Global Veterinaria 8 (1): 08-14, 2012
ISSN 1992-6197


# Biochemical Changes in Specific-Pathogen-Free Chicks Infected with Infectious Bursal Disease Virus of Malaysian Isolate

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**Abstract:** A study was conducted to determine the biochemical changes following very virulent infectious bursal disease virus (vvIBDV) infection of Malaysian isolate. One-hundred-ten, 32-day-old specific-pathogen-free (SPF) chicks were divided into two groups namely, IBD (n=55) group inoculated with vvIBDV and control (n=55) group which served as uninoculated control. The chicks in the IBD group were examined for clinical signs and blood samples were collected for biochemical assay. Typical clinical signs of acute IBD were noted as early as 2 day post-inoculation (pi) in the IBD group. The serum samples revealed significant ($p<0.05$) reduction in total protein (TP), albumin (Alb) and albumin to globulin (A:G) ratio at days 5 and 7 pi leading to hypoalbunaemia meanwhile, significantly ($P<0.05$) increased aspartate aminotransferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), gluteral dehydrogenase (GLDH), alkaline phosphatase (ALP) and creatine kinase (CK) at day 3 and/or 5 and 7 pi were observed. The concentrations of uric acid was significantly ($P<0.05$) increased at days 5 and 7 pi, while the cholesterol level was significantly ($P<0.05$) decreased at day 7 pi. Further, significant ($P<0.05$) hypocalcemia, hyponatermia, hyperchloremia and hyperkalemia at days 5 and/or 7 pi in the IBD group were observed compared with the control group. These changes correspond to the clinical signs observed, mainly the anorexia, diarrhoea and dehydration and also reflected an insult to those organs that produce, metabolize or excrete them, among others liver and kidney. By and large, the present study revealed the vvIBDV of Malaysian isolate produced biochemical changes consistent with its pathogenicity and these changes could be used in the clinico-pathological study and further understanding of pathogenesis of the disease.

**Key words:** Vvibdv Infection · SPF Chicks · Biochemical Changes · Clinico-Pathology

## INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious, acute viral disease of poultry caused by IBD virus (IBDV). The causative agent is a bisegmented, double stranded RNA virus that belongs to the genus *Avibirinavirus* family *Birnaviridae*. Two distinct serotypes of IBDV namely, serotypes 1 and 2 are identified. Serotype 1 is pathogenic to chicks and classified as classical (c), variant (va) and very virulent (vv) IBDV [1], while serotype 2 is not pathogenic to chicks.

Infectious bursal disease has worldwide distribution and the effects of the disease are economically significant to the commercial poultry industry [2] through the mortality, reduced weight gain and condemnation of carcasses due to marked haemorrhage in the skeletal muscle [1-4] and secondary losses due to immunosuppression [5, 6].

Among the pathogenic strains, the vvIBDV causes an acute form of the disease characterized by severe clinical signs and mortality in susceptible chicks. Besides, it exhibits several pathological changes as part of the pathogenesis of the disease which could essentially be explained in biochemical changes in relation to the effect of the virus in several organs among others, liver and kidney [6-8]. Few studies have tried to determine those biochemical changes in IBDV infection [7, 9, 10] and found variable biochemical profiles.

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In Malaysia although the disease has been previously known to occur, mortalities with clinical signs and pathological changes associated with acute IBD have been confirmed for the first time during the middle of 1991 [11] and there was no attempt to date to investigate the biochemical changes following infection of chicks with IBDV of local isolate. Therefore, it is the objective of this paper to elucidate biochemical changes that are pertinent to vvIBDV infection of a Malaysian isolate in specific-pathogen-free (SPF) chicks.

## MATERIALS AND METHODS

**Virus Isolates:** Malaysian isolate namely UPM0081 which is characterized as vvIBDV (Gen Bank, with an accession number of AY791998 [12], was used in the present study.

