International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Antibodies Production Comparison against SRBC between Normal and Probiotic Treated Chicks

Shaik Haseenabi[1], Dr. Kondapalli Kasturi[2]

[1, 2]Department of Biotechnology, Acharya Nagarjuna University, India

Abstract: ***The present study was stated that the antibody production comparison against sheep red blood cells between probiotic treated and normal feed treated chicks. The totals of 3 batches were used for this experiment. First batch chicks were fed with normal feed treated as control and fed and 2nd group was treated with probiotics and feed diet and 3rd group was treated with normal diet but inoculation sample was formaldehyde-treated sheep red blood cells. When blood samples were taken as week intervals and go for the hemagglutination inhibition assay. The results were observed at weekly intervals, and the last week the antibody titer was observed highest in probiotic treated group shows the highest dilution at 1: 5120. Where as normal feed treated group contains dilutions of 1:1280 higher than the inactivated sheep red blood cells inoculated group of 1: 640. Inoculated sample quantity was increased as weeks increased as 0.5ml to 2ml at final week. The final large quantities of samples were stored in conical flasks for future purpose.***

Keywords: Hemagglutination inhibition test, SRBC, Probiotics, Antibody

1. Introduction

The nucleic acids of various viruses encode with surface proteins that agglutinate red blood cells from the hemagglutinin. which bound to sialic acid receptors on cells. The virus was l also bound to erythrocytes (red blood cells), caused the formation of a lattice. This phenomenon was called hemagglutination and the basis of a rapid assay to determined the levels of influenza virus present in a sample. The conducted assay, was two-fold serial dilutions of a virus were prepared, mixed with a specific amount of red blood cells, To this compound have been added to the wells of a plastic tray. The red blood cells and influenza settled in the bottom. The blood mixed with the virus this formed the agglutination and form the Lattice. The assay was formed for 30minutes.

HAI test was also one of the best methods used for viral infections. One of the viral infections like Infectious bursal disease (IBD) observed majorly in the poultry industry worldwide. It was an acute, highly contagious viral infection of young chicks. The economic importance of the disease was manifested in two ways; firstly some virus strains caused up to 20-30% mortality in three weeks age of older chickens. Hemagglutination inhibition tests were simple, and rapid and often the method of choice for assaying antibodies to influenza A virus. The test relied on the hemagglutination activity of virus HA and the ability of HA-specific antibodies to inhibit the virus from agglutinating erythrocytes Hemagglutination inhibition antibodies defined subtype-specific antigens on the virus particle, Also, HI assays have found wide application in the analysis of antigenic differences between strains in equine and human influenza surveillance.

Microflora of gut played an important role in boosting the immune system (Diarra et al., 2011). Intestine bacteria primarly Contact with the cells of the gut associated immune system (Haghighi et al., 2005), Stimulation of phagocytic activity (Matsuzaki et al 1998), Improvement of immunity by oral dose (Starvic, 1987)and Panda ***et al.*** (2008), Basophilic Hypersensitivity (Panda ***et al.*** (2003)., Immunoglobulin production to antigenic stimuli (Nahashon ***et al.,*** 1994), Antibodies production against SRBC (Haghighi ***et al***., 2005*).* (Huang ***et al***., 2004). Li ***et al.*** (2009). Higgins ***et al***. (2007)stated that improved the number of macrophages in the caecum as well as increased the phagocytic activities against *Salmonella enteritidis*.

In this study compared the production of antibodies in 3 groups. These are group treated with sheep red blood cells and inactivated sheep red blood cells and normal feed and probiotic feed treated batches.

2. Literature Survey

An indication of stimulated the mucosal immune system, which secreted immunoglobulin (IgA) in response to antigenic stimuli (Nahashon ***et al.,*** 1994) done by supplementation of probiotics in layers increased cellularity of Payer's patches in the ileum, High significantly more serum antibody (IgM) against SRBC (sheep red blood cells) Probiotic-treated birds have than birds that were not treated with probiotics (Haghighi ***et al***., 2005*). L.acidophilus* and *L. casei* in inactivated form enhance IgA titers in the serum of broiler chicks (Huang ***et al***., 2004). Li ***et al.*** (2009). Higgins ***et al***. (2007) stated that improved the number of macrophages in the caecum as well as increased the phagocytic activities against *Salmonella enteritidis*.

