



Effect of Delayed Feeding with synbiotics on Nutrient Digestibility, Carcass Characteristics and Gut Morphology of Broiler Chickens

Minnat Patel^{1*}, Safimahmad Vahora², Rajpura Rais³, Prashant Dabhi⁴, Dharmik Desai¹ and Mohsin M. Pathan⁵

¹Research Scholar, Department of Animal Nutrition,

Collage of Veterinary Science, Kamdhenu University, Anand (Gujrat), India.

²Research Scientist, Animal Nutrition Research Station,

Collage of Veterinary Science, Kamdhenu University, Anand (Gujrat), India.

³Assistant Professor, Department of Animal Science, Anand Agricultural University, Anand (Gujrat), India.

⁴Assistant Professor, Department of Pathology, Kamdhenu University, Anand (Gujrat), India.

⁵Assistant Professor, Department of Physiology and Biochemistry,

Collage of Veterinary Science, Kamdhenu University, Anand (Gujrat), India.

(Corresponding author: Minnat Patel*)

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ABSTRACT: The study was conducted to investigate the effect of delayed feeding with synbiotics on nutrient digestibility, carcass characteristics and gut morphology of broiler chickens. A total of 108 commercial broiler chickens were divided into three groups, each with 4 replicates containing 9 birds. The control group received water with synbiotics and pre-starter feed upon arrival at the farm from the hatchery, continuing for the first 24 hours. In the Treatment D12 group, synbiotics-supplemented water and pre-starter feed were provided 12 hours after the chicks arrived at the farm and in D24 after 24 hours. For the treatment groups, synbiotics (1g/liter) were added to the drinking water for the first 24 hours. Early access to feed and synbiotics added water group (control) resulted in numerically higher dry matter retention compared to delayed synbiotics supplemented group. The positive N balance was found to be improved significantly ($P < 0.05$) in D12 group as compared to Control and D24 groups. The carcass percentage, abdominal fat percentage, giblet percentage, small intestine weight and length and large intestine weight were found statistically similar in early and delay synbiotics supplementation groups. Gizzard weight was significantly higher ($P < 0.05$) in D24 group. D24 groups observed significantly higher ($P < 0.05$) villus height to crypt depth ratio in duodenal gut morphology compared to Control and D12 groups. Non-significant results in livability and other data carcass characteristics and gut morphology showed that there was no anyadverse effect of early or delay feeding in broilers.

Keywords: Delayed feeding, nutrient digestibility, carcass characteristics, gut morphology.

INTRODUCTION

Broiler feeding in the first days of life is one of the priority factors that could effect on growth, feed efficiency, uniformity and finally economic benefit. After chicks hatch out of the incubation machine, there is an interval in the hatching window during which chicks are deprived of feeds and water for up to 24 h (Liu *et al.*, 2020). Besides, owing to farm transportation and other factors, chicken feed deprivation time can attain 48 h or even 72 h (Boyner *et al.*, 2021). However, feed early acquisition can effectively stimulate the absorption of residual yolk in chicks, especially the digestion and utilization of hydrophilic compounds such as glucose and protein (Wang *et al.*, 2020), and then ameliorate gastrointestinal development (Reicher *et al.*, 2020), which is conducive to the growth and development of chicks and the maintenance of

homeostasis (Jha and Yadav 2019; Lingens *et al.*, 2021). Since the gastrointestinal tract is the main location for nutrition absorption, rapid gut growth is essential for increasing body weight and overall performance. It has been demonstrated that intestine growth, slaughter weight, and carcass yield are negatively impacted by delayed post-hatch availability to feed (Halevy *et al.*, 2000; Noy & Uni 2010). A meta-analysis study done for the effect of post-hatch feed and water deprivation (PHFWD) shows significant sub normality in the small intestine segment with reduced length and relative weight of duodenum, jejunum, and ileum, and shorter villus height and crypt depth during the first week of age (de Jong *et al.*, 2017). In the post-hatch period, there is the rapid development of intestinal length, weight, and its enzymatical activities, where delay in feeding causes a reduction in development and expression of nutrient transporters

affecting absorption of nutrients (Yegani *et al.*, 2008). This study was undertaken with the objective to evaluate the effect of delayed feeding on nutrient digestibility, carcass characteristics and gut morphology of broiler broilers.

