of feed during 22-37 weeks of age to the commercial White Leghorn layers has influenced significantly ($P<0.05$) and more effective among all the dietary treatments in terms of major parameters such as mean Percent Hen Day Egg Production, Feed Conversion Ratio (feed consumed per Kg egg mass and per dozen eggs), Yolk Index, Broken Eggs and Soiled Eggs.
- The supplementation of Prome Max @ 100 g per ton of feed during 22-37 weeks of age to the commercial White Leghorn layers has also influenced significantly ($P<0.05$) and effective among the dietary treatments in terms of parameters such as Haugh Unit, Albumen Index, Yolk Index, Shell Percentage, Shell Strength & Net Profit.
- The supplementation of Probiotic at different inclusion rates influenced Net Profit per Dozen Eggs and is highest in Sporich-Total @ 100 g per ton of feed (Rs. 9.43) compared with control (Rs.8.67) during the 22-37 weeks of age.
- During the laying between 22-37 weeks of age, the broken eggs and soiled eggs production were within in the limitation and lowest in Sporich-Total @ 100g per ton of feed.
- Mortality rate was within the limits & one, only mortality recorded in Competitor-BS @ 100g per ton of feed, and no specific disease was recorded in all the treatments.
- It can be concluded that, supplementation of Multi Strain Probiotic Sporich-Total @ 100 g per ton of feed & Prome Max @ 100 g per ton of feed can be used as an alternative to AGP / Antibiotics (BMD) and have significantly better performance compared to the Single Strain Probiotic (Competitor-BS) and Control.
- Sporich-Total @ 100 g per ton of feed has exhibited better production performance of commercial White Leghorn layer birds during the 22-37 weeks of age.

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SPORICH™-total

In-Vivo Studies

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VENVI AGRO-INDUSTRIAL VENTURES CORPORATION
LAYER DEPARTMENT
Sporich Trial (June 4-July 6, 2018)

NECROPSY REPORT

I.

ANIMAL INFORMATION	
AGE: 63 wks	BUILDING: #7
BREED: Shaver	DATE OF NECROPSY: June 11, 2018
FEED TYPE: AL2 rev G	LIGHTING HOURS: 5am-7:30pm

II.

HISTORY:

Clinical Signs:

- **Wet droppings**

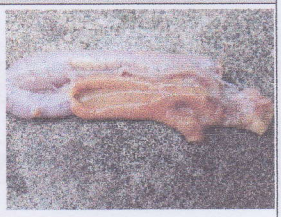
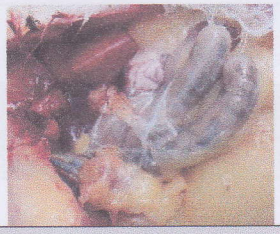
Medication/Vaccination:

- Norfloxacin 10 for 7 days
- Norfloxacin 200 for 5 days
- Vitamin C and Cotixine (Electrolytes) for 3 days
- Linco-spectin for 7 days(on-going)
- Acid Pak Medication
- Natustat Feed Premix (March 19-April 19,2018)

III.

POST-MORTEM EXAMINATION:	
EXTERNAL/INTEGUMENTARY SYSTEM	
• No gross lesion found	
RESPIRATORY SYSTEM	
• No gross lesion found	

MUSCULO-SKELETAL SYSTEM	
*No gross lesion found	

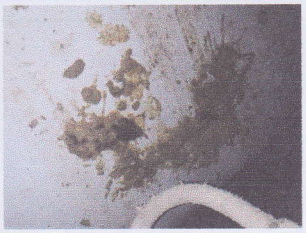
DIGESTIVE SYSTEM	
STOMACH: • *Filled with abdominal fats* **PROVENTRICULUS & GIZZARD:** • *Filled with fats* • *Erosion both sample 1 and 2* • *Thickened proventriculus* **SMALL INTESTINES** • *Accumulation of mucous* • *Petechial hemorrahges* **CECUM** • *Dark frothy content* • *Petechial hemorrhages in the cecal junction*	  

NERVOUS SYSTEM	
*Not Examined	

IV.