**IBDV Inoculums:** The vvIBDV isolate acquired from the bursa of fabricus (BF) of infected chickens during a 2004 IBD outbreaks in Selangor, Malaysia, [13] was kept at-20°C (Senyo, Japan) as a stock virus. The isolate was then passaged 3 times in chorioallantoic membrane (CAM) of 10-day-old embryonated SPF chicken eggs prior to inoculation by the method described by Senne [14]. The harvested CAM was freezed and thawed three times and ground separately using sterile pestle and sands to make 1:2 (w/v) dilution of each in sterile phosphate buffer saline (PBS) with pH 7.4. Processed samples was centrifuged at 3000 rpm for 15 min at 4°C (MSE, Mistral 4L, Germany). The supernatant were collected through filtration by 0.45 µm pore filter syringe and treated with antibiotic-antimycotic solution (GIBCO Lab., USA) in 1:10 (v:v). The viral inoculums were kept at -20°C (Senyo, Japan) until used.

**Virus Titration and Inoculation:** The titration of the virus was carried out in 10-day-old SPF embryonated chick eggs based on method described by Reed and Muench [15]. Accordingly the titer of the virus was determined as $10^{4.8}$ EID50/mL. Each chick in the IBD group was inoculated with 0.1 ml of vvIBDV inoculum, via the oral route using a 1.0 ml syringe.

**Experimental Chicks:** Day-old embryonated chick eggs were obtained from specific pathogen free (SPF) single comb white Leghorn flocks, hatched and reared in an experimental unit. The flock was reared on slated wire-floored isolation unit in a closed house that had been fumigated three times with 100% formaldehyde prior to rearing of the chickens. They were provided feed and water *ad-libitum.*

**Experimental Design:** One-hundred-ten, 32-day-old SPF chicks were distributed into two groups, namely the IBD and control groups. The IBD group was inoculated orally with 0.1 ml per chick with the virus titter of $10^{4.8}EID_{50}$ unit/mL. The control group remained un-inoculated. The chicks were monitored for any clinical abnormalities for at least twice daily and were given feed and water *ad-libitum.* Blood was collected for biochemical examinations from both groups. Sampling of chicks was scheduled at 1 hour (h), 3h, 6h, 12h, 1 day (d), 3d, 5d, 7d, 10d and 14d post-inoculations (pi) from the control and the IBD group and equal number of 5 chicks each was sampled. But because of the death of 6 chicks in the IBD group at days 3, 4 and 5 pi, the trial was terminated at day 7 pi.

**Serum Biochemistry Determination:** Three mL of blood was collected from each chick via jugular vein or intra-cardiac route using a 23 G needle and 3.0 ml syringe and were immediately placed into non-heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min. and serum obtained was stored at-20°C until analysis. Total protein (TP), albumin (ALB), globulin (Glb), glucose, cholesterol, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), glutamate dehydrogenase (GLDH), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP) and creatine kinase (CK), sodium ion ($Na^+$), chloride ion ($Cl^-$), potassium ion ($K^+$) and calcium ion ($Ca^{++}$) were measured. Globulin was determined by subtracting albumin (Alb) from the total protein. These serum biochemistry components were measured by an automatic analyzer (Hitachi: 902, Japan) using standard reagents (RoCHE, Switzerland).

**Statistical Analysis:** Means were obtained and subjected to statistical test of significance using one-way analysis of variance (ANOVA) for equal replication using SPSS software version 15.0 (SPSS Inc. Chicago IL., USA) [16]. Values were expressed as mean ± standard deviation (SD) and statistical significance ($P<0.05$) among the means were determined using Duncan's multiple range test.

## RESULTS

The chicks in the infectious bursa disease (IBD) group showed signs of depression, weakness diarrhoea and vent picking at day 2 pi and became recumbent and mortality was recorded beginning on day 3 pi. Accordingly, 2, 3 and 1 chicks died at days 3, 4 and 5 pi.

The concentration of protein (TP) and albumin (Alb) in both the IBD and control groups was fairly close to each other and showed no significant difference ($p>0.05$) between the two groups during this time. Significantly ($p<0.05$) decreased TP and Alb was recorded at days 5 and 7 pi in the IBD group compared with the control group. On the contrary, there was no significant difference ($p>0.05$) in the concentration of globulin (Glb) between the control and the IBD groups throughout the trial. The albumin to globulin (A:G) ratio, was significantly ($p<0.05$) decreased in the IBD group at days 5 and 7 pi with respect to the control group (Table 1).

With regard to the serum enzymes analyzed in the present study the glutamate dehydrogenase (GLDH) and alanine phosphatise (ALP) concentration in the IBD group showed significant increase ($P<0.05$) at days 5 and 7 pi and day 7 pi respectively when compared with the control group (Table 2). On the other hand, the aspartate transferase (AST), alanine transferase (ALT),

Table 1: The TP, Ab, Glb in u/L, A:G ratio of the control and IBD groups throughout the trial period

| | TP (u/L) | | ALB (uL) | | GLB (u/L) | | A:G | |
|---|---|---|---|---|---|---|---|---|
| Sampling time (pi) | Control | IBD | Control | IBD | Control | IBD | Control | IBD |
| 1 hr | 23.74±2.04 | 23.12±2.22 | 9.80±0.70 | 10.00±1.39 | 13.94±2.43 | 13.12±2.50 | 0.73±0.18 | 0.79±0.24 |
| 3 hrs | 23.74±1.82 | 24.72±1.97 | 10.02±1.49 | 10.40±1.92 | 13.72±2.21 | 14.32±0.94 | 0.75±0.18 | 0.73±0.15 |
| 6 hrs | 23.04±2.05 | 25.84±1.88 | 9.42±1.40 | 11.44±1.72 | 13.62±1.08 | 14.40±1.02 | 0.69±0.10 | 0.79±0.13 |
| 12 hrs | 22.56±1.81 | 25.70±1.61 | 9.42±2.11 | 12.04±1.57 | 13.14±0.93 | 13.66±1.20 | 0.72±0.19 | 0.89±0.15 |
| Day 1 | 22.80±1.15 | 25.28±2.00 | 9.62±1.95 | 11.68±1.93 | 13.18±1.16 | 13.60±1.49 | 0.74±0.22 | 0.87±0.19 |
| Day 3 | 24.26±1.96 | 25.52±0.33 | 10.08±1.15 | 11.96±0.83 | 14.18±2.72 | 13.56±0.55 | 0.75±0.26 | 0.88±0.10 |
| Day 5 | 24.78±1.72 | 15.98±0.88[a] | 10.46±1.29 | 4.40±1.53[a] | 14.32±1.67 | 11.58±1.17 | 0.74±0.14 | 0.37±0.06[a] |
| Day 7 | 23.14±2.06 | 15.70±2.22[a] | 9.26±0.70 | 3.48±1.07[a] | 13.88±1.49 | 12.22±2.23 | 0.78±0.05 | 0.28±0.11[a] |

[a] *statistically significant compared with the control group ($p<0.05$)*

Table 2: The ALT, ALP and AST in u/L, of the control and IBD groups throughout the trial period