3. Materials and Methods

3.1 Experimental design

Newly hatched commercial broiler chicks of 0-6 days old used in this experiment. Total 30 chicks were used these were fed with normal diet and water.30 chicks were randomly divided into three groups in each group contains 10 chicks. The first group treated with Sheep red blood cells and probiotics and the second group treated with Formaldehyde treated sheep red blood cells with probiotics and third group treated as control fed with normal feed.

3.2 SRBC processing and used as antigen:

Erythrocytes from one sheep (SRBC), were collected weekly under sterile conditions and washed by centrifugation three times with 40 volumes of sterile Phosphate buffer solution. and bovine serum albumin homogenized as a 10% W/V suspension in phosphate-buffered saline and formaldehyde solution. The suspension was clarified by centrifugation at 3000 rpm for 10 minutes. The formaldehyde-treated sheep red blood cells were used for inoculation.

3.3 Inoculation process

0.5 ml of SRBC and bovine serum albumin was inoculated to all 30 chicks of 3 groups. The first week inoculated with 0.5ml of sheep red blood cells and 2 nd week increased the volume of the sheep red blood cells to 1ml and 3rd week increased the quantity of inoculum to 1.5ml and 4th week onwards to up to 70days with week interval add the 2ml of inoculum to all 30 chicks. Blood samples collection done for every week intervals up to 70days and allowed for hemagglutination inhibition test. At the final day collected the large quantity of blood sample and stored for future purpose.

3.4 Raising of hyperimmune serum

A total of 30 healthy chicks of 3batches were used for raising hyperimmune serum. All the chicks were de-wormed with albendazole at the dose rate of 5 mg per kg body weight and their serum was checked by indirect hemagglutination (IHA) test (Hussain ***et al.***, 2003) for antibodies against IBD, which was found to be zero. The study was conducted in 3 groups.

3.5 Hemagglutination Testing procedure

A commercially available virapur kit was used for this test for every week intervals.

3.5.1 Chickens RBC preparation:

1) 4 ml of blood is pipetted into a 15 ml conical and topped off with PBS.
2) Centrifuge the sample at 800 rpm for 10 minutes.
3) Discard the supernatant without disturbing the blood cells.
4) 12ml of phosphate buffer solution added and put in inverting.
5) Wash two more times by spin at 800 rpm for 5 minutes.
6) Discard the supernatant after final wash and add enough PBS to make a 10% solution of red blood cells. This solution is useable for one week.
7) Finally, concentration makes the working solution of 0.5% RBCs in PBS.

3.6 Sheep red blood cells Assay

1) Total 96 numbers of round-bottomed or Flat-bottomed plates were also worked but need to be placed at an incline to develop.
2) Add 50 µl PBS to each well.
3) Add 50 µl of SRBC sample to the first column.
4) Transfer 50 µl to the next well on its right and mix it well. Repeat mixing and transferring 50 µl to the well. Discard 50 µl from the last well.
5) Add 50 µl of 0.5% red blood cell working solution to each well. Mix gently. Leave it for 30 to 60mins.

3.7 Interpretations

- A dots in the center of round-bottomed plates formation indicates that result negative.
- A uniformly reddish color across the well indicates the positive results.
- The sheep red blood cells HA titer is a simple number of the highest dilution factor that produced a positive reading.

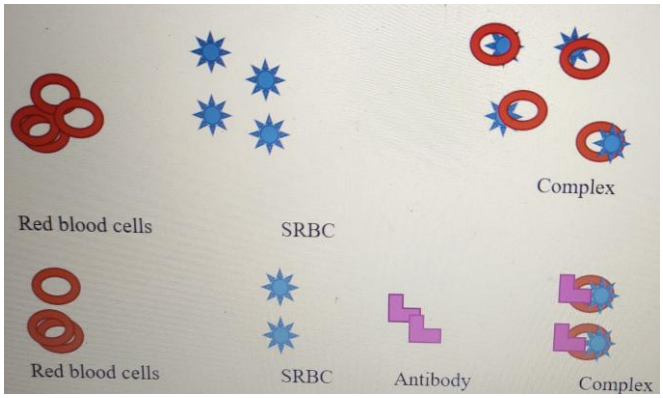


Figure 1: The procedure how the sheep red blood cells agglutinate with red blood cells

