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Experimental Induction of Subclinical Necrotic Enteritis using C. Perfringens Field Isolate in Male Layer Chickens

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SUMMARY

Oral infection of chicken groups at 31 day of age with field isolate of toxogenic C. *perfringens* Type A resulted in subclinical necrotic enteritis as diagnosed with post-mortem and histopathological lesions in intestine and liver from the 3rd day post infection with C.*perfringens* culture. The only detected clinical signs were general signs of low feed intake and low conversion rate in infected groups as compared with the negative control group.

Aflatoxins and /or coccidia vaccine act as predisposing factors for NE. Weekly and total body weight gain (BWG), feed intake (FI) and conversion rate (CR) at the 1st wpi in aflatoxin and coccidia vaccine group was the lowest,followed by that of coccidia vaccine group ; while that of aflatoxin group was the highest.

Post mortem, microscopical lesions and lesion score in sacrificed chickens treated with aflatoxin and /or coccidia vaccine were more severe than culture infected group. Bursal and thymic microscopic lesions have been detected in all infected groups.

Experimentally, subclinical NE was induced in presence of aflatoxin and/or coccidia vaccine as predisposing factor by a single dose of C. *perfringens* broth culture.

INTRODUCTION

Necrotic enteritis (NE) is a worldwide poultry disease caused by the alpha toxin-producing bacterium *Clostridium perfringens*. C. *perfringens* can cause both clinical and

subclinical disease in poultry (Engström *et al.*, 2003; Saif *et al.*, 2003; Williams, 2005). Toxogenic strains have isolated from both diseased and healthy chickens (Timbermont *et al.*, 2009). The disease risk factors including concurrent coccidial infection and the dietary use of cereal grains high in nonstarch polysaccharides (NSP), such as wheat, barley, rye, and oats (Saif *et al.*, 2003; Jia *et al* .,2009 *and* Palliyeguru *et al.* ,2010). Coccidial vaccine had also been reported as a cofactor in induction of NE (Gholamiandehkordi *et al.*, 2007; Pedersen *et al.*, 2008). Immunosuppressive causes played a role in induction of NE (McReynolds *et al.*, 2004); as aflatoxicosis increased susceptibility to infectious disease in chickens (Bryden *et al.* 1980 and Pramanik and Bhattacharya, 1987) including cecal coccidiosis (Edds *et al.*, 1973), that act as predisposing factor for NE . NE in chickens caused economic losses due to mortalities, low growth rate and feed conversion (Lavland and Kaldhusdal, 2001) as well as costs associated with disease prevention. On the other hand; it is difficult to determine the prevalence of the mild infection in chickens that cause higher condemnation rates in broilers due hepatitis (Lavlandand and Kaldhusdal, 1999).

This work was carried out to study the effect of infection by toxogenic C. *perfringens* isolate culture administered orally with the presence of predisposing factors such as coccidia vaccine and/or aflatoxin.

Key words: Necrotic enteritis- chickens- C. *perfringens*- Coccidia vaccine- Mycotoxin.

MATERIALS AND METHODS

Ration:

Commercial balanced ration from El-Ahram poultry Rations Company was used for feeding the experimental chickens. The used ration was free from feed additives and mycotoxins.

Chicks:

One handed and fifty, 1- day old commercial male chicks obtained from El Wadi group, Giza, Cairo were used for experimental infection during this work.

Clostridial culture:

A 48 hours culture in the cooked meat medium was prepared from *C. perfringens* toxogenic field isolate and identified by HAMOUDA, *et al.* (2010) was used for experimental infection of the used chicks. The culture suspension was centrifuged for one hour at 4000 rpm, Gram stained smears made from the sediment were examined microscopically to ensure purity. The sediment was washed three times in saline, and then resuspended in thioglycollate

medium. The plates count technique was used for determination of the viable count of cells/ ml of suspension (Cruikshank *et al.*, 1975).

Experimental infection:

At the age of 31 day chickens of infected groups were orally inoculated each with 3ml /chicks of broth whole *C. perfringens* culture containing ($3x10^9$ CFU/ml).

Pathological lesions:

Gross lesions of liver and intestine of infected chickens were given scores according to AL-Sheikly and Truscott (1977) as - : Grossly Normal organ, 1+: Mild infection, 2+ Congested and 3+: Necrotic lesions.

Histopathological examination:

Tissue samples were taken at 3 days intervals from 3- 15 days post infection (dpi) from infected and control chicken groups immediately after cervical dislocation. Organ specimens were obtained from the different parts of the small intestine 1 to 5 cm long, pieces about 0.5 – 2 gm from liver, thymus and bursa. All the specimens were fixed in 10% formol saline at room temperature for at least 2 days before processing. Sections 5 µm thick were routinely stained with hematoxyline and eosin and examined microscopically for histopathological lesions as compared with non treated controls.