POST MORTEM ANALYSIS:
After trial, we conducted necropsy and it shows that there is an erosion in the gizzard and a thickened proventriculus. There's still an accumulation of mucous and a petechial hemorrhages in the small intestines. A dark frothy content and a petechial hemorrhage in the cecum were also found.

V.

EFFECT ON MANURE		
After 2 weeks of trial we observed that there's already changes in manure consistency. But after the 30 days trial wet droppings resumed.	Before:	
	During:	
	After:	

VENVI AGRO-INDUSTRIAL VENTURES CORPORATION
LAYER DEPARTMENT
Sporich Trial

PRODUCTION REPORT

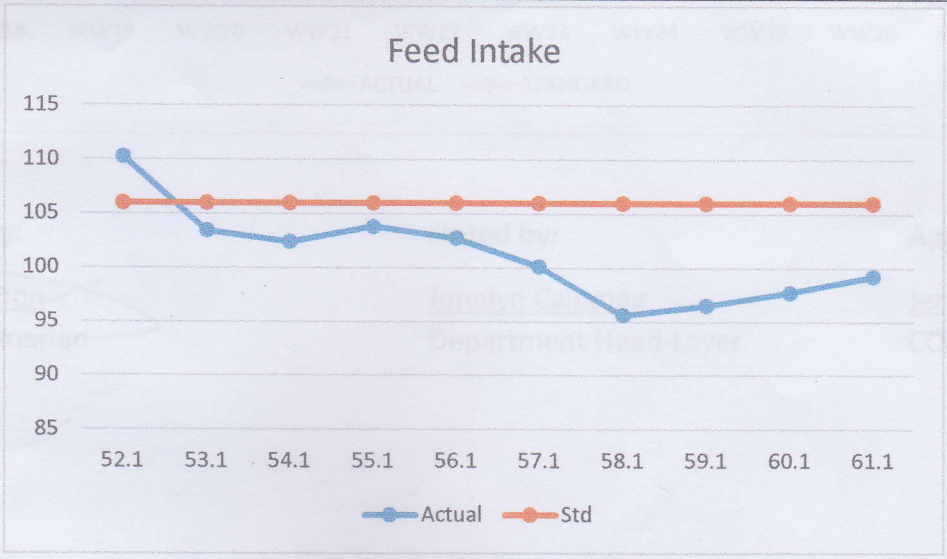
I.

ANIMAL INFORMATION	
AGE: 63 wks	BUILDING: #7
BREED: Shaver	LIGHTING HOURS: 5am-7:30pm
FEED TYPE: AL2 rev G	