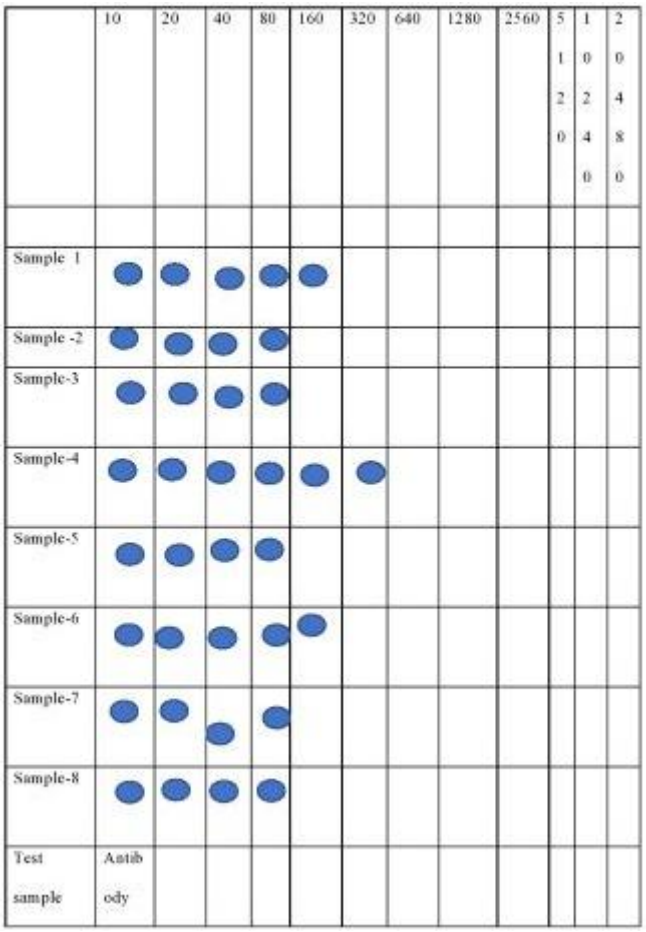
4. Results and Discussion

Table 1: Hemagglutination test procedure the arrow shows that the 2 fold serial dilution if dilution increase the antibodies production also shows in higher concentration.

- **Table 38 Hemagglutination test procedure The arrow shows that the 2 fold serial dilution If dilution increase the antibodies production also shows in higher concentration**

2 fold serial dilution →

	10	20	40	80	160	320	640	1280	2560	5120	10240
A											
B											
C											
D											
E											
F											
G											
H											
Test sample	Antibody										



Sample 1	160	Sample 5	80
Sample 2	80	Sample 6	160
Sample 3	80	Sample 7	80
Sample 4	320	Sample 8	80

- Table 2 example of the hemagglutination test Example of HI titers: The HI titer value is the last dilution factor of antibody showing completely inhibited hemagglutination. If antibodies bind to the antigen particles, the antigen is effectively blocked from causing hemagglutination.
- Table 3: Inoculation schedule formaldehyde-treated and direct SRBC in different groups of chicks. The table shows that from zero to56 th day when the concentration of the SRBC increases the antibody production also increases in probiotic treated group than control group.

Inoculation day	Concentration of SRBC	SRBC inoculated with normal feed	SRBC with formaldehyde-treated	SRBC with probiotic and feed
Zero-day	0.5	0.5	0.5	0.5
7	1.0	1.0	1.0	1.0
15	1.0	1.0	1.0	1.0
21	1.5	1.5	1.5	1.5
35	1.5	1.5	1.5	1.5
42	2	2	2	2
56	2	2	2	2
70	2	2	2	2

Table 4: HIU dilutions of SRBC at different groups. SRBC with formaldehyde -treated group shows antibody production from zero to 70 days as 1280 to 640.Concentration of the SRBC increase as 0.5 to 2 ml The antibodies production was also high as 1280 to 5120

Inoculation day	Concentration of SRBC/ml	SRBC inoculated with normal feed	SRBC with formaldehyde-treated	SRBC with probiotic and feed
Zero-day	0.5	Nill	Nill	Nill
7	1.0	320	1280	1280
15	1.0	320	640	1280
21	1.5	320	640	2560
35	1.5	640	640	2560
42	2	640	640	5120
56	2	1280	640	5120
70	2	1280	640	5120

Table 5: Shows that HAU in log units shows highest numbers in group of probiotic with SRBC treated group than another two groups of formaldehyde treated and control feed treated

Inoculation day	Concentration of SRBC	SRBC inoculated with normal feed	SRBC with formaldehyde-treated	SRBC with probiotic and feed
Zero-day	Nill	Nill	Nill	Nill
7	0.5	Log 6	Log 8	Log8
15	1	Log 6	Log7	Log8
21	1.5	Log6	Log 7	Log9
35	1.5	Log7	Log 7	Log9
42	2	Log7	Log 7	Log10
56	2	Log8	Log 7	Log10
70	2	Log8	Log7	Log10