Aflatoxin:

Contaminated corn with 20 mg / 1 kg aflatoxin was kindly supplied by Prof. Dr M. M. Amer professor of poultry diseases, Faculty of Veterinary Medicine, Cairo University. The contaminated corn was added to chicken ration in dose of 5 g/kg.

Vaccines:

1- Coccidial vaccine: Live commercial coccidiosis vaccine (Coccivac-D) produced by Schering Plough animal, health, and Millsboro, Delaware, USA lot No 167/08. Coccidia vaccine was given by eye drop instillation of 0.05 ml/chicks containing 10 immunizing dose.

2- C. perfringens Vaccine:

Chicken NE gel vaccine was obtained from Vet, Serum and vaccine research Inst., Abassia, Cairo. The producer instruction for vaccine use was followed where the 1st dose was 0.5 ml at 2 weeks an 2nd 1ml at 2 month of age via s. c injection.

Experimental design:

The used chicks (150 one day old chicks) were floor reared and fed commercial balanced ration and water ad libitum. At the 4th day of age the chicks were randomly divided into 5 equal separate groups; 30 chicks each. At the 4th day of age, goups 2 and 4 were given

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coccidia vaccine. Chickens of groups 3 and 4 were given aflatoxin at a dose of 5µg/kg in ration from the 24th day of age to the end of the experiment. At age of 31 days birds of groups 2-5 were orally inoculated, while birds of group 1 were left as negative control.

All groups were subjected to daily observation for clinical signs and/or mortalities. From the 0 day and every 3 days till the 15th dpi, average weekly BWG and FI for calculation of FCR. One bird /group was randomly sacrificed at 3, 6, 9 and 12 days as well as 10 birds at the 15th dpi for post-mortem examination with collection of tissue samples for histopathological examination. Obtained results are shown in tables (1 and2), figs (9-12) and plates (7-15).

RESULTS

No marked clinical signs or mortality could be detected during this experiment. The recorded average (BW) of control negative group was 40.02g, 81.90 g, 131.5g and 203.75g at 1, 9, 15, and 21day of age, respectively.

Average BW of all chickens groups at the 1st and 4th day of infection was nearly the same without marked difference between treated groups and control negative one (Table1,Fig1) ; while aflatoxin treated group (3) showed the highest BWD values over all treated and control groups till the 15th dpi. Average BW in coccidia vaccine group (2) was the lowest from the 4th till the 15th dpi. Birds of group 5, those received C. *perfringens* culture, was higher than group 2 and 4 at the 7th dpi and lower than control negative group at the 1st, 11th and 15 th dpi.

The total BW of treated groups was lowest in the group 2, followed by that of group 4 and 3 (Table 2); while the control positive was lower than group 3 and higher than group 2 and 4 in the 1st week. In the 2nd week it was lower than group 3 and 4 and higher than group 2.

The control negative group was lower than group 3 in the first week and higher than all groups in the total body weight gain.

Recorded total FCR of treated groups showed that, the group 4 was higher than group 2 and 3 in the 1st week and lower than group 3 and 2 in the 2nd week (Table 2, Fig 2); while the control positive group was lower than group 2 and 4 and higher than group 3 in the 1st week, in the 2nd week it was higher than group 3 and 4 and lower than group 2. The FCR of control negative group was the lowest, while total FCR the was the best.

Control negative birds (Gr.1) showed no detectable lesions. Chickens of group (2), which was given coccidia vaccine and infected with C. *perfringens* culture, showed liver

necrosis, serosal intestinal haemorrhages (Plate 1.A) and mucosal thickness with haemorrhages (Plate1.A1) at 3rd dpi , necrotic foci and intestinal thickness with hemorrhage (Plate1.B) at 6th dpi, while additionally ballooned intestine was seen at the12th dpi (Plate1.C) and 15th dpi (Plate 1.D).

Chickens of group 3, which were given Aflatoxin and infected with C. *perfringens* culture showed liver necrosis, hemorrhages in muscles, nephritis and necrotic foci in intestinal wall at the 3rd dpi (Plate 2.A); necrotic foci, haemorrhages and thickness in intestinal mucosa at 6th and 12th dpi (plate 2B, C); distended ceci was seen at 12thand 15th dpi (Plate 2 C, D). Sacrificed birds at the 15th dpi showed also nephritis (Plate 2D).

Chickens of group 4 given 367 occidian vaccine and aflatoxin and infected with C. *perfringens* culture showed liver necrosis, severe nephrosis and haemorrhages in intestinal wall (plate 3A) at 3rd dpi; massive haemorrhage and necrotic foci in intestinal mucosa at 11th dpi (plate 3 B); while the most additional lesion at 15th dpi was enlarged ocher discoloration, haemorrhages in the capsular surface of liver (plate 3C).