| | GLDH (u/L) | | ALP (u/L) | | CK (u/L) | |
|---|---|---|---|---|---|---|
| Sampling time | Control | IBD | Control | IBD | Control | IBD |
| 1 hr | 24.00±3.35 | 21.00±2.11 | 199.90±25.40 | 210.00±24.59 | 223.26±28.23 | 221.40±27.77 |
| 3 hrs | 22.20±1.68 | 22.80±1.99 | 210.80±28.36 | 200.00±29.51 | 205.34±22.11 | 210.88±33.07 |
| 6 hrs | 23.40±2.40 | 24.00±3.23 | 180.10±29.76 | 191.50±36.67 | 212.62±28.22 | 200.94±36.02 |
| 12 hrs | 20.40±3.37 | 22.00±3.33 | 200.10±37.23 | 202.70±30.88 | 201.86±19.31 | 212.94±29.30 |
| Day 1 | 22.80±4.33 | 19.80±2.01 | 204.50±35.51 | 210.00±44.53 | 199.12±37.25 | 199.64±29.66 |
| Day 3 | 22.20±3.34 | 20.40±1.89 | 208.80±39.27 | 220.20±37.65 | 201.62±42.88 | 195.68±17.22 |
| Day 5 | 24.60±3.56 | 33.60±1.75[a] | 219.90±25.79 | 224.90±37.03 | 211.78±27.43 | 217.72±10.51 |
| Day 7 | 23.40±3.60 | 36.00±1.93[a] | 222.60±30.37 | 263.20±21.00[a] | 201.78±18.13 | 198.46±18.16 |

[a] *statistically significant compared with the control group ($p<0.05$)*

Table 3: The GGT, LDH, ALT and AST in u/L, of the control and IBD groups throughout the trial

| | GGT (u/L) | | LDH (u/L) | | ALT (u/L) | | AST (u/L) | |
|---|---|---|---|---|---|---|---|---|
| Sampling time (pi) | Control | IBD | Control | IBD | Control | IBD | Control | IBD |
| 1 hr | 21.40±1.95 | 21.40±1.82 | 168.54±45.68 | 169.50±38.51 | 3.80±0.52 | 4.10±1.10 | 189.36±14.54 | 185.26±25.61 |
| 3 hrs | 22.60±3.05 | 23.00±0.71 | 173.32±33.19 | 180.19±24.85 | 4.02±0.76 | 4.58±1.20 | 191.34±13.19 | 185.98±11.90 |
| 6 hrs | 23.20±0.84 | 25.00±1.81 | 179.90±28.18 | 188.06±33.40 | 3.80±0.71 | 3.82±0.84 | 182.28±13.93 | 188.76±3.70 |
| 12 hrs | 24.20±0.84 | 25.20±1.30 | 188.09±24.03 | 199.05±37.56 | 3.01±0.78 | 2.96±0.57 | 186.64±13.84 | 190.52±12.45 |
| Day 1 | 25.60±1.37 | 26.00±1.54 | 191.41±35.16 | 194.06±38.23 | 3.08±0.62 | 3.12±0.62 | 188.62±9.61 | 193.32±11.44 |
| Day 3 | 25.80±1.19 | 42.00±1.22[a] | 184.01±25.16 | 240.65±25.04[a] | 3.42±1.37 | 5.18±0.98[a] | 183.58±11.06 | 300.82±7.04[a] |
| Day 5 | 26.60±1.14 | 37.40±4.20[a] | 189.00±30.01 | 265.30±28.03[a] | 3.56±0.38 | 6.78±0.86[a] | 183.22±18.75 | 353.22±17.24[a] |
| Day 7 | 26.80±1.39 | 46.20±5.20[a] | 198.80±28.18 | 370.37±23.33[a] | 3.94±0.64 | 13.68±1.87[a] | 180.28±12.40 | 405.22±20.20[a] |

[a] *statistically significant compared with the control group ($p<0.05$)*

Table 4: The concentration of creatine, uric acid, cholesterol and glucose in mmol/L of the control and IBD groups and throughout the trial