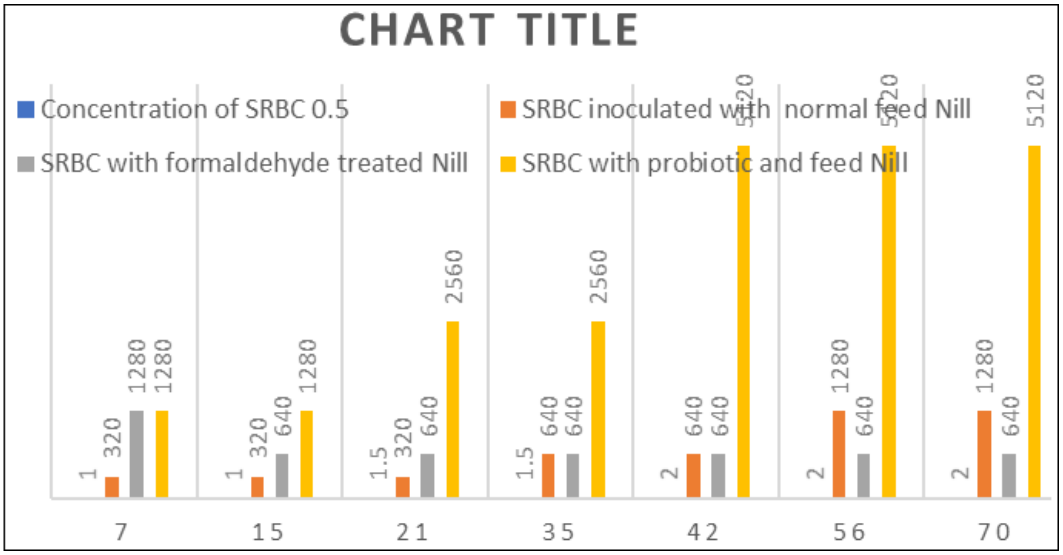


Figure 2: Shows that Concentration of antibodies at different groups. Shows highest concentration of the antibodies of 5120 observed at probiotic feed treated group than the formaldehyde and control group

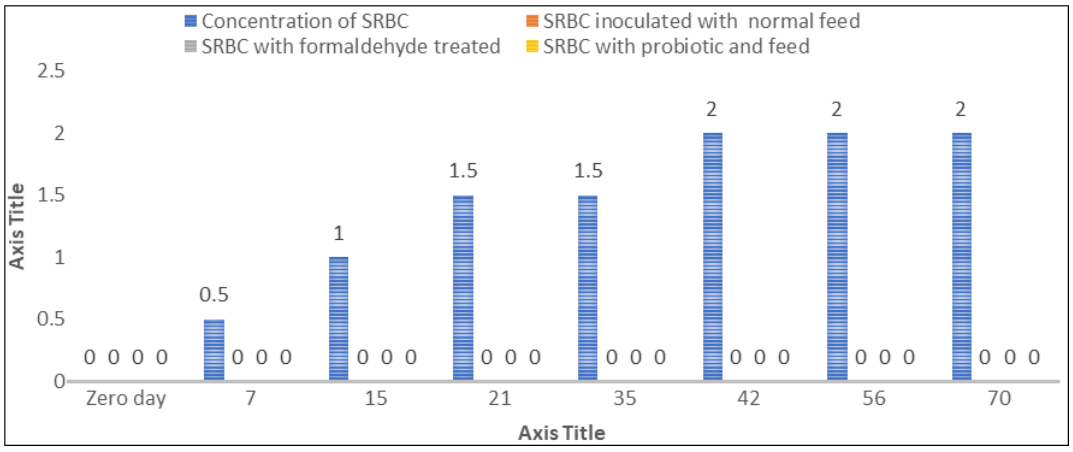


Figure 3: Shows that HAU in log units. Observed that the when the concentration of the SRBC increases as the production of the antibodies also increases. Highest observed at 2ml concentration of the antigen treated group

Table-1 explained the hemagglutination inhibition test procedure in table form. Table:2 was an example of the hemagglutination inhibition test. Table -3 that the concentration of the inoculum was inoculated to the 3 groups for week intervals all 3 groups were inoculated with the same concentration of inoculum and there was no change in concentration. Table -4 showed that the final dilution of the antibody titer the group control was showed that the dilution of antibodies low than the dilution titer of the inactivated sheep red blood cells inoculated sample and showed the highest dilution in the group of the probiotic treated group.Figure -1Procedurical diagram how the sheep red blood cells agglutinate with antibodies.Figure-2 showed that concentration of the antibodies at different concentration of inoculum added at week interval. The highest concentration of the antibodies showed at last week at 2ml of inoculum concentrations.Figure-3 showed that the concentration of the antibodies at log levels at different at concentrations of log 10 at a final concentration of 2ml at the final week of the group treated with probiotics as a feed, then inactivated sheep red blood cells inoculated group than normal feed.

5. Results and Conclusion

According to the above experiment, I conclude that the concentration of antibodies by Hemagglutination inhibition test was showed that highest concentration of antibodies present in the probiotic treated group than the control and inactivated sheep red blood cells inoculated group because of probiotics were induced the immunity naturally than the control group.

6. Future Scope

This data compailed to show variation in antiboies production bet ween the probiotic treated group chick and non probiotic treated chicks.I found the success in this experiment found that highest antibodies production in probiotic treated chicks.

References

[1] Diarra.Youcef, Mehdi, Marie -Pierre, Letourneau-Montminy, Marie-Lou gaucher, Younes Chorfi, Gayatri Suresh, tarek Rouissi, Satinder kaur Barar, Caroline Cote, Antonio Avalos Ramirez, Stephane Godbout.

(2011), Use Of antibiotics in broiler production Global Impacts and alternatives, 4 (2):170-178.

[2] Haghighi HR1, Gong J, Gyles CL, Hayes MA, Sanei B, Parvizi P, Gisavi H, Chambers JR, Sharif S. (2005) Modulation of antibody-mediated immune response by probiotics in chickens. Clin Diagn Lab Immunol. 2005 Dec;12 (12):1387-92.

[3] Huang and Lin, Ockerman and Basu, B. K. An, B. L. Cho, S. J. You, H. D. Paik, H. I. Chang1, S. W. Kim2, C. W. Yun1 and C. W. Kang* (2004), Growth performance and antibody Response of Broiler Chicks Fed Yeast Derived Beta -Glucan and Single -Strain probiotics, *Asian-Aust. J. Anim. Sci.* 21 (7):1027-1032.

[4] Hussain Asma Latif1, Zunaira Akhtar2, Riasat Wasee Ullah1*, Aamer Bin Zahur1, Aman Ullah1, Hamid Irshad1, Adnan Rashid Malik3, Munib Hussain1, Khawar Mahboob4, Shahida Afzal4 Evaluation of Haemagglutination Assay (HA) for the Detection of Peste des Petits Ruminants Virus (PPRV) in Faecal Samples of Recovered Goats.Latif et al (2014). Evaluation of HA for PPRV Diagnosis 11, ISSN 2308–2798

[5] Higgins, Huang, Timmerman, Vicente. (2007). Effect of Probiotic on Salmonella Enteritidis infection on broiler chickens, A journal of Egypt Chemistry. 2 (2):298:314.

[6] Matsuzaki.s, Hao Li, Man-Li, Ma, Hui-Jun Xie, Jian Kong, (1998) Biosafety evaluation of bacteriophages for treatment of diarrhea due to intestinal pathogen *Escherichia coli* 3-2 infection of chickens, World Journal of Microbiology and Biotechnology, 28 (1):1-6.

[7] Nashashon, Abdulrahim, (1994) Effect of probiotic on laying hen diets on yield performance and serum and egg yolk cholesterol. Article Food Additives and Contaminants, 21 (9):817-23.

[8] Panda, Liopis, Cao, X.J Yang. (2008) Effect of probiotic (Lactobacillus sporogenes) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. Journal of the Science of Food and Agriculture, 88 (1):43 – 47.

[9] Panda, Jin, Kalavathy. (2003)Dietary Supplementation Of probiotic On growth, Serum cholesterol and gut microflora of broilers, The Indian Journal of animal sciences, 71 (5):488-490.

[10] Starvic.V, Leonchuk, Tsimokh, Chowdhary. (1987), Effect of probiotic (Saccharomyces cerevisiae) addition to diets on Intestinal Microflora and Performance Of Hy-Line Layers Hens. Journals Of American Science, 6 (11).

[11] Li, A. Sarsenbek, *† T. Wang, *1 J. K. Zhao, † and W. Jiang † (2009), Comparison of carcass yields and meat quality between Baicheng-You chickens and Arbor Acres broilers, A journal of Poultry science92:2776-2782.