Sacrificed chickens of group 5 infected with C. *perfringens* culture showed haemorrhages in intestinal wall at 3rd dpi (Plate 4A), massive hemorrhage and necrotic foci in intestinal mucosa (Plate 4 B) at 6th dpi; liver necrosis with hemorrhages in intestinal wall was detected at 9th and 12th dpi (plate 4 C, D); necrosis in intestinal wall was additionally seen at 12th dpi (plate 4 D). Intestinal lesions at 15th dpi were very mild.

Table (1): Average body weights of treated infected chicken groups and their control.

Gr No.	Treatment	Weight post infection				
		1 day	4 days	7 days	11 days	15 days
		M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
1	Control –ve	331.4 28.4	362.9 31.6	403.9 31.5	453.7 32.6	539.2 38.8
2	Coccidia vaccine	325.9 36.62	363.9 44.7	403.0 57.5	430.4 59.3	460.0 69.0
3	Aflatoxin	347.6 27.9	396.2 33.5	455.4 33.9	513.0 41.9	563.1 49.5
4	Coccidia vaccine + aflatoxin	332.4 29.6	376.2 34.1	400.0 51.7	465.4 51.7	507.6 63.4
5	C. p culture	324.8 34.1	364.8 36.9	410.7 42.0	452.9 47.8	514.4 60.4

C. P : *Clostridial perfringens*

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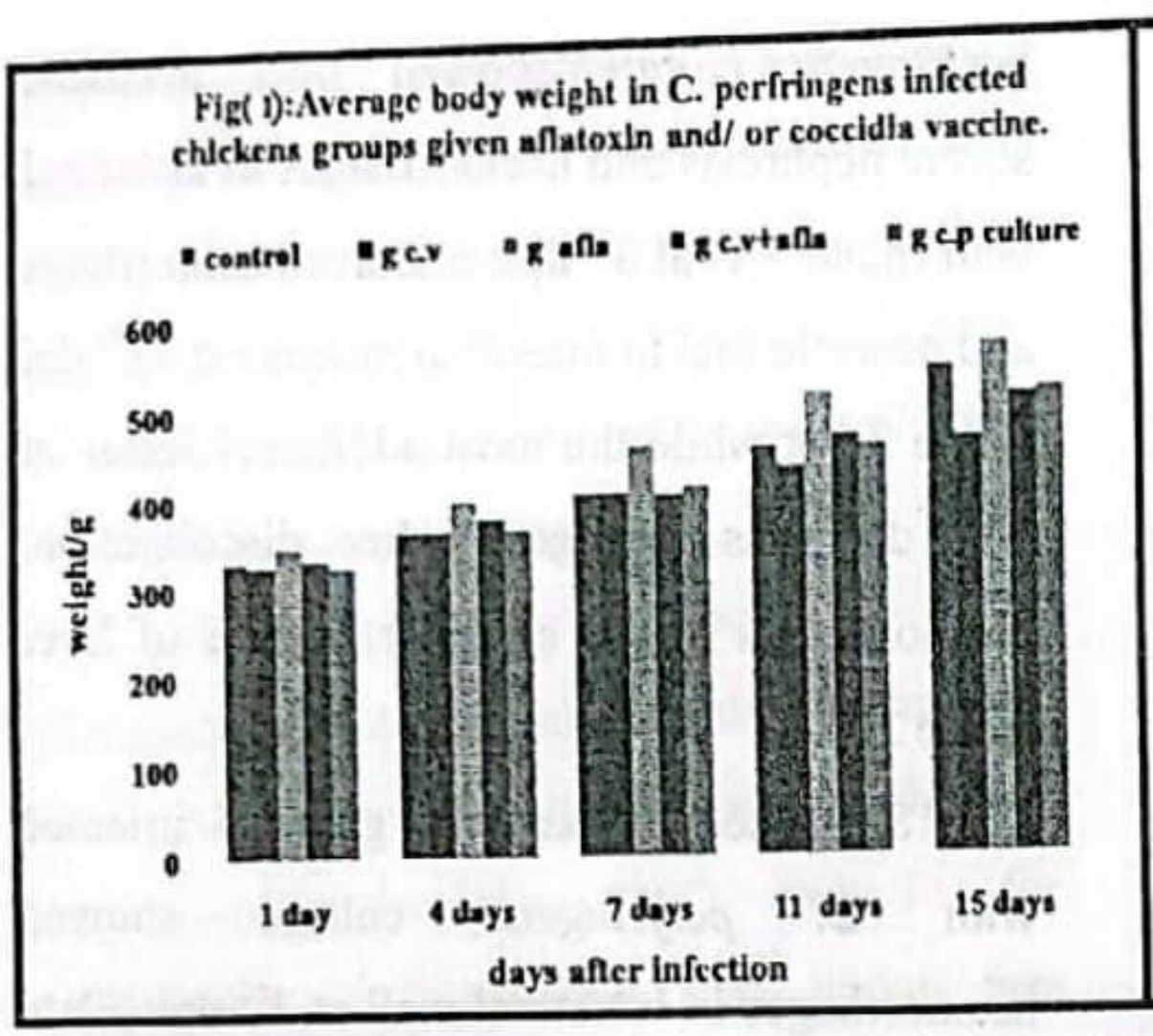