| Sampling time (pi) | Creatine (mmol/L) | | Uric acid (mmol/L) | | Cholestrol (mmol/L) | | Glucose (mmol/L) | |
|---|---|---|---|---|---|---|---|---|
| | Control | IBD | Control | IBD | Control | IBD | Control | IBD |
| 1 hr | 23.80±1.64 | 22.40±1.82 | 214.08±78.95 | 227.12±67.98 | 3.60±0.29 | 3.49±0.36 | 16.32±0.93 | 15.96±1.02 |
| 3 hrs | 24.80±1.17 | 24.00±2.35 | 261.78±52.47 | 242.74±47.25 | 3.44±0.45 | 3.19±0.35 | 16.18±2.28 | 15.42±0.80 |
| 6 hrs | 23.00±1.16 | 23.80±3.27 | 241.78±52.40 | 274.42±57.59 | 3.21±0.46 | 3.04±0.35 | 15.64±1.76 | 15.10±0.93 |
| 12 hrs | 25.40±1.99 | 25.40±1.95 | 262.56±78.91 | 251.54±69.67 | 3.47±0.54 | 3.13±0.44 | 15.58±1.19 | 15.02±1.20 |
| Day 1 | 26.40±2.70 | 22.60±1.52 | 212.50±64.09 | 225.10±73.84 | 3.19±0.59 | 3.03±0.22 | 15.76±1.63 | 14.58±0.63 |
| Day 3 | 23.80±1.48 | 24.20±1.19 | 232.40±67.31 | 245.06±41.61 | 3.07±0.27 | 2.97±0.20 | 13.30±0.52 | 14.12±1.27 |
| Day 5 | 24.20±1.39 | 28.40±2.71 | 216.80±79.21 | 352.60±80.54[a] | 3.11±0.49 | 1.41±0.32 | 13.76±1.51 | 13.94±1.05 |
| Day 7 | 27.60±2.66 | 29.80±2.88 | 246.42±67.31 | 412.84±105.56[a] | 3.97±0.12 | 1.60±0.02[a] | 13.14±1.78 | 12.96±0.63 |

[a] *statistically significant compared with the control group (p<0.05)*

Table 5: The concentration of $Ca^{++}$, $Na^{+}$, $CL^{-}$ and $K^{+}$ in mmol/L the IBD and control groups through out the trial

| Sampling time (pi) | $Ca^{++}$(mmol/L) | | $Na^{+}$ (mmol/L) | | $CL^{-}$ (mmol/L) | | $K^{+}$ (mmol/L) | |
|---|---|---|---|---|---|---|---|---|
| | Control | IBD | Control | IBD | Control | IBD | Control | IBD |
| 1 hr | 1.79±0.24 | 1.72±0.19 | 133.12±4.13 | 138.86±4.92 | 99.96±6.38 | 100.92±2.07 | 3.88±0.89 | 3.98±1.13 |
| 3 hrs | 1.80±0.29 | 1.69±0.13 | 139.78±8.50 | 137.14±1.68 | 100.52±2.35 | 101.20±3.29 | 3.16±0.54 | 3.46±0.49 |
| 6 hrs | 1.67±0.11 | 1.55±0.10 | 134.46±0.82 | 134.92±0.99 | 100.72±1.37 | 101.26±0.88 | 3.86±0.46 | 3.64±0.19 |
| 12 hrs | 1.73±0.12 | 1.69±0.18 | 136.42±0.87 | 137.04±0.70 | 101.82±0.89 | 100.16±2.00 | 3.92±0.18 | 3.86±0.44 |
| Day 1 | 1.80±0.22 | 1.77±0.14 | 136.86±0.95 | 136.36±1.15 | 102.14±0.90 | 100.80±1.75 | 3.74±0.39 | 3.36±0.42 |
| Day 3 | 1.68±0.26 | 1.80±0.20 | 136.18±1.16 | 136.62±2.08 | 102.16±1.50 | 103.10±2.53 | 3.12±0.57 | 3.14±0.42 |
| Day 5 | 1.76±0.31 | 0.67±0.29[a] | 138.02±0.91 | 69.60±6.02[a] | 103.02±3.32 | 154.60±14.28[a] | 3.21±0.42 | 3.98±0.41 |
| Day 7 | 1.83±0.19 | 0.59±0.06[a] | 139.94±2.89 | 79.80±6.02[a] | 103.54±2.14 | 152.88±18.90[a] | 3.54±0.30 | 4.98±0.58[a] |

[a] *statistically significant compared with the control group (p<0.05)*

γ-glutamyl transferase (GGT) and lactate dehydogenase (LDH) concentrations in the IBD group, all have shown a significant (P<0.05) increase at days 3, 5 and 7 pi when compared with the control group, while there was no significant (P>0.05) difference between the control and the IBD groups in the concentration of these enzymes during the early phase of the disease from 1 hr to day 1 pi (Table 3). There was no significant difference (p>0.05) in the concentration of creatine kinase (CK) throughout the trial.