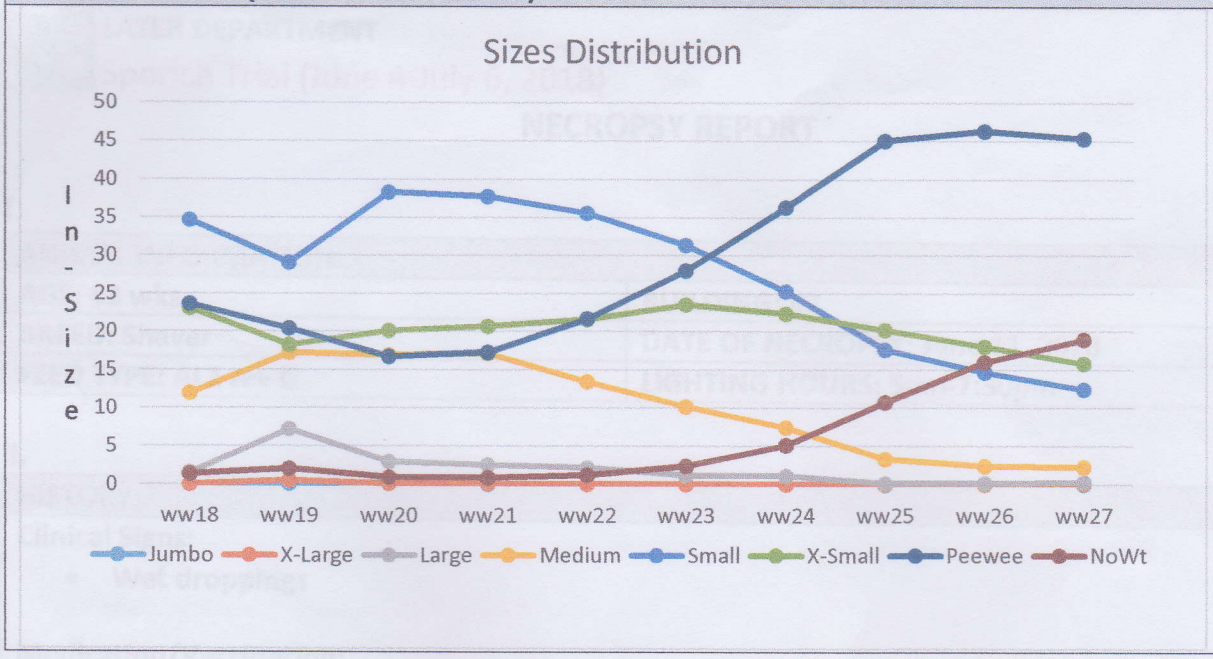
II.

Production (Trial starts at 57.1wk)

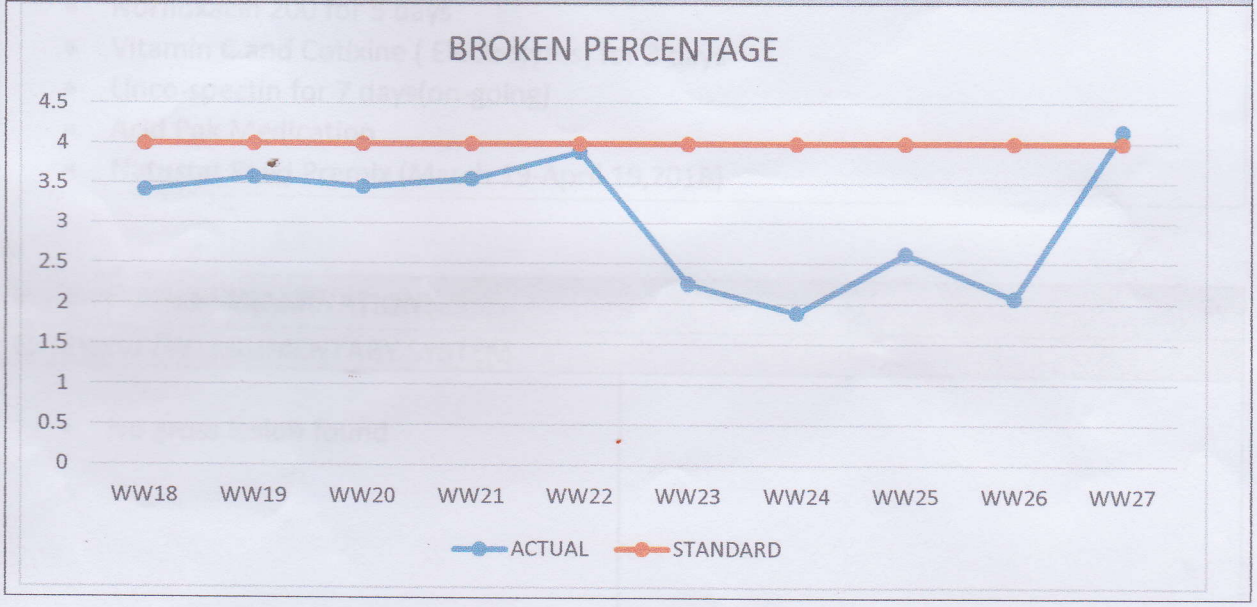
Feed Intake



Size Distribution (Trial starts at WW23)



Broken Percentage



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サンザイム
Sanzyme Biologics

Effects of SANZYME BIOLOGIC's, **Sporich™- Total** on Body Weight, Feed Conversion Ratio, Litter Condition & Foot Health of Broiler Chicken

Study **Location:** Broiler Farm - Hyderabad

Study **Period:** May - Jun 2017 (40 days)

Experimental Design

Groups	Description	
T1	Practical Control	PC
T2	PC + **Sporich™-Total**	PC + SPT

Controlled Inputs

1	T1 & T2 were fed Mash Feed throughout the Production Cycle
2	T2 was supplemented with **Sporich™-Total** from Day "0" to Day "7" through drinking water @ 50 g / 1000 Birds / Day
3	T2 was supplemented with **Sporich™-Total** from Day "8" to Day "40" through Mash Feed @ 100 g / Ton
4	**Sporich™-Total** is a proprietary blend of *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB • Potency used for experiment = 4 Billion CFU/g

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Growth Performance

Groups	Description	Body Weight	FCR
T1	PC	2.92	1.719
T2	PC + SPT	2.98	1.699

Litter Condition on Day of Culling

Foot Health on Day of Culling

Conclusions:

1. **Sporich™-Total** increases Body Weight by 60g as compared to Control
2. **Sporich™-Total** improves FCR by 1.18% as compared to Control
3. **Sporich™-Total** helps maintaining Dry Litter Conditions
4. **Sporich™-Total** helps maintaining Good Foot Health of the Birds and prevents associated pathologies

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Effects of SANZYME BIOLOGIC's, **Sporich™- Total** on 1st Week Litter Condition of Broiler Chicks

Study **Location:** Broiler Farm - Hyderabad

Study **Period:** July' 2022 (7 days)

Controlled Inputs

1	Chicks were fed Pre Starter Crumbles
2	**Sporich™-Total** from Day "0" to Day "7" through drinking water @ 50 g / 1000 Birds / Day
3	**Sporich™-Total** is a proprietary blend of *Bacillus subtilis* SAN 144BS + *Bacillus coagulans* SAN 135BC + *Bacillus licheniformis* SAN 136BL + *Saccharomyces boulardii* SAN 158SB • Potency used for experiment = 4 Billion CFU/g

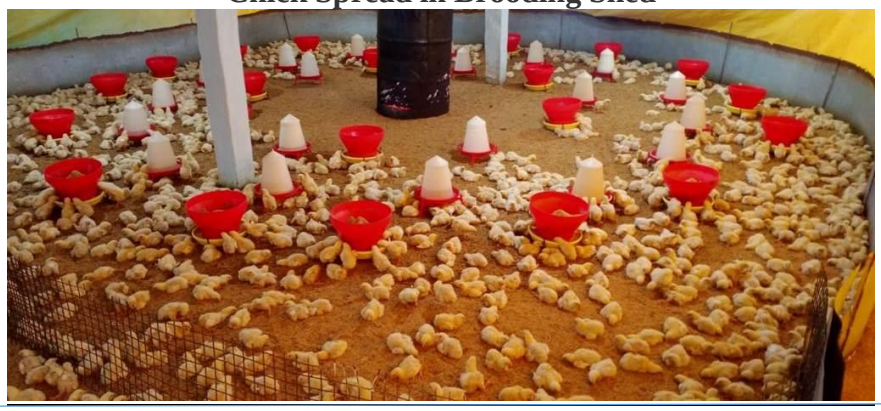
Litter with Silver Capping

3rd Day

4th Day

5th Day

Chick Spread in Brooding Shed



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