Fig(1):Average body weight in C. perfringens infected chickens groups given aflatoxin and/ or coccidia vaccine.

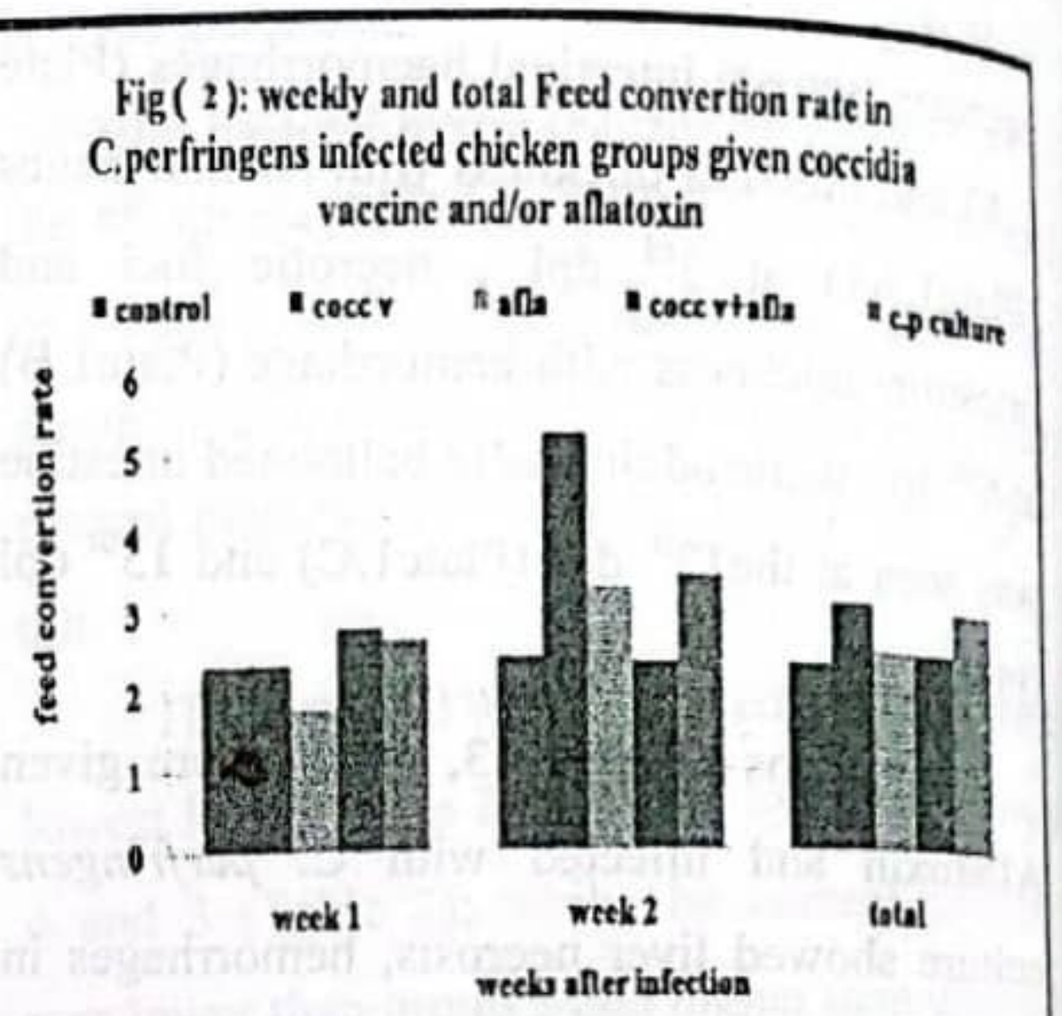

Fig (2): weekly and total Feed convertion rate in C.perfringens infected chicken groups given coccidia vaccine and/or aflatoxin

Table (2): Weekly and total body weight gain, feed intake and conversion rate in chicken groups infected with C. *perfringens* after administration of coccidia vaccine and/or aflatoxin.