From the metabolites analyzed, only cholesterol and uric acid showed significant changes in the IBD group when compared with the control group. In this regard, the cholesterol concentration was significantly (p>0.05) decreased at days 5 and 7 pi the IBD group. On the contrary, the uric acid concentration of the IBD group showed a paramount change at the later stage of the disease where it was significantly (p<0.05) increased at days 5 and 7 pi when compared with the control group. In the present study, the glucose and creatine concentration didn't show a significant change between the two groups throughout the trial (Table 4).

All the electrolytes in the present study showed significant differences (p<0.05) between the IBD and control groups towards the end of the trial. Accordingly, the calcium ($Ca^{++}$) and sodium ($Na^{+}$) concentration was reduced in the IBD groups at days 5 and 7 pi, while the concentration of chloride ($CL^{-}$) and potassium ($K^{+}$) were significantly (P<0.05) increased at days 5 and 7 pi and day 7 pi, respectively, when compared with the control group (Table 5).

## DISCUSSION

The results of this study on serum chemistry confirmed the biochemical evidence of liver and kidney damage of chickens infected with infectious bursal disease virus. Moreover, these biochemical changes agreed with the typical clinical signs such as anorexia, dehydration and diarrhoea as well as the pathological changes such as hemorrhagic, necrotic and oedematous lesions in quite a number of organs, observed in acute IBDV infection [13, 17, 18].

A significant reduction in the total protein and albumin at days 5 and 7 pi were noticed in the present study. These could be related to decrease synthesis by the impaired liver or loss of proteins through damaged kidney which has been reported previously [7, 8]. Because liver is the main site for the synthesis of protein, impaired liver morphology could lead to hypoprotenemia,

while damaged kidney would lead to loss of small molecular size and osmotic sensitivity protein (albumin) leading to hypoalbuminaemia [19]. The anorexia in the IBD infected chicks is another possible cause of the hypoprotenemia. The exhaustion of proteins in the process of recruiting the body need for proteins for tissue repair, white blood cell and antibody production, maintenance of plasma osmolality as well as enzyme production [20] should also be considered because vvIBDV potentially causes aplastic bone marrow and lymphoid necrosis in the bursa of fabricus. The diarrhoea in the present study could also contribute for the hypoprotenemia.

The reduced A:G ratio at days 5 and 7 pi in the IBD group is most likely due to decreased albumin in the face of unchanged globulin. Similarly, a decreased in A:G ratio was previously reported in IBDV infection [10]. The A:G ratio has been used extensively in analysis of sera as an indicator of infection and antibody response [21]. This dysproteinaemia has a bigger diagnostic value than the determination only of total protein [22]. The significant reduction in A:G ratio in IBDV infection has also been attributed to hypo-albuminaemia and hyper-gamma-globulinaemia [23]. Nevertheless, in the present study, there was no change in the globulin level in the present study. This finding was in full agreement with Afaleq [9]. On the other hand increased in gamma-globulin [7, 24] and total globulin [10] following IBDV infection has been described. These discrepancies could be related to the age, strain of the virus and immune status in the affected chicks. Moreover, the true serum gamma-globulin concentration might have been masked by neutralization of the antibody by the virus [25] hence less participation of the gamma-globulin fraction in determining the total globulin concentration.

The elevation in serum concentration of AST, ALT, GLDH and, LDH, GGT and ALP at day 3 pi and/or days 5 and 7 pi in the IBD group suggested pathological involvement of liver and kidney which are common sequels in IBDV infection especially following secondary viremia [7, 8, 13, 26, 27, 28,]. These hepatocellular and kidney injuries are postulated to result from hypoxic state caused by aplastic bone marrow following IBDV infection [8]. GLDH is situated in the mitochondrion of the hepatocytes and its elevation in the serum is associated with severe necrosis of the hepatocytes [23]. Ley *et al.* [7] have reported increased LDH and AST that corresponds with pathological changes in kidney and liver. Though elevation of LDH could also imply damage to the muscle [29] there was no report of muscle damage in IBDV infection. Besides the absence of changes in creatinine kinase (CK), a muscle specific enzyme [30], in the present study exclude muscle damage. These changes in serum enzymes fairly elaborate the hypoprotenemia and hypoalbunemia due to liver and kidney pathology discussed above.

The increased in uric acid (hyperurecemia) and creatinine at days 5 and 7 pi might be explained by the prevailing dehydration [23] and impaired kidney function in the chicks suffering from IBD. The serum uric acid concentration is an indicator of renal function in chickens, because uric acid is the major nitrogenous end product of chickens and is excreted into the urine through the renal tubules [31]. In a clinical chemistry observation study carried out by Ley *et al.* [7], it was observed that several chickens at 3 days pi had serum uric acid concentration higher than the control groups. Moreover, deposition of urate crystalloids in the tubules of kidney following IBDV infection was associated with the severe dehydration in acute form of the disease [32]. Ley *et al.* [33] have observed immune complex involvement in the pathogenesis of IBDV infection and suggested an immune-mediated glomerulonephrities compatible with immune-complexemia [7].

The increase in cholesterol concentration might be related to liver damage, because most of the cholesterol is metabolized in liver [34]. The mobilisation of lipids due to physiological stress is expected to raise cholesterol concentrations [35], lipid metabolism might have been impaired due to the damage to the liver. The decreased cholesterol could also be related to anorexia and diarrhoea causing reduced availability and absorption of fatty acid. Further, given the strong correlation between lipid and albumin, the hypoalbuminaemia might have resulted in low level of albumin bound lipids in the vvIBDV infected chicks leading to low level of serum cholesterol [36]. This finding was contrary to a previous report [10]; however, who have reported an increased in cholesterol concentration. This discrepancy could be related to the strain of the virus and the extent of liver damage.

The decreased $Ca^{++}$ (hypocalcemia) in the serum could be related to a decrease in intestinal absorption of dietary calcium due to anorexia and diarrhoea. The dehydration and anorexia in IBDV infection are also stressors that are supposed to induce corticosteroid secretion [9] which in turn leads to reduced serum calcium [37]. Moreover, hypoalbuminaemia is said to reduce the quantity of bound calcium and result in a decreased total serum calcium concentration [37, 38].

Similarly, the decreased sodium $Na^+$ concentration (hyponatermia) in the IBD group could be related to the dehydration, anorexia and decreased water intake [39] that were evident in the present study starting at day 2 pi. The diarrhoea is another possible cause to be considered because excess water loss can cause reduction in the Na level [38]. On the other hand, the increased chloride $CL^-$ (hyperchloridemia), decreased potassium $K^+$ (hypokalemia) could be associated with the diarrhoea [23]. Hypokalemia could result from kidney damage [37] which is a common pathology in IBDV infection.

In conclusion, the result in this study presented biochemical changes in vvIBDV infection which were consistent with the pathogenicity of IBDV infection. By and large, these biochemical changes in vvIBDV infection are mostly related to damage to the liver and kidney following the localization of the virus in these organs during secondary viremia along the course of the disease and they can be used to assess the damage and its extent in these organs prior to necropsy or death. Furthermore, these parameters are essential in clinco-pathological assessment of the disease and also potentially contribute to the understanding of the pathogenesis of IBD.

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