

The levels of infectious bursal disease virus antibodies and histopathology of bursa of Fabricius in broilers

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ABSTRACT

The present study aimed to investigate the antibody titers to infectious bursal disease and histopathology of the bursa of Fabricius in broilers on field trips. A total of 225 Ross 308 chickens were randomly selected from ten farms with different diets and medical care programs. Birds were sacrificed at 1, 7, 14 and 28 days old to determine the bursa/body weight ratio. Bursal tissues were fixed in 10% buffered formalin and used to evaluate bursa histological properties. Blood samples were analyzed by ELISA for the presence of antibody against infectious bursal disease (IBD) virus. Results showed that all birds had protective antibody titers against IBVD from 1 to 14 days old, except birds at day 14 of farms 3 and 5 (245.6 and 342.2, respectively). Likewise, no birds in this study were protective antibody titers against IBVD on day 28, except farms 6 and 10 (821.8 ± 452.2 and 425.1 ± 229.4 , respectively). In the present study, the bursa weights of broilers gradually increased from 1 to 28 days old. At 28 days of age, the lowest bursa weight and bursa index were 1.61 g and 0.12%, respectively, while the highest ones were 2.70 g and 0.21%, respectively. The histopathological score of the bursa was not significantly different across all farms. In general, antibody titers against IBD and histopathological score of the bursa do not appear to be affected by dietary supplementation in broiler chickens under various husbandry practices examined in this study, therefore, it is difficult to explain whether feed supplementation on broiler farms can boost antibodies to IBD disease.

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1. Introduction

Infectious bursal disease (IBD) is an acute, highly contagious viral disease of young chickens at three to six weeks of age. It is characterized mainly by immunosuppression in birds and causes severe lesions in the bursa of Fabricius (BF) (Saif, 1994). This disease is caused by the infectious bursal disease virus (IBDV) belonging to the genus *Birnavirus*, subgenus *Avibirnavirus*, family *Birnaviridae*. Clinical signs are inflammation of the BF, hemorrhages in skeletal muscles, and death. Morbidity in fully IBDV-infected flocks can reach up to 100%, and mortality rates with classic strain may range from 1 - 60% (Murphy et al., 1999). The presence of coliforms, *Staphylococcus* spp., and *Clostridium perfringens* in broiler houses can significantly impact poultry health and productivity, potentially reducing the effectiveness of the IBDV vaccine. In various husbandry practices examined in this study (unpublished data), large quantities of coliforms and *Staphylococcus* spp. were detected in swab samples collected from walls, ceilings, fan plates, feeders, drinkers, and heaters across 10 farms. In addition, *Clostridium perfringens* was identified in samples from farms 2, 4, 6, 7, 8, and 10, but was not detected in farms 1, 3, and 5. Previous study showed that the utilization of probiotic microorganisms, prebiotic substrates, and organic acids has been demonstrated to promote the proliferation of beneficial bacterial populations, promote immune response, and reduce the risk of antibiotic resistance, resulting in reduced pathogen load and improved animal health status (Ayalew et al., 2022). Wang et al. (2020) reported that *Lactobacillus salivarius* results in enhanced immune response after IBDV vaccine immunization, enhanced specific antibody and IFN- γ production, and lymphocyte proliferation in layers. However, Talebi et al. (2008) reported that no positive effect was found on the impact of probiotics on antibody responses to infectious

bursal disease virus vaccination in broiler chickens. Furthermore, it is crucial to consider that dietary supplements frequently increase feed costs, necessitating a careful evaluation of the cost-benefit ratio of feed additives. Therefore, this study was undertaken to investigate IBDV antibody titers and histopathological alterations in the bursa of Fabricius (BF) of broiler chickens utilizing probiotics, prebiotics, organic acids, or their combinations under field conditions.

2. Materials and Methods

2.1. Animals

A total of 250 Ross 308 broilers from ten different farms located in Hoc Mon district (Ho Chi Minh City), Ba Ria - Vung Tau, Binh Duong, and Dong Nai provinces. Chickens were reared on intensive indoor farms which were supplied with water and commercial feeds (De Heus Vietnam Feed Company. Ltd, Vietnam) *ad libitum* by nipple drinkers and plastic feeders. In this study, group A including farms 1, 2, 6, 7 and 9 received probiotics and prebiotics; group B including farms 4 and 8 received probiotics, prebiotics, and organic acids; and group C including farms 3, 5 and 10 received probiotics.