MATERIALS AND METHODS

Location. The experiment was conducted at poultry research station, collage of Veteinary science, Kamdhenu university, Anand and analysis work was carried out at animal nutrition research station and department of pathology, collage of veterinary science, Kamdhenu university, Anand.

Treatment groups and feeding standards. The experimental birds were randomly allotted into three treatment groups: Control, D12 and D24. A total of 108 broiler birds divided into three treatments which consist

of four replicates of 9 birds each. In the control group, birds received synbiotics-supplemented water and pre-starter feed immediately upon arrival at the farm from the hatchery for the first 24 hours. In Treatment D12, synbiotics supplemented water and pre-starter feed were given 12 hours after the chicks arrived at the farm. In Treatment D24, synbiotics supplemented water and pre-starter feed were offered 24 hours after the chicks arrived at the farm. The experimental feed for all groups was prepared according to the BIS (2007) standards for broiler chickens, divided into pre-starter (0 to 7 days), starter (8 to 21 days), and finisher (22 to 42 days) phases (Table 1). 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.

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

I. Nutrient retention and balance study

During the 6th week, a metabolic trial was performed on one bird per replicate, involving a 2-day adaptation period followed by a 3-day collection period. Birds were housed in individual deep litter systems to ensure precise control over feeding and watering. Data on feed intake, refusals, and excreta output were collected to assess nutrient utilization. Excreta samples were gathered using plastic sheets, with a portion preserved in concentrated H₂SO₄ for nitrogen analysis, while the remainder was oven-dried for dry matter estimation. These samples were pooled and analyzed for proximate composition, with nitrogen content determined using the Kjeldahl method (AOAC, 2000). Proximate analysis, along with calcium and phosphorus estimation, was conducted at the Animal Nutrition Research Station. Samples were ground to a 1 mm particle size using a cyclone mill. The proximate analysis of feed, leftovers, and dried excreta followed AOAC (2000) guidelines. Calcium estimation was performed according to ISI (1962) standards, and phosphorus was measured using a BIOMATE 3S spectrophotometer (Thermo Fisher) as per AOAC (2000) protocols.

II. Carcass characteristics

At 42 days of experiment, one bird from each replicate was randomly chosen, fasted for 12 hours, and then slaughtered using standardized methods. The pre-slaughter weight was recorded. After complete bleeding, the carcass was processed by removing feathers, skin, and eviscerating the bird. Organs such as the liver (excluding the gall bladder), heart (excluding the pericardium), and gizzard were collected and weighed separately. The dressed carcass and abdominal fat were also weighed. Giblet weight was calculated by summing the weights of the liver, heart, and gizzard. The dressing percentage was determined based on the pre-slaughter weight, while gillet and abdominal fat percentages were calculated relative to the dressed weight.

III. Histomorphological study of small intestine

On the 7th, 21st, and 42nd days of the experiment, one bird from each replicate in each treatment group was sacrificed. Intestinal segments (duodenum and jejunum) were collected to measure villi height, crypt depth, and the villi height to crypt depth ratio. These samples were preserved in 10% formalin. After evisceration, 2 to 3 cm sections of the middle duodenum and jejunum were removed, rinsed with PBS, and fixed in 10% neutral buffered formalin. The samples were processed using paraffin embedding for histomorphological examination. Sections were cut to 4-5 microns thickness using an automatic microtome (Leica, Germany) and stained with Haematoxylin and Eosin (H&E) stains (Luna, 1968). The H&E-stained slides were observed under a light microscope, and measurements were taken using ImageJ software. Intact lamina propria was used for villus selection. Villi height (VH) was measured from the villus crypt junction to the villus tip, crypt depth (CD) from the

base of the villus to the invagination between two villi, and villus width (VW) as an average of the apical and basal widths.

IV. Livability

Livability percentage was calculated by determining the proportion of surviving birds in each treatment group, accounting for any mortality during the experimental period.