Group No	Treatment	Weekly avenage						Total		
		1st Week			2nd Week					
		BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR
1	Control	82.5	191.37	2.31	135.5	325.92	2.4	218	517.29	2.37
2	Coccidia Vaccine	78.1	182.14	2.33	57	300	5.26	135.1	428.14	3.16
3	Aflatoxines	107.8	190	1.76	107.7	357.14	3.31	215.5	547.14	2.53
4	Cocc.v.+afla	69.6	194.82	2.79	107.6	251.85	2.34	177.2	446.67	2.52
5	C. p Culture	85.9	227.58	2.64	103.7	359.25	3.46	189.6	586.83	3.09

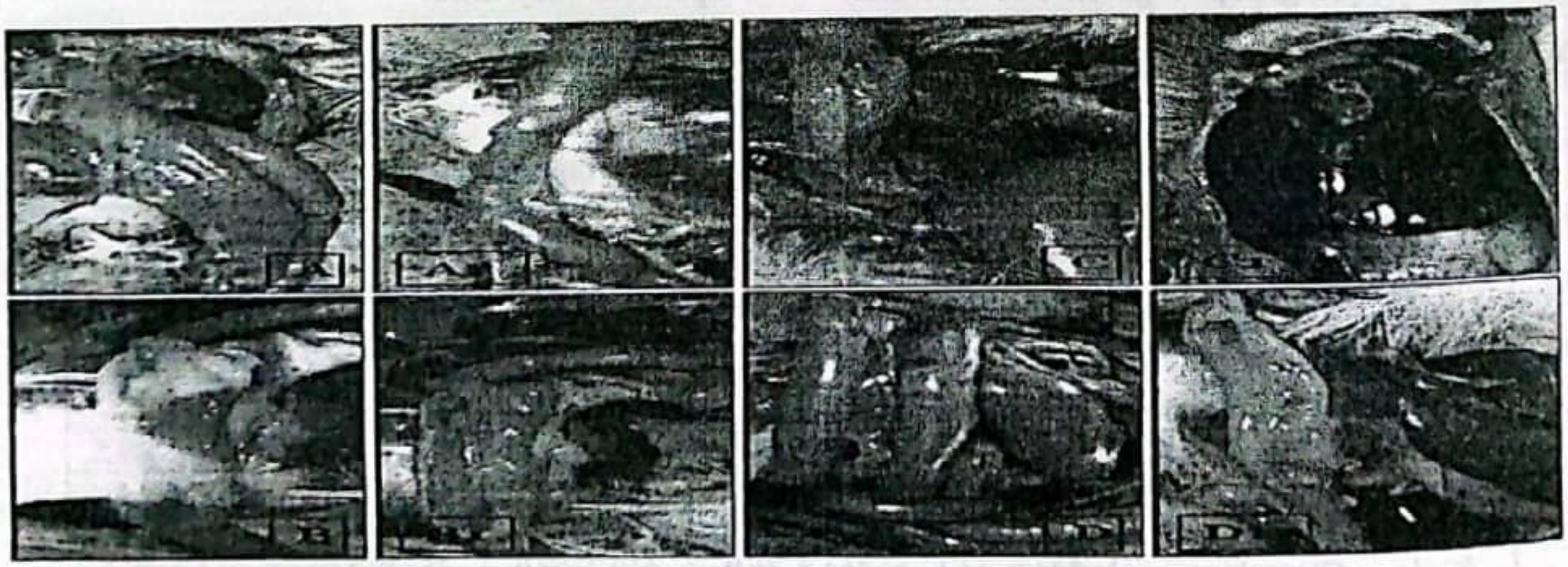

Plate (1): Liver and intestinal lesion in sacrificed chickens given coccidia vaccine and infected with C. *perfringens*:

A: 6 dpi; Liver necrosis and serosal intestinal haemorrhages
A1: Mucosal thickness with haemorrhages
B: 9 dpi; necrotic foci necrosis and mucosal thickness with haemorrhages
B1: Liver necrosis and mucosal thickness with haemorrhages
C: 12 dpi; Liver necrosis, ballooned intestine and mucosal thickness with haemorrhages
C1: Liver necrosis
D: 15 dpi; Ballooned cecum, serosal intestinal haemorrhages
D1: Liver necrosis, and mucosal thickness with haemorrhages