2.2. Supplementation, additives and vaccination schedules

Chickens in group A were raised with the same diet containing prebiotics consisting of 45% beta glucans, 27.3% malto-oligosaccharides, > 20% d-Galactosamine, and < 10% d-Glucosamine (Celmanax® Liquid, USA) and probiotics (Clostat® SP Dry, USA, consisting of at least 4.0×10^{11} CFU *Bacillus subtilis*/kg); while, group B were supplied with prebiotics (Celmanax® Liquid, USA); probiotics (Clostat® SP Dry, USA); and organic acids including aqueous formaldehyde 37% solution and propionic acid (Sal curb®, USA); and (3)

group C including farm 3 was supplied with probiotics (Nutrilaczym, Vietnam) contained at least 3×10^{10} CFU *Bacillus subtilis*/kg; Farm 5 was supplied probiotics (Pnbio-superzym, Vietnam) containing at least 1×10^8 CFU *Bacillus spp.*/g, at least 1×10^6 CFU *Lactobacillus sp.*/g, and at least 1×10^7 CFU *Saccharomyces sp.*/g; and farm 10 was supplemented with probiotics (Sakan-B.Complex, Sakan,

Vietnam) containing at least 1.2×10^8 CFU *Bacillus subtilis*, 1.2×10^8 CFU *Lactobacillus acidophilus*, 1.2×10^8 CFU *Aspergillus oryzae*, and 1.2×10^8 CFU *Saccharomyces cerevisiae*. Chickens were vaccinated at the hatchery according to the standard vaccination schedule for broiler chickens. Meanwhile, the birds were vaccinated with the infectious bursal disease described in Table 1.

Table 1. Vaccination schedules used in ten farms

Days of age	Vaccines (route)	Types of vaccines	Farms
9	IBDV (SC)	Live immune complex infectious bursal disease virus vaccine, Winterfield 2512 strain + antibodies against Infectious Bursal Disease Virus	4, 8 and 10
12	IBDV (DW)	Live vaccine strain Cu-1M	3 and 5
14	IBDV (DW)	Live vaccine strain Cu-1M	1, 2, 6, 7 and 9

IBDV: infectious bursal disease virus; SC: subcutaneous; DW: drinking water.

2.3. Antibody titers against IBDV and histopathological examination

Ten chicks of mixed sex (male and female) on 1 day and five chickens of mixed sex (male and female) on 7, 14, and 28 days were randomly collected in each farm. Each bird was weighed before sample collection. A blood volume of 0.2 to 2 mL was collected from the wing vein or jugular vein of each bird and serum was subsequently collected. Serum samples were collected separately and stored at -20°C until analysis. There were 225 samples in total that were used to detect antibodies against IBDV by using the IDEXX IBD-XR ELISA kit (IDEXX, Labs Inc., Westbrook, Maine, USA). All samples were tested with ELISA immunoassays according to the manufacturer's instructions.

On days 1, 7, 14 and 28 of age, three birds of each farm were killed and the bursa removed. These bursa tissues were fixed in 10% formalin in PBS (pH 7.6) for histological analysis and pathological scoring system as described by previous studies (Muskett et al., 1979; Henry et al., 1980; Williams & Davison, 2005). The bursa/body weight ratio (B/BW ratio) was determined as described by Heckert et al. (2002) and histopathological changes in the BF were scored from 0 to 5 based on a scoring index (Williams & Davison, 2005).

2.4. Statistical analysis

Data on antibody titers against IBDV, histopathological lesion scores, body weight, and bursa weight were demonstrated in this study.