V. Statistical analysis

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

RESULTS AND DISCUSSION

Nutrient Digestibility (Retention). The data pertaining to nutrient intake and utilization is presented in table 3. The dry matter retentions recorded as percentage values for the treatments as control, D12 and D24 were 71.31 ± 5.65 , 70.00 ± 6.01 and 69.07 ± 5.33 , respectively. likewise organic matter retention values were 75.39 ± 4.26 , 79.53 ± 2.74 and 81.73 ± 4.90 for respective groups. It was found that early or late access to feed and water with synbiotics supplementation resulted in similar retention values for dry matter and organic matter. The dry matter retention numerically increased upon early access to feed and water with synbiotics supplementation control compared to D12 and D24. Crude fat retention recorded as a percentage of feed intake for treatments control, D12 and D24 was determined to be 80.81 ± 3.06 , 81.20 ± 2.96 and 80.45 ± 5.84 , correspondingly. Similarly, 44.00 ± 3.98 , 40.41 ± 2.69 and 39.83 ± 4.21 values for crude fiber retention as a percentage of treatments control, D12 and D24, respectively. The findings of Ojebiyi *et al.* (2022) are in agreement with the findings of the present study. The immediate supply of feed and water did not significantly affect the retention of dry matter, crude fat and crude fiber. The current study contradicts the findings of Obun and Osaguona (2013), who observed a significant ($P < 0.05$) increase in dry matter and crude fiber retention in the 12, 24 and 36-hour fasting groups compared to the 48, 60 and 72-hour fasting groups.

Balance Studies. The results indicate (Table 3) that the average daily positive nitrogen balance (g/bird) for control, D12 and D24 was 2.67 ± 0.22 , 5.20 ± 0.31 and 3.03 ± 0.13 . The average daily positive calcium balance values were 0.65 ± 0.09 , 0.75 ± 0.06 and 1.14 ± 0.15 , whereas for positive Phosphorus balance values were 0.20 ± 0.02 , 0.40 ± 0.06 and 0.25 ± 0.06 for treatments control, D12 and D24, respectively. The positive N balance and positive Phosphorus values were found to be improved significantly ($P < 0.05$) D12 group as compared to Control and D24 groups. Whereas positive Ca balance found statistically similar. Obun and Osaguona, (2013) observed a decrease in ileal digestibility of crude protein due to delay access to feed and water of broilers which support our findings of a significant ($P < 0.05$) decrease in N-balance observed in the D24 group. Vieira *et al.*, (1999) suggested that the

initial fasting period after hatching increases which leads to decrease in the level of trypsin enzyme in intestine. However, the revelations of the present study with respect to 24 hours delay access to feed and water

(D24) are in contradiction with the findings of Ojebiyi *et al.* (2022). They observed a non-significant change in crude protein availability due to the delay access to feed and water.

Table 2: Average nutrient retention (%) of experimental broilers during metabolic trail.

Nutrient	Control	D12	D24	CD	SE
Dry Matter	71.31 ± 5.65	70.00 ± 6.01	69.07 ± 5.33	NS	2.97
Organic Matter	75.39 ± 4.26	79.53 ± 2.74	81.73 ± 4.90	NS	2.27
Crude Fat	80.81 ± 3.06	81.20 ± 2.96	80.45 ± 5.84	NS	2.09
Crude Fiber	44.00 ± 3.98	40.41 ± 2.69	39.83 ± 4.21	NS	2.01

Table 3: Means for balance (g/day/bird) of nitrogen, calcium and phosphorus of experimental broilers under feeding experiment.

	Control	D12	D24	SE	CD
Nitrogen					
Total Intake	3.72 ^b ± 0.09	6.34 ^a ± 0.35	3.94 ^b ± 0.21	0.38	0.98
Excreted in Faeces	1.05 ± 0.22	1.15 ± 0.13	0.91 ± 0.23	0.11	NS
Balance	2.67 ^b ± 0.22	5.20 ^a ± 0.31	3.03 ^b ± 0.13	0.36	0.89
Calcium					
Total Intake	1.12 ± 0.04	1.42 ± 0.18	1.16 ± 0.10	0.07	NS
Excreted in Faeces	0.47 ± 0.11	0.51 ± 0.07	0.41 ± 0.13	0.06	NS
Balance	0.65 ± 0.09	0.92 ± 0.16	0.75 ± 0.06	0.07	NS
Phosphorus					
Total Intake	0.56 ± 0.02	0.71 ± 0.09	0.58 ± 0.05	0.04	NS
Excreted in Faeces	0.36 ± 0.00	0.31 ± 0.04	0.32 ± 0.09	0.03	NS
Balance	0.20 ^b ± 0.02	0.40 ^a ± 0.06	0.25 ^{ab} ± 0.06	0.04	0.16

Carcass characteristics. The data pertaining to the average carcass characteristics per bird are presented in table 4. The dressing percentage recorded for treatments control, D12 and D24 were 60.83 ± 0.87, 61.51 ± 0.51 and 60.51 ± 1.52. The observed dressing percentage for various treatments was statistically similar, indicating that delay access to feed and water with synbiotics supplementation after hatching did not significantly affect the dressing percentage of broiler birds. Similar to our findings Alireza *et al.* (2022) observed that no difference in dressing percentage in early synbiotics supplementation birds' group than control group birds. Mahapatra *et al.* (2017) also supported result with present study that they found no significant in dressing percentage in one day early probiotic supplemented group than control group. These findings are divergent from Arulnathan *et al.* (2019), Abousekken *et al.* (2017) and Kadam *et al.* (2009) where they found significantly increased ($P < 0.05$) dressing percentage in early access to feed and water group compared to delay access to feed and water group.

For treatments control, D12 and D24 giblet percentage was 8.55 ± 0.67, 7.87 ± 0.37 and 9.69 ± 1.83, respectively, likewise abdominal fat percentage was 2.39 ± 0.45, 2.85 ± 0.28 and 2.42 ± 0.28 and 2.27 ± 0.08, respectively. there was no significant difference found in giblet and abdominal fat percentage. This finding of present study was agreement with Mahapatra

et al. (2017) and Alireza *et al.* (2022). They found not change significantly in abdominal fat percentage between the groups supplemented with early life synbiotics and those provided with a basal diet. The gizzard weight was significantly higher ($P < 0.05$) in D24 group (68.24 ± 3.78) compared to control (43.68 ± 2.06) and D12 (43.50 ± 1.40) groups.

For treatments control, D12 and D24 small intestine weight (g) was 74.39 ± 3.58, 64.56 ± 1.81 and 72.22 ± 3.48, likewise small intestine length (cm) was 142.75 ± 2.93, 162.5 ± 2.87 and 163.00 ± 10.23, respectively. The weight (gm) of the large intestine was in 8.81 ± 0.60, 9.45 ± 0.55 and 8.89 ± 0.19 in treatments control D12 and D24, respectively. There was no any difference observed in SI and LI weight and SI length in treatment groups. Khadem *et al.* (2018) investigated that there was non-significant difference in proportion of small and large intestine in early and delay access to feed and water groups at the age of 14th day, which shows similar results to the present study. In contrast to our findings, Ganjali *et al.* (2015) found significantly ($P < 0.05$) higher weight of gastrointestinal tract (percentage of live body weight) in 6 hours post hatch feeding group compared to 12 hours and 18 hours post hatch feeding groups. There was no any adverse effect found delay feeding and synbiotics supplementation in water. Synbiotics supplementation in water might be nullify the adverse effects of delay feeding.

Table 4: Carcass characteristics of experimental broilers under feeding experiment.