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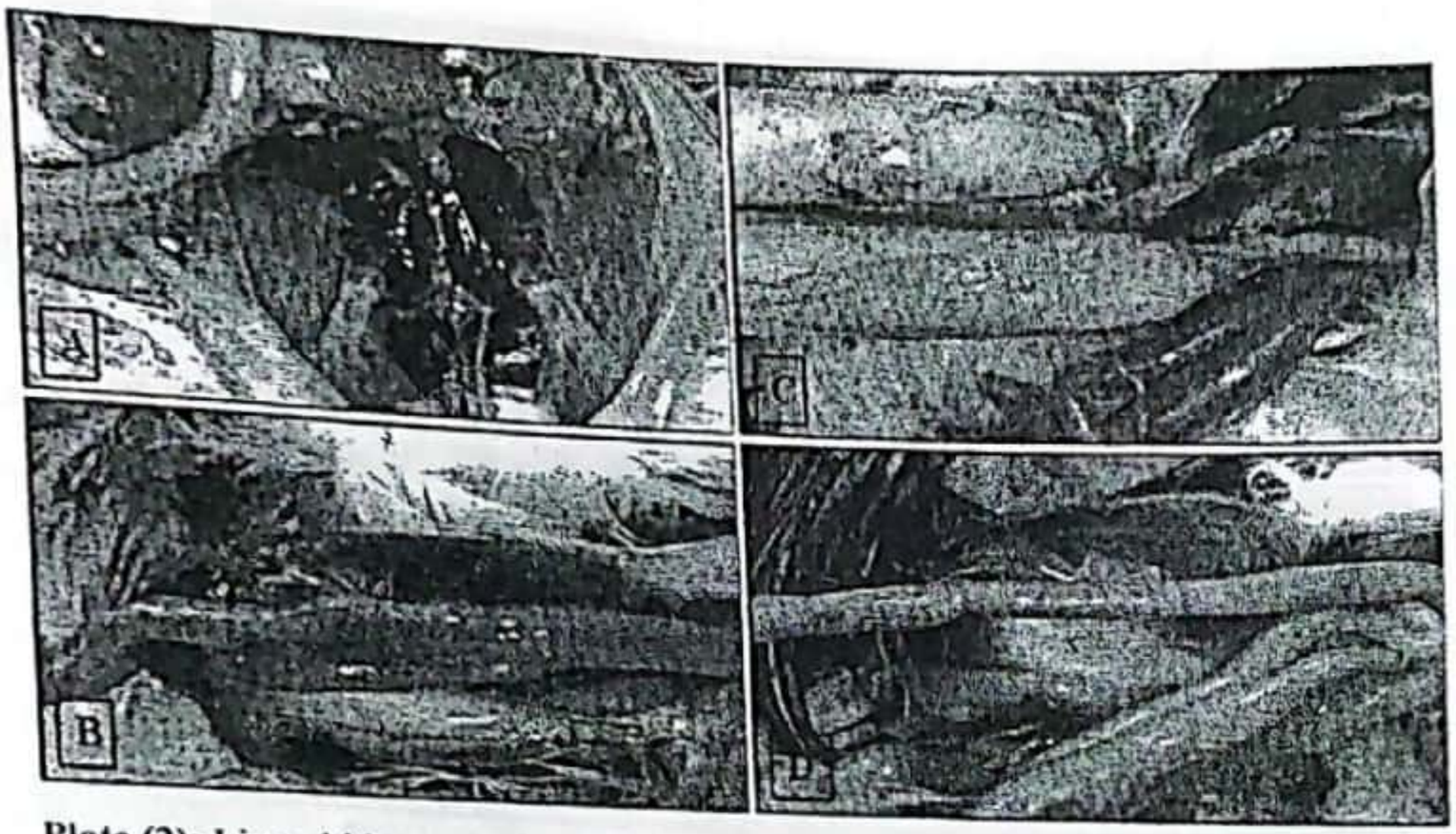

Plate (2): Liver, kidney, muscle and intestinal lesion in sacrificed chickens given aflatoxin and infected with C. *perfringens*

A: 3 dpi; haemorrhages in muscles, Nephritis and necrotic foci in intestinal wall.
B: 6 dpi; Necrotic foci, haemorrhages and thickness in intestinal mucosa.
C: 12 dpi; Liver necrosis, distended ceci, s and necrotic foci in intestinal mucosa with haemorrhages.
D: 15 dpi; Nephritis, Distended ceci, and haemorrhage in intestinal serosa.