3. Results and Discussion

Table 2. Average value of antibodies titers against IBDV of broilers based on day(s) of age

Days of age (days)	A				B			C		
	1	2	6	7	9	4	8	3	5	10
Mean	4,619.1	6,058.3	4,297.8	4,314.2	5,774.2	4,878.3	3,752.2	4,659.5	5,547.9	6,014.1
SD	793.4	1,592.1	1,597.2	1,047.1	1,757.9	618.8	1,575.7	433.3	1,145.3	1,076.6
Min.	3,707	2,238	2,506	2,716	3,450	3,608	1,939	1,981	3,944	4,232
Max.	6,070	7,457	7,161	5,946	8,351	5,537	7,144	7,543	7,446	7,473
CV (%)	17.2	26.3	37.2	24.3	30.4	12.7	42.0	9.3	20.6	17.9
Protection (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mean	2,927.0	2,291.6	2,901.6	2,919.6	2,024.6	3,362.6	1,846.0	1,406.6	1,996.8	4,925.4
SD	779.2	1,217.0	408.4	566.7	1,210.2	408.7	764.2	511.5	601.5	931.3
Min.	1,682	1,018	2,421	2,171	957	2,889	1,213	848	1,332	3,841
Max.	3,837	4,169	3,267	3,621	3,892	3,868	3,080	2,243	2,675	5,906
CV (%)	26.6	53.1	14.1	19.4	59.8	12.2	41.4	36.4	30.1	18.9
Protection (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

A: farms 1, 2, 6, 7 and 9 received probiotics and prebiotics; B: farms 4 and 8 received probiotics, prebiotics, and organic acids; C: farms 3, 5 and 10 received probiotics.

Table 3. Average value of antibodies titers against IBDV of broilers based on day(s) of age

Days of age (days)	A				B			C		
	1	2	6	7	9	4	8	3	5	10
Mean	502.4	574.2	1,360.0	1,116.8	1,234.0	1,874.0	935.6	245.6	342.2	3,315.0
SD	356.8	589.7	873.5	785.9	519.7	572.7	338.4	149.7	176.7	1,356.4
Min.	120	80	521	446	911	1,062	521	39	156	1,660
Max.	899	1,546	2,744	2,224	2,151	2,658	1,435	457	536	5,035
CV (%)	71.0	102.7	64.2	70.4	42.1	30.6	36.2	61.0	51.6	40.9
Protection (%)	40.0	60.0	100.0	100.0	100.0	100.0	100.0	20.0	40.0	100.0
Mean	157.4	181.4	821.8	230.6	141.6	205.6	207.4	180.6	175.2	425.1
SD	35.0	45.7	452.2	105.4	45.4	86.0	93.6	64.3	95.1	229.4
Min.	118	114	473	111	89	118	87	122	87	145.4
Max.	205	237	1,589	356	200	334	315	262	319	705.3
CV (%)	22.2	25.2	55.0	45.7	32.1	41.8	45.1	35.6	54.3	54.0
Protection (%)	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	40.0

A: farms 1, 2, 6, 7 and 9 received probiotics and prebiotics; B: farms 4 and 8 received probiotics, prebiotics, and organic acids; C: farms 3, 5 and 10 received probiotics.

According to the IDEXX IBD-XR ELISA kit (IDEXX, Labs Inc., Westbrook, Maine, USA), if the value of antibody titers against IBDV is greater than or equal to 396, the sample is considered positive for IBDV antibodies. All birds had protective antibody titers against IBDV from 1 to 14 days old (Table 2). This result was consistent with Kreider et al. (1991) that the values of maternal antibodies against IBDV were from 3,000 to over 6,000.

However, birds at 14 days of the age of farms 3 (245.6 ± 149.7) and 5 (342.2 ± 176.7) antibody titers were not satisfied with the guidelines of the IDEXX IBD-XR ELISA kit. Likewise, at 28 days of age, no birds in this study were antibodies against IBD (except for birds of farms 6 and 10). Based on vaccination schedules used in ten farms in Table 1, the results of antibody titers against IBDV of birds at 28 days old were in disagreement with Shrestha et al. (2003), the significant uptrend of IBD antibody titers of birds at 28 days old was found when chicks were vaccinated against

IBDV around day 14 (Table 3). Islam et al. (2008) indicated that some of the reasons attributed to IBD vaccination include improper handling and administration of the vaccine, virus antigenic differences, live attenuated vaccine virus potency, residual maternal antibody interference with the vaccine virus and leaving the birds susceptible to IBD. In this study, it is hard to explain whether the supplementation of probiotics, prebiotics and/or organic acid in broilers in these farms can enhance antibody to infectious bursal disease, the same downtrend of IBD antibody titers during the study and these farms had different schedules of feed-supplementation were found. This leads to consider the effect of treatment of supplementation in broilers in this study. Khalifa et al. (2014) reported that probiotic use in broiler diets improves the immune status and humoral immune response against IBD in chickens. In contrast, Balevi et al. (2001) reported that the addition of probiotics via drinking water had no significant effect on the antibody responses to IBD vaccination.

Table 4. Bursa weight, bursa/body weight ratio (B/BW), and histopathological lesion scores of broilers in all farms based on day(s) of age

Days of age (day)/factors		Variables	A				B		C			
			1	2	6	7	9	4	8	3	5	10
1	Bursa weight (g)	Mean	0.06	0.07	0.03	0.03	0.04	0.06	0.05	0.06	0.03	0.04
		SD	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
		Min.	0.03	0.05	0.01	0.01	0.01	0.03	0.02	0.04	0.01	0.02
		Max.	0.09	0.08	0.07	0.07	0.06	0.09	0.07	0.07	0.07	0.06
	B/BW (%)	Mean	0.14	0.15	0.09	0.09	0.07	0.13	0.11	0.15	0.09	0.09
		SD	0.04	0.03	0.06	0.06	0.04	0.04	0.05	0.03	0.05	0.03
		Min.	0.08	0.12	0.03	0.03	0.02	0.06	0.04	0.10	0.03	0.05
		Max.	0.20	0.20	0.17	0.17	0.13	0.19	0.16	0.20	0.19	0.14
	HLS	Mean	0.93	1.07	1.00	1.00	1.00	0.07	1.87	0.87	1.00	1.00
		SD	0.42	0.77	0.37	0.63	0.37	0.25	0.34	0.62	0.37	0.63
7	Bursa weight (g)	Mean	0.29	0.39	0.26	0.28	0.21	0.29	0.23	0.25	0.27	0.26
		SD	0.05	0.06	0.05	0.04	0.05	0.06	0.04	0.08	0.04	0.04
		Min.	0.20	0.35	0.21	0.24	0.16	0.22	0.19	0.19	0.22	0.21
		Max.	0.33	0.49	0.31	0.35	0.26	0.38	0.29	0.38	0.32	0.31
	B/BW (%)	Mean	0.15	0.21	0.28	0.26	0.13	0.13	0.18	0.15	0.15	0.15
		SD	0.02	0.03	0.05	0.02	0.02	0.02	0.03	0.03	0.02	0.02
		Min.	0.11	0.18	0.23	0.23	0.11	0.11	0.13	0.12	0.13	0.12
		Max.	0.18	0.26	0.35	0.28	0.16	0.16	0.21	0.20	0.18	0.18
	HLS	Mean	1.00	1.00	1.00	1.00	1.00	1.00	1.75	0.93	1.00	1.00
		SD	0.73	0.97	0.97	0.63	0.37	0.37	0.58	0.68	0.97	0.37
14	Bursa weight (g)	Mean	1.22	0.75	0.69	0.83	0.93	1.02	0.98	1.14	0.92	0.91
		SD	0.08	0.10	0.13	0.13	0.28	0.16	0.32	0.09	0.22	0.20
		Min.	1.13	0.61	0.52	0.72	0.67	0.80	0.65	0.99	0.73	0.72
		Max.	1.31	0.86	0.84	1.05	1.35	1.15	1.48	1.22	1.18	1.17
	B/BW (%)	Mean	0.24	0.16	0.18	0.19	0.18	0.18	0.22	0.24	0.28	0.28
		SD	0.03	0.01	0.04	0.03	0.06	0.03	0.08	0.03	0.09	0.08
		Min.	0.21	0.14	0.13	0.17	0.11	0.15	0.12	0.20	0.19	0.19
		Max.	0.27	0.18	0.21	0.24	0.25	0.23	0.35	0.27	0.39	0.39
	HLS	Mean	1.07	1.67	1.00	1.00	1.00	0.87	1.87	0.93	1.13	1.00
		SD	0.70	0.72	0.38	0.65	0.38	0.52	0.35	0.70	0.35	0.65
28	Bursa weight (g)	Mean	1.94	2.70	1.61	1.85	2.41	2.60	2.37	2.55	2.02	2.02
		SD	0.57	0.55	1.15	0.65	0.76	0.87	0.52	1.33	0.38	0.34
		Min.	1.40	2.10	0.75	0.94	1.73	1.70	1.61	1.24	1.60	1.60
		Max.	2.60	3.30	3.34	2.66	3.57	3.70	2.95	4.13	2.50	2.50
	B/BW (%)	Mean	0.13	0.21	0.12	0.14	0.16	0.15	0.18	0.18	0.21	0.21
		SD	0.04	0.03	0.08	0.05	0.05	0.06	0.04	0.10	0.04	0.03
		Min.	0.09	0.18	0.06	0.08	0.12	0.09	0.13	0.08	0.16	0.17
		Max.	0.17	0.27	0.26	0.20	0.22	0.21	0.24	0.30	0.25	0.25
	HLS	Mean	1.80	1.20	1.60	1.73	1.80	1.07	1.27	1.73	1.00	1.13
		SD	0.41	0.86	0.74	0.46	0.56	0.80	0.70	0.70	0.65	0.74

A: farms 1, 2, 6, 7 and 9 received probiotics and prebiotics; B: farms 4 and 8 received probiotics, prebiotics, and organic acids; C: farms 3, 5 and 10 received probiotics; B/BW: bursa/body weight ratio; SD, standard deviation; HLS, histopathological lesion scores.

According to Bolis et al. (2003), the evaluation of bursal weight, bursal index (bursa weight:body weight ratio), and histopathological lesion scores are the most used models to estimate the protection rate given by vaccines against IBD. In the present study, the bursa weights of all farms gradually increased from 1 to 28 days old. At 28 days of age, the low bursa weight and bursa index in farm 6 (1.61 g; 0.12) and the high bursa weight and bursa index in farm 2 (2.70 g; 0.21) were found (Table 4; Figures 1 and 2). In agreement with Latif et al. (2014), in the experiment of birds raised with basal diet, the average bursa weight of Ross 308 broiler and bursa index were 1.719 ± 0.087 g, 0.203 ± 0.034 , respectively. Likely, the positive effect of supplementation of probiotics, prebiotics, and organic acids on the parameters of bursa was hard to determine in this research. Awad et al. (2009) and Morales-López et al. (2009) reported that dietary inclusion of organic acids, probiotics, and prebiotics did not influence the weights of the BF. In contrast, Abdel-Fattah et al. (2008), confirmed that the birds on fed organic-acid-supplemented diet had heavier BF weight. Between 7 days old and 28 days old, the bursa indices of birds of all farms were over 0.1 which

was in agreement with the results of Cazaban et al. (2015) showed that an ideal B/BW ratio potential of 0.11 or above could be observed in healthy Cobb 500 commercial broilers from 7 to 42 days of age that were housed under ideal conditions (minimum stress, and no diseases). Agboola et al. (2015) indicated that supplementation of organic acids could reduce the bursal index. Likewise, sick or stressed birds have small bursa sizes; while healthy birds have large bursa sizes (Yegani & Korver, 2008). According to Murmu et al. (2014), the severity of lesions in the BF is proportionally related to the degree of viral pathogenicity inoculated or infected naturally; however, it is noticed that bursal lesions in vaccinated chickens were histopathologically characterized as either normal follicles with or without mild to moderate lymphoid depletion without follicular atrophy or the development of cystic follicles. Moreover, the histopathological lesions observed in the bursa of vaccinated chickens did not mean the vaccine breaks or immunosuppression, because the lesions stated here might be developed by the vaccine virus (Islam et al., 2008).



Figure 1. Bursal lesion scoring in field IBDV infected broiler chickens (H & E staining, 10X). A: normal architecture; B: minor lymphocyte depletion (+1) and C: lymphocyte depletion from most follicles and hyperaemia (+2).

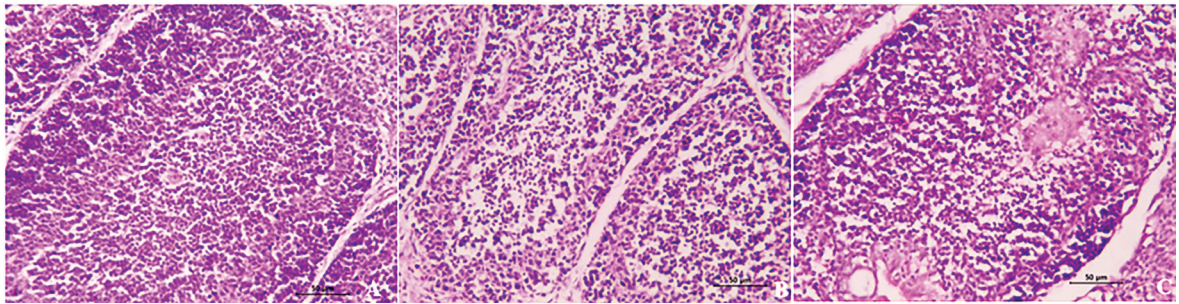


Figure 2. Bursal lesion scoring in field IBDV infected broiler chickens (H & E staining, 40X). A: normal architecture; B: minor lymphocyte depletion (+1) and C: lymphocyte depletion from most follicles and hyperaemia (+2).

4. Conclusions

All birds were protective antibody titers against IBDV from 1 to 14 days old (except for farms 3 and 5). On the contrary, at 28 days of age, no birds in this study were antibodies against IBD (except for farms 6 and 10). Bursa/body weight ratios and histopathological lesion scores of the BF remained consistent across farms. Under various husbandry practices, with the presence of coliforms, *Staphylococcus* spp., and *Clostridium perfringens* in broiler houses, the positive effects of supplementing diets on antibody levels against IBD and bursal parameters were difficult to ascertain in this research. Further studies with control plots should be conducted to determine whether supplementation with probiotics, prebiotics, organic acids, or their combinations are necessary for broilers.

Conflict of interest

All authors declare that they have no conflicts of interest.

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References

- Abdel-Fatt, S. A., El-Sanhour, M. H., El-Mednay, N. M., & Abdel-Azee, F. (2008). Thyroid Activity, Some Blood Constituents, Organs Morphology and Performance of Broiler Chicks Fed Supplemental Organic Acids. *International Journal of Poultry Science* 7(3), 215-222. <https://doi.org/10.3923/ijps.2008.215.222>.
- Agboola, A. F., Omidwura B. R. O., Odu O., Popoola I. O., & Iyayi, E. A. (2015). Effects of organic acid and probiotic on performance and gut morphology in broiler chickens. *South African Journal of Animal Science* 45(5), 494-501. <https://doi.org/10.4314/sajas.v45i5.6>.
- Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. *Poultry Science* 88(1), 49-55. <https://doi.org/10.3382/ps.2008-00244>.
- Ayalew, H., Zhang, H., Wang, J., Wu, S., Qiu, K., Qi, G., & Chanie, D. (2022). Potential feed additives as antibiotic alternatives in broiler production. *Frontiers in Veterinary Science* 9, 916473. <https://doi.org/10.3389/fvets.2022.916473>.

- Balevi, T., Uçan, U. S., Coskun, B., Kurtoglu, V., & Çetingül, S. (2001). Effect of dietary probiotic on performance and humoral immune response in layer hens. *Archiva Zootechnica* 42(4), 456-461. <https://doi.org/10.1080/00071660120073133>.
- Bolis, D. A., Paganini, F. J., Simon, V. A., Zuanaze, M., Scanavini, N. H., & Correa, A. (2003). Gumboro disease: Evaluation of serological and anatomopathological response vaccinated broiler chicken challenged with very virulent virus strain. *Brazilian Journal of Poultry Science* 5(2), 137-146. <https://doi.org/10.1590/S1516-635X2003000200008>.
- Cazaban, C., Masferrer, N. M., Pascual, R. D., Espadamala, M. N., Costa, T., & Gardin, Y. (2015). Proposed BF weight to body weight ratio standard in commercial broilers. *Poultry Science* 94(9), 2088-2093. <https://doi.org/10.3382/ps/pev230>.
- Heckert, R. A., Estevez, I., Russek-Cohen, E., & Pettit-Riley, R. (2002). Effects of density and perch availability on the immune status of broilers. *Poultry Science* 81(4), 451-457. <https://doi.org/10.1093/ps/81.4.451>.
- Henry, C. W., Brewer, R. N., & Edgar, S. A. (1980). Studies on infectious bursal disease in chickens. 2. Scoring microscopical lesions in the bursa of Fabricius, thymus, spleen, and kidney in gnotobiotic and battery reared white leghorns experimentally infected with infectious bursal disease virus. *Poultry Science* 59(5), 1006-1017. <https://doi.org/10.3382/ps.0591006>.
- Islam, M. N., Rashid, S. M. H., Hoque, M. F., Juli, M. S. B., & Khatun, M. (2008). Pathogenicity of IBDV related to outbreaks in the vaccinated flocks and the causes of vaccination failure. *Journal of Innovation and Development Strategy* 2, 22-30.
- Khalifa, E., Gomaa, E., Abd El-Mageed, A. E. R., Atef, N., & Abd El-Latif, K. (2014). Effect of probiotics and *Escherichia coli* infection on performance of some viral vaccination in broiler chicks. *Animal Health Research Journal* 2, 69-78. <https://doi.org/10.2141/jpsa.43.296>.
- Kreider, D. L., Skeeles, J. K., Parsley, M., Newberry, L. A., & Story, J. D. (1991). Variability in a commercially available enzyme-linked immunosorbent assay system. I. Assay variability. *Avian Disease* 35(2), 276-287.
- Latif, K., Majed, M., & Sahar, H. (2014). Determine the weight of thymus, bursa of Fabricius and spleen and its ratio to body weight in some diseases of broilers. *MRVSA* 3(1), 8-14. <https://mrvsa.com/upload>.
- Morales-López, R., Auclair, E., García, F., Esteve-García, E., & Brufau, J. (2009). Use of yeast cell walls; beta-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. *Poultry Science* 88(3), 601-607. <https://doi.org/10.3382/ps.2008-00298>.
- Murmu, R., Islam, M. N., Juli, Khan, A. S., & Harun-ur-Rashid, S. M. (2014). Pathogenicity and immunosuppressive properties of GM-97 strain of infectious bursal disease virus in commercial broiler chickens. *Journal of Advanced Veterinary and Animal Research* 1(1), 1-7. <https://doi.org/10.5455/javar.v1i1p1-7>.
- Murphy, F. A., Gibbs, E. P. J., Horzinek, M. C., & Studdert, M. J. (1999). Paramyxoviridae. In Murphy, F. A., Gibbs, E. P., Horzinek, M. C., & Studdert, M. J. (Eds.). *Veterinary virology* (3rd ed., 411-428). Cambridge, UK: Academic Press.

- Muskett, J. C., Hopkins, I. G., Edwards, K. R., & Thornton, D. H. (1979). Comparison of two infectious bursal disease vaccine strains: efficacy and potential hazards in susceptible and maternally immune birds. *Veterinary Record* 104, 332-334. <https://doi.org/10.1136/vr.104.15.332>.
- Saif, Y. M. (1994). Antigenicity and immunogenicity of infectious bursal disease virus. *International symposium on infectious bursal disease and chicken infectious anaemia* (21-24). Retrieved September 1, 2018, from <https://doi.org/10.1080/03079459508419076>.
- Shrestha, P., Ahsan, M. M., Islam, K. M. D., Billah, M. M., Mehedi, M., Mitre, S., & Islam, M. R. (2003). Seroprevalence of infectious bursal disease virus (IBDV) specific antibody in chicken. *Pakistan Journal of Biological* 1240.
- Talebi, A., Amirzadeh, B., Mokhtari, B., & Gahri, H. (2008). Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathology Journal of The W.V.P.A* 37(5), 509-512. <https://doi.org/10.1080/03079450802356995>.
- Wang, G., Yang, S., Sun, S., Si, Q., Wang, L., Zhang, Q., Wu, G., Zhao, J., Zhang, H., & Chen W. (2020). *Lactobacillus rhamnosus* strains relieve loperamide-induced constipation via different pathways independent of short-chain fatty acids. *Frontiers in Cellular and Infection Microbiology* 10, 423. <https://doi.org/10.3389/fcimb.2020.00423>.
- Williams, A. E., & Davison, T. F. (2005). Enhanced immunopathology induced by very virulent infectious bursal disease virus. *Avian Pathology* 34, 4-14. <https://doi.org/10.1080/03079450400025364>
- Yegani, M., & Korver, D. R. (2008). Factors affecting intestinal health in poultry. *Poultry Science* 87(10), 2052-2063. <https://doi.org/0.3382/ps.2008-00091>.