Treatment	Control	D12	D24	SE	CD
Pre-Slaughter Wt (g)	2143.75 ± 15.33	2093.75 ± 16.75	2146.25 ± 91.55	29.36	NS
Dressed Wt (g)	1303.90 ± 17.03	1267.37 ± 39.34	1321.20 ± 65.67	24.59	NS
Dressing %	60.83 ± 0.87	61.51 ± 0.51	60.51 ± 1.52	0.56	NS
Liver Wt(g)	57.40 ± 6.35	45.45 ± 2.86	48.05 ± 6.04	3.18	NS
Heart Wt(g)	10.33 ± 0.59	10.5 ± 0.66	10.03 ± 0.60	0.33	NS
Gizzard Wt (g)	43.68 ^b ± 2.06	43.50 ^b ± 1.40	68.24 ^a ± 3.78	4.20	13.48
Giblet Wt(g)	111.40 ± 8.50	99.45 ± 3.48	126.31 ± 21.13	5.36	NS
Giblet %	8.55 ± 0.67	7.87 ± 0.37	9.69 ± 1.83	0.46	NS
Abdominal Fat Wt (g)	31.08 ± 5.61	35.98 ± 3.05	32.25 ± 4.80	2.49	NS
Abdominal Fat %	2.39 ± 0.45	2.85 ± 0.28	2.42 ± 0.28	0.19	NS
SI Wt (g)	74.39 ± 3.58	64.56 ± 1.81	72.22 ± 3.48	4.37	NS
SI Length (cm)	142.75 ± 2.93	162.5 ± 2.87	163.00 ± 10.23	2.60	NS
LI Wt (g)	8.81 ± 0.60	9.45 ± 0.55	8.89 ± 0.19	1.53	NS
Livability %	94.44	97.22	94.44	2.76	NS

Gut Morphology. The information on the average gut structure of the duodenum and jejunum for each bird at days 7th, 21st, and 42nd is shown in Tables 5, 6 and 7. There was non-significant difference (P>0.05) observed regarding villi height, crypt depth, villi width and villi height to crypt depth ratio of duodenum and jejunum between Control, D12 and D24 groups, at the age of 7th and 21st days.

At 42nd day of age, duodenal crypt depth in Control group (441.16 ± 18.31) was significantly higher (P<0.05) compared to D12 (394.01 ± 2.17) and D24 (394.65 ± 2.54) groups. D24 groups (6.71 ± 0.12) observed significantly higher (P<0.05) villus height to crypt depth ratio in duodenal gut morphology compared to Control (6.15 ± 0.15) and D12 (6.37 ± 0.11) groups.

The control group (2577.11 ± 41.36) had significantly lower (P<0.05) jejunal villus height compared to the D12 (2789.53 ± 23.11), whereas D24 groups (2666.01 ± 51.02) was at par. Jejunal gut morphology revealed non-significant (P>0.05) difference in Villus width, crypt depth and villus height to crypt depth ratio among the treatment groups at 42nd day of age.

The findings of Li *et al.* (2022) remained contradict to that of the study where it was found that chicks

received feed immediately after hatching resulted in a significantly increased duodenal villi height of broilers as compared to chicks received feed after 24 hours of hatching. Arulnathan *et al.* (2019) also observed that there was significantly (P<0.05) increased villi height and crypt depth of growth promoter supplemented group compared to basal diet supplementation group. Similarly, Adeleye *et al.* (2018), where they found significantly (P<0.05) increased villi height in early fed group compared to 48 hours delayed fed group, but the crypt depth of present study is similar to them. The findings of Liu *et al.* (2020) align with the present study, where they found no significant difference in jejunal villus height between delayed access to feed for the initial 24 hours and immediate access to feed after hatching. Delayed access to feed post-hatch been shown to adversely affect intestinal development and gut morphology (Halevy *et al.*, 2000; Noy & Uni, 2010). However supplementation of synbiotics in water to delay feeding groups (D12 and D24) might be prevent adverse effects on intestinal development and gut morphology (Liu *et al.*, 2020, Arulnathan *et al.* 2019).

Table 5: Histomorphological observation of Duodenum and Jejunum (µm) of experimental broiler under feeding experiment at the age of 7 Days.

	Control	D12	D24	CD (0.05)	SE
Duodenum					
Villi Height	597.23 ± 13.43	588.99 ± 5.32	577.77 ± 9.68	NS	5.78
Villi Width	81.90 ± 5.75	74.64 ± 3.99	75.20 ± 4.33	NS	2.69
Crypt Depth	109.54 ± 5.04	112.65 ± 2.72	123.20 ± 3.88	NS	2.81
Villi Height: Crypt Depth	5.50 ± 0.31	5.25 ± 0.12	4.71 ± 0.12	NS	0.16
Jejunum					
Villi Height	490.99 ± 2.10	489.93 ± 1.89	486.93 ± 3.43	NS	1.46
Villi Width	85.47 ± 1.96	86.02 ± 2.72	85.85 ± 2.32	NS	1.24
Crypt Depth	91.43 ± 3.70	88.98 ± 1.55	87.62 ± 1.78	NS	1.44
Villi Height: Crypt Depth	5.42 ± 0.21	5.54 ± 0.11	5.60 ± 0.10	NS	0.08

Table 6: Histomorphological observation of Duodenum and Jejunum (µm) of experimental broiler under feeding experiment at the age of 21 Days.

	Control	D12	D24	CD (0.05)	SE
Duodenum					
Villi Height	1353.61 ± 23.68	1387.62 ± 23.91	1425.19 ± 22.6	NS	15.07
Villi Width	242.67 ± 11.14	233.01 ± 13.33	236.91 ± 10.71	NS	6.21
Crypt Depth	236.82 ± 7.11	231.82 ± 9.68	230.46 ± 2.12	NS	3.77
Villi Height: Crypt Depth	5.74 ± 0.28	5.99 ± 0.24	6.19 ± 0.13	NS	0.13
Jejunum					
Villi Height	1285.15 ± 56.58	1225.51 ± 70.72	1140.92 ± 40.03	NS	34.78
Villi Width	170.90 ± 2.59	168.20 ± 1.91	171.10 ± 2.29	NS	1.25
Crypt Depth	202.25 ± 6.03	212.92 ± 14.46	219.58 ± 8.91	NS	5.84
Villi Height: Crypt Depth	6.37 ± 0.34	5.84 ± 0.55	5.22 ± 0.25	NS	0.25

Table 7: Histomorphological observation of Duodenum and Jejunum (µm) of experimental broiler under feeding experiment at the age of 42 Days.

	Control	D12	D24	CD (0.05)	SE
Duodenum					
Villi Height	2707.48 ± 68.11	2510.65 ± 54.24	2650.64 ± 61.22	NS	40.64
Villi Width	357.79 ± 11.65	367.27 ± 8.95	358.71 ± 25.53	NS	8.97
Crypt Depth	441.16 ^a ± 18.31	394.01 ^b ± 2.17	394.65 ^b ± 2.54	29.13	8.71
Villi Height: Crypt Depth	6.15 ^b ± 0.15	6.37 ^b ± 0.11	6.71 ^a ± 0.12	0.37	0.10
Jejunum					
Villi Height	2577.11 ^b ± 41.36	2789.53 ^a ± 23.11	2666.01 ^{ab} ± 51.02	163.21	33.62
Villi Width	332.63 ± 14.24	348.67 ± 9.26	340.36 ± 16.15	NS	8.50
Crypt Depth	372.78 ± 28.31	330.04 ± 11.42	356.22 ± 26.34	NS	13.26
Villi Height: Crypt Depth	7.06 ± 0.64	8.48 ± 0.28	7.57 ± 0.41	NS	0.30

Livability. At the end of 42 days livability percentage was recorded (Table 4) for treatment treatments Control, D12 and D24 were found to be 94.44, 97.22 and 94.44, respectively. Non-significant results in livability showed that there was no any adverse effect of early or delay feeding and watering with synbiotics on livability. This finding is consistent with Mahapatra *et al.* (2017), who observed no differences ($P>0.05$) in livability between chicks supplemented with glucose and probiotics early in life than basal diet supplemented group. Kadam *et al.* (2009) discovered a notable discrepancy with the present study, as they observed significantly higher ($P<0.05$) livability rates in the groups supplemented with 6 g and 8 g of poly herbal feed compared to the control group.

CONCLUSIONS

It can be concluded from the study that delay feeding with synbiotics had no any adverse effect on nutrient digestibility, carcass characteristics, gut morphology and livability in broilers.

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