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Effect of early post-hatch feeding with synbiotics on immunity of broilers

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Abstract
Early feeding is vital for the rapid growth and immune system development of newly hatched chicks, greatly enhancing their resistance to diseases. A total of 144 day-old chicks were randomly divided into four treatment groups, each consisting of 9 birds per replicate, with 4 replicates per group. The Control group received synbiotics in water and pre-starter feed for 24 hours upon arrival at the farm. The Negative Control group was given water and pre-starter feed without synbiotics. In the Treatment Transport group, birds received synbiotics via beak dip at the hatchery and continued with synbiotics at the farm for 24 hours. The Treatment On-farm group (OF) provided birds with pre-starter feed and water containing synbiotics immediately after hatching on the farm for 24 hours. The mean log2 values of HI antibody titer and the mean percentages of heterophils, lymphocytes, monocytes, eosinophils, and basophils showed no significant statistical differences in blood samples collected on the 7th, 21st, and 42nd days across the Control, Negative Control, Transport, and OF treatment groups. On the 42nd day of biochemical investigation, the mean albumin (g/dL) and the albumin-to-globulin ratio were significantly lower ($p<0.05$) in the Control group compared to the Negative Control, Transport, and OF groups, whereas globulin value was significantly lower ($p<0.05$) in Control group compared to Negative Control, Transport and OF groups. The spleen, thymus, and bursa maintained their normal histomorphological structure on days 7, 21, and 42.

Keywords: Early post-hatch feeding, synbiotics, immunity

Introduction

In hatcheries, it is common to remove all chicks once the majority have hatched, leading to early-hatched chicks being deprived of feed for extended periods. Additional hatchery procedures such as sexing, vaccination, packaging, and Transportation further delay feeding (Batal, A. B., and Parsons, C. M, 2002) [6]. Prolonged post-hatch holding periods can result in dehydration and energy loss, negatively impacting chicks. Delayed feeding is associated with reduced weight gain, increased mortality, poor vaccination response, slower development of the gastrointestinal tract and immune system, decreased disease resistance, and long-term performance issues (Panda *et al.*, 2015) [21]. Early feed intake is crucial for the rapid development of the immune system in newly hatched chicks. The transition from yolk sac nutrients to exogenous feeds supports the functional and structural development of the gut (Geyra, *et al.*, 2001) [9]. Nutrient substrates from feed are essential for the growth of primary and secondary lymphoid organs (Dibner, J. J., and Richards, J. D, 1998) [8]. The mucosal immune system, in particular, requires oral feed intake for rapid development (Bar Shira *et al.*, 2005) [5]. Without adequate feed, chicks are highly susceptible to infections, leading to increased early mortality due to reduced disease resistance (Juul-Madsen *et al.*, 2004; Mast and Goddeeris, B 1999; Yunis *et al.*, 2000) [12, 18, 25]. Early dietary restriction can impair systemic immune competence due to metabolic changes and reduced growth of lymphoid organs (Juul-Madsen *et al.*, 2004, Hulsewe *et al.*, 1999; Nathan *et al.*, 1977) [12, 11, 20]. The thymus, a primary lymphoid organ, is particularly sensitive to feed deprivation, resulting in a rapid decline in cellularity and weight gain (Klasing, K, 2007) [14]. Thus, early feeding is vital for the optimal growth and immune development of broiler chicks.

Materials and Methods

Broiler birds, feeding, management and dietary treatments

The trial was conducted in the months of February and March with the approval of the Institutional Animal Ethics Committee of the Veterinary College, Anand, Gujarat, India (No. 417/AN/23). The experimental birds were reared at the Veterinary College, Anand, and samples were analyzed there as well. The experimental birds were randomly allotted into four treatment groups: Control, Negative Control, Transport, and OF. Each group had 36 birds, divided into four replicates of 9 birds each.

The experimental feed for all groups was prepared according to the BIS (2007) [7] standards for broiler chickens, divided into pre-starter (0 to 7 days), starter (8 to 21 days), and finisher (22 to 42 days) phases. These diets were given based on daily requirements. Synbiotics were added to the drinking water (1g/liter) for the first 24 hours in the treatment groups. After this period, normal feeding and watering resumed for all groups.

In the Control group, birds received synbiotics-supplemented water and pre-starter feed upon arrival at the farm from the hatchery for the first 24 hours. The Negative Control group received pre-starter feed and water without synbiotics upon arrival. In the Treatment Transport group, synbiotics were administered via the beak dip method at the hatchery and continued with synbiotics-supplemented water and pre-starter feed at the farm for the first 24 hours post-hatch. In the Treatment On-farm group, synbiotics-supplemented water and pre-starter feed were provided as soon as the chicks hatched on the farm.

Table 1: Proportion of feed ingredients (%) used in pre-starter, starter and finisher diets

	Ingredients	Name of Mash		
		Pre-starter	Starter	finisher
		Qunt. (kg)	Qunt. (kg)	Qunt.(kg)
1	Maize	56.00	57.00	60.00
2	Soyabean DOC	37.60	35.50	30.00
3	Deoiled Rice Bran	1.50	1.19	2.19
4	Calcite Powder	1.37	1.36	1.36
5	DCP	0.96	1.00	1.00
6	Vitamins	0.05	0.05	0.05
7	Vitamin-B12	0.01	0.01	0.01
8	Trace Minerals	0.10	0.10	0.10
9	Choline Chloride-60%	0.10	0.10	0.10
10	Lysine	0.05	0.05	0.05
11	Methionine	0.15	0.13	0.13
12	Phytase-5000	0.01	0.01	0.01
13	Enzymes	0.05	0.05	0.05
14	Salt	0.25	0.25	0.25
15	Sodium Bicarbonate	0.10	0.10	0.10
16	Livertonic	0.10	0.10	0.10
17	Immunomodulator	0.05	0.05	0.05
18	Toxin Binder	0.10	0.10	0.10
19	Emulsifier	0.05	0.05	0.05
20	Probiotic	0.05	0.05	0.05
21	Anticoccidial	0.05	0.05	0.05
22	Oil	1.30	2.70	4.20
	Total	100.00	100.00	100.00
	Calculated Crude protein	23.50	22.45	20.28
	Calculated Metabolizable Energy (ME kcal/kg feed)	2986.67	3098.76	3199.88

Measures of traits

1. Antibody Titer against ND Vaccine: The effects of early post-hatch feeding with synbiotics on immune response were evaluated following standard procedures. Blood samples were collected on the 7th, 21st, and 42nd days of age to analyze the antibody titers against the Newcastle disease vaccine (NDV).

2. Haematological Investigation (Differential Leucocyte Counts): Blood samples were taken from one bird per replicate in each treatment group on the 7th, 21st, and 42nd days. Immediately after collection, thin blood smears were prepared on clean, grease-free slides, dried at room temperature, and fixed in alcohol. Subsequently, the smears were stained using field's stain, allowed to dry, and then examined microscopically under an oil immersion objective (100X) to perform differential leucocyte counts

3. Histological Examination of Immune Organs (Spleen, Thymus and Bursa of Fabricius): Throughout the experimental period, on the 7th, 21st, and 42nd days, one bird per replicate from each treatment group was humanely sacrificed. The thymus, spleen, and bursa of Fabricius were collected for histological examination and preserved in a 10% formalin solution. Tissue sections, cut to a thickness of 4-5 microns using an automatic microtome (Leica, Germany), were stained with Haematoxylin and Eosin (H&E) stains [15] for microscopic analysis.

Statistical analysis

Data from the Completely Randomized Design were analyzed using the methods of Snedecor and Cochran (2014) [23]. The means of replicates within each treatment were analyzed using SPSS software (version 20).

Result and Discussion

Antibody titer against NDV vaccine

The HI log2 values (Mean±S.E.) for the ND vaccine in different groups at weeks 1, 3, and 6 are shown in table 2. The mean log2 values of HI antibody titer on 7th, 21st and 42nd days non-significant difference in Control, Negative Control, Transport and OF groups. Martins *et al.* (2023) [17] observed no significant differences in serum IgY titers against the NDV vaccine between groups subjected to fasting (with or without feeding) and those supplemented with CLA (0% or 0.025%). Similarly, Alireza *et al.* (2022) [3] reported no significant difference in antibody titers between early and delayed synbiotics supplemented groups within the first 24 hours of life. Kadam *et al.* (2009) [13] found no significant differences (p>0.05) in antibody titers against the ND vaccine among Control and polyherbal supplemented groups (6 & 8 grams) at 7, 21, and 35 days. Contrarily, Arulnathan *et al.* (2019) [4] reported significantly higher (p<0.05) HI antibody titers against the NDV vaccine in early feed supplemented groups compared to the Control group.

Table 2: Determination of antibody titer against ND vaccine of experimental broilers under feeding experiment

PERIOD	Control	Negative Control	Transport	OF	CD	SE
Day 7	4.50±0.29	4.50±0.29	5.75±0.85	4.75±0.25	NS	0.26
Day 21	6.50±0.29	6.25±0.25	6.50±0.29	7.00±0.41	NS	0.16
Day 42	7.25±0.48	7.50±0.50	7.75±0.48	7.75±0.48	NS	0.22

Biochemical investigations

Serum samples were collected to measure total protein, albumin, and globulin on days 7, 21 and 42. The results are shown in table 3. At 7-day albumin (g/dl) values observed significantly lower (p<0.05) in Transport group compared to control, Negative control and OF groups. On the 21st day albumin (g/dl) values showed significantly lower (p<0.05) in OF and Control groups compared to Transport and Negative Control. On the 42nd day also Globulin and Albumin to Globulin ratio followed same trend as 21st day. Higher level of globulin and lower level of Albumin to globulin ratio indicate an active immune response. Early feeding with synbiotics increases immune response. The findings of Hassan *et al.* (2023) [10], Mustafa (2021) [19], and Abousekken *et al.* (2017) [1] contrast with the present study, as they reported no significant differences (p<0.05) in total protein and albumin levels between early and delayed feeding groups. Adeleye *et al.* (2018) [2] findings are agreement with present study as they found significant difference (p<0.05).

Table 3: Biochemical Investigations (Total Protein, Albumin, globulin and Albumin: Globulin) of experimental broilers under feeding experiment

	Control	Negative Control	Transport	OF	SE	CD
Biochemical Investigations of 7th Day						
Total Protein	4.75±0.12	4.81±0.10	5.14±0.25	4.26±0.48	0.13	NS
Albumin	3.02^{ab}±0.14	3.16^{a}±0.22	2.14^{b}±0.38	2.56^{a}±0.42	0.19	0.96
Globulin	1.73^{b}±0.19	1.65^{b}±0.29	3.00^{a}±0.42	3.78^{a}±0.57	0.22	1.21
A/G	1.83^{ab}±0.29	2.25^{a}±0.66	0.81^{b}±0.24	0.73^{b}±1.03	0.33	1.20
Biochemical Investigations of 21st Day						
Total Protein	5.32±0.21	4.58±0.08	5.07±0.37	5.28±0.21	0.14	NS
Albumin	2.04^{b}±0.14	2.94^{a}±0.29	3.15^{a}±0.12	2.08^{b}±0.33	0.15	0.78
Globulin	3.28^{a}±0.27	1.65^{b}±0.22	1.92^{b}±0.30	3.20^{a}±0.35	0.21	0.83
A/G	0.65^{b}±0.09	1.92^{a}±0.35	1.44^{a}±0.26	0.71^{b}±0.48	0.19	0.71
Biochemical Investigations of 42nd Day						
Total Protein	5.47±0.19	5.22±0.07	5.13±0.05	5.4±0.08	0.10	NS
Albumin	1.51^{c}±0.19	2.93^{a}±0.15	2.64^{ab}±0.13	2.11^{b}±0.23	0.16	0.55
Globulin	3.96^{a}±0.29	2.30^{b}±19	2.49^{b}±0.10	3.30^{a}±0.26	0.21	0.69
A/G	0.40^{b}±0.08	1.32^{a}±18	1.07^{a}±0.10	0.66^{b}±0.24	0.12	S

Haematological investigation

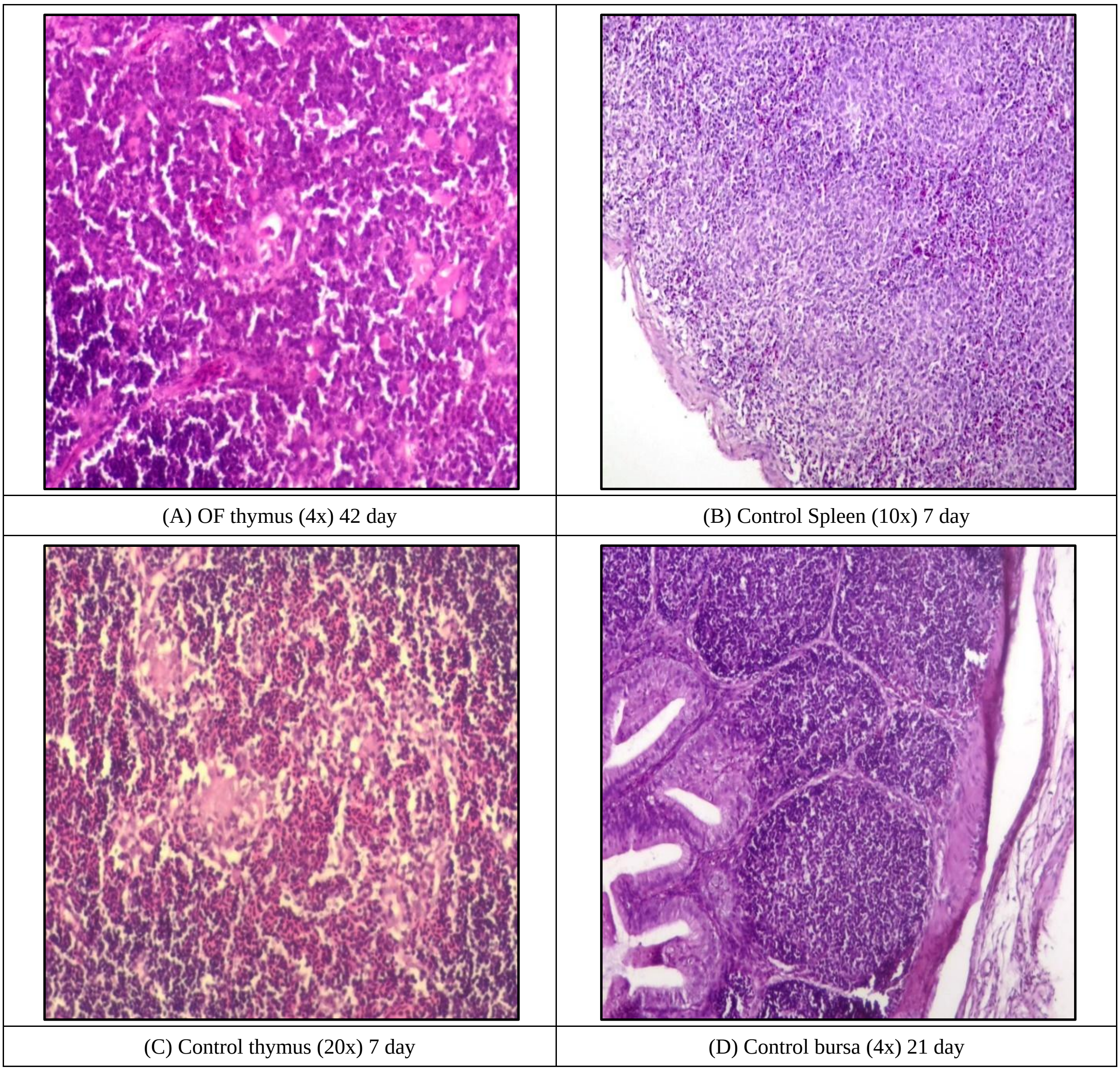
Data of DLC counts are presented in table 4. The mean percentages of heterophils, lymphocytes, monocytes, eosinophils, and basophils showed no significant statistical differences in blood samples collected on the 7th, 21st and 42nd days across the Control, Negative Control, Transport, and OF treatment groups. The findings of Madej *et al.* (2024) [16] are consistent with the present study, as they also observed no significant differences in the percentages of heterophils and lymphocytes between the HatchCare hatcher group and the standard hatcher chambers group at 7th days of age. In contrast to the present study, Tamboli *et al.* (2018) [24] reported a significantly higher (p<0.05) percentage of heterophils in early feed supplemented groups than delayed feed supplemented groups. Sarlak *et al.* (2016) [22] found significantly higher (p<0.05) heterophils counts and significantly lower (p<0.05) lymphocytes counts in early feed supplemented groups than delayed feed supplemented groups.

Table 4: Haematological investigation (Differential Leucocyte Counts) of experimental broilers under feeding experiment

	Control	Negative Control	Transport	OF	SE	CD
Day 7 DLC counts						
Heterophils (%)	20.13±1.19	19.93±1.10	20.89±0.7	19.11±2.39	0.68	NS
Lymphocytes (%)	66.32±1.13	66.66±1.60	69.23±1.53	65.89±1.8	0.76	NS
Monocytes (%)	4.16±0.47	4.70±0.65	4.55±0.5	5.84±0.46	0.29	NS
Eosinophils (%)	1.29±0.46	1.67±0.37	1.62±0.45	1.68±0.4	0.19	NS
Basophils (%)	0.40±0.46	0.74±0.32	0.39±0.16	0.47±0.03	0.10	NS
Day 21 DLC counts						
Heterophils (%)	33.83±0.82	32.25±0.32	31.50±0.72	31.48±1.46	0.48	NS
Lymphocytes (%)	59.75±1.00	62.03±0.36	62.33±1,44	61.68±0.8	0.51	NS
Monocytes (%)	3.62±0.10	3.33±0.17	3.50±0.17	3.42±0.49	0.13	NS
Eosinophils (%)	2.32±0.10	2.33±0.09	2.19±0.14	2.22±0.08	0.05	NS
Basophils (%)	1.00±0.12	0.81±0.26	0.50±0.09	0.89±0.06	0.08	NS
Day 42 DLC counts						
Heterophils (%)	39.68±0.77	37.33±0.35	37.33±0.43	37.50±1.66	0.50	NS
Lymphocytes (%)	52.92±0.56	57.15±0.45	57.42±0.92	55.48±2.61	0.79	NS
Monocytes (%)	3.17±0.21	3.40±0.14	4.26±0.16	3.57±0.26	0.14	NS
Eosinophils (%)	2.42±0.31	2.43±0.27	2.64±0.39	2.70±0.47	0.17	NS
Basophil (%)	0.50±0.09	1.00±0.14	0.67±0.15	0.69±0.09	0.07	NS

Histopathological Examination

All the birds were sacrificed on 7th, 21st and 42nd day of experiment Tissues like bursa of Fabricius, thymus and spleen were collected for histopathological examination, which depicted in Figure 1. During necropsy, no appreciable gross changes were observed in bursa of Fabricius, thymus and spleen of any experimental birds. On histopathological examination, normal architecture was observed in the sections of bursa of Fabricius from birds of group Control, Negative Control, Transport and OF. Normal architecture was also observed in the sections of thymus and spleen from birds of groups Control, Negative Control, Transport and OF on histopathological evaluation and are depicted in figure 1. Madej *et al.* (2024) [16] also found normal architecture structure in spleen, thymus and bursa of Fabricius in HatchCare hatchers birds and standard hatchers birds.



(A) OF thymus (4x) 42 day | (B) Control Spleen (10x) 7 day

(C) Control thymus (20x) 7 day | (D) Control bursa (4x) 21 day

Fig 1: Histomorphological observation of spleen, thymus and bursa of fabricius

Conclusion

Early post-hatch feeding with symbiotics leads to higher levels of globulin and lower level of Albumin to globulin ratio. Histopathological examination of bursa of Fabricius, spleen and Thymus were found to be normal architecture. Looking to this we concluded that early post-hatch feeding with synbiotics activates the immune response.

Application of Research

This research can be applied to rear commercial broilers by early post-hatch feeding with symbiotics for better health and prevention of diseases.

Research Category

Early post-hatch feeding with symbiotics

Abbreviations

OF-on farm, A/G-Albumin to Globulin ratio

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