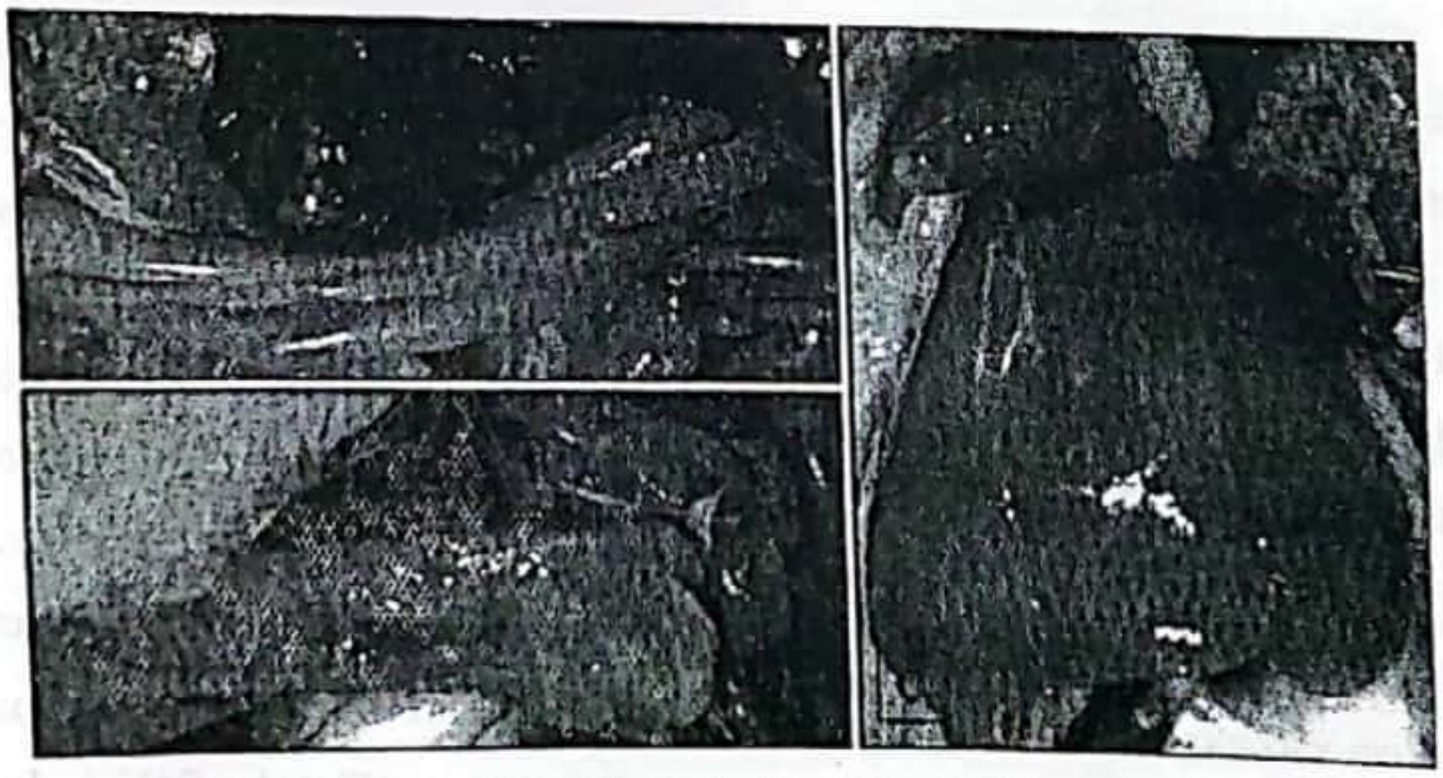

Plate (3): Liver, kidney and intestinal lesion in sacrificed chickens given coccidia vaccine and aflatoxin, infected with C. *perfringens*

A: 3 dpi ; liver necrosis, severe nephrosis and haemorrhages in intestinal wall.
B: 12 dpi; massive haemorrhage and necrotic foci in intestinal mucosa.
C: 15 dpi; enlarged ocher discoloration, haemorrhages in the capsular surface of liver.

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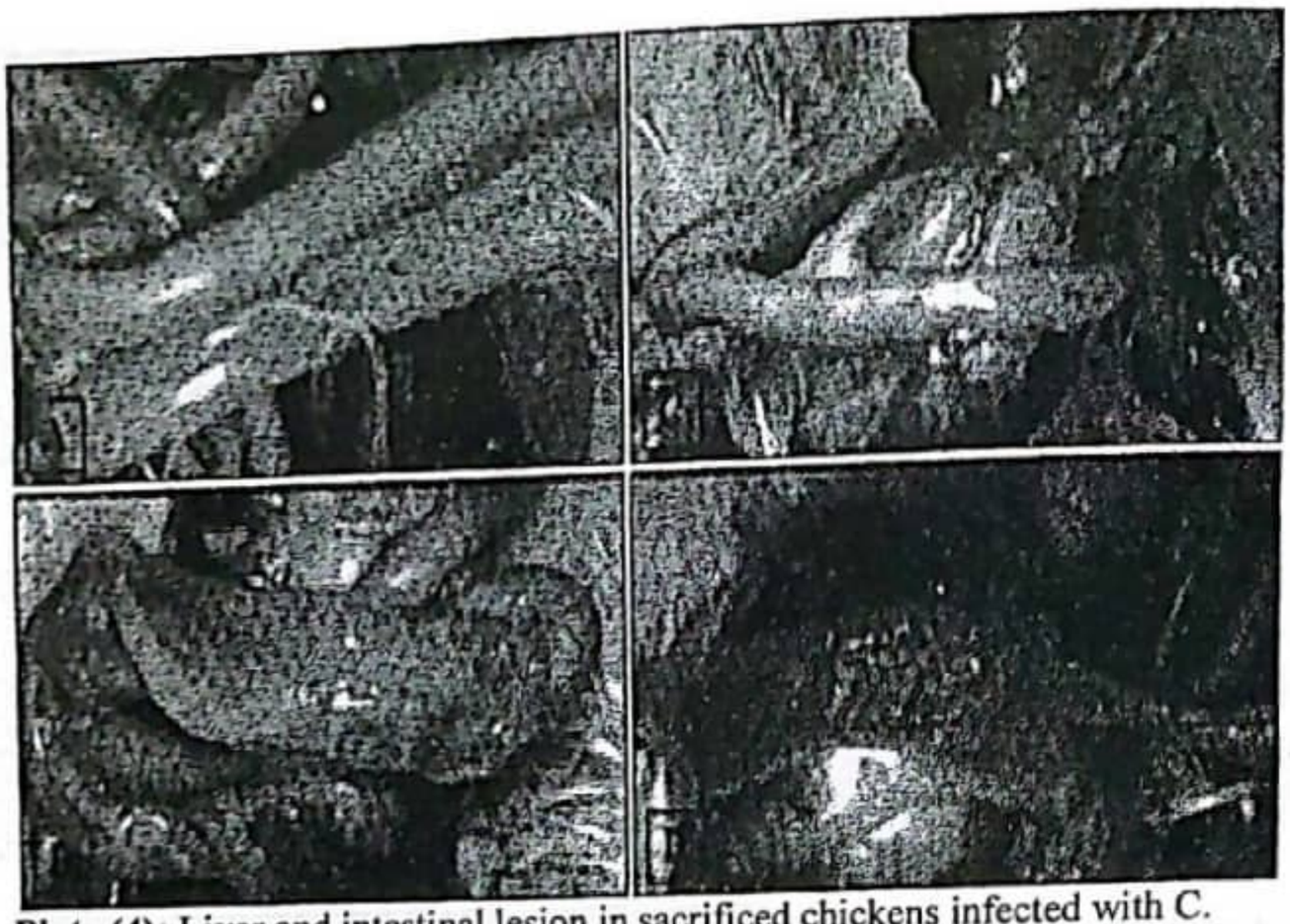

Plate (4): Liver and intestinal lesion in sacrificed chickens infected with C. *perfringens*
A: 3 dpi; haemorrhages in intestinal wall
B: 6 dpi; massive haemorrhage and necrotic foci in intestinal mucosa
C: 9 dpi; liver necrosis and haemorrhages in intestinal wall
D: 12 dpi; haemorrhages and necrosis in intestinal wall.

Lesion score indicated that C. *perfringens* infected groups 2 and 3 showed the same increasing intestinal score from 1+ at 3rd dpi to reach 2+ at 9th dpi while group 4 showed score 2+ at 3rd dpi. The effect of previous treatment on lesion score was markedly seen at the 10 th dpi, where groups 4 and 5 showed 3+. Results of 15th dpi proved that the group 2, which are given coccidian vaccine showed 3+ as well as group 5, which are given C. *perfringens* culture. Liver lesion score was 2+ at 3rd dpi and from 9th to 15th dpi in group 4.

Generally speaking, coccidia vaccine and/or aflatoxin increased lesion score as compared with that in infected control (group 5).

Microscopically examined tissue sections in group 1 (control negative) showed apparently healthy liver, intestine, bursa and thymus tissues at all intervals.

Birds of group 2 showed liver congestion of the portal vessels with formation of newly formed bile ductules at 6-9 dpi (Plate1, 2); while at12-15 dpi liver showed focal haemorrhage and focal area of coagulative necrosis infiltrated by leucocytes (Plate1, 2a). Intestine showed vacuolated enterocytes and submocosal congestion at 3-15 (Plate1, 2). Bursa showed atrophy of follicles and interfollicular edema and congestion at 6-9 dpi (Plate 2, 2); as well as necrosis of lymphoid follicles 12-15 (Plate 2, 2a). Thymus showed diffuse cortical

haemorrhage at 6-9 dpi (Plate3, 2). And cortical atrophy with medullary haemorrhage at 12-15 dpi (Plate 4, 2a).

Sacrificed chickens at 3 dpi in group 3 showed portal congestion and fibrosis (plate1, 3),and focal area of necrosed hepatocytes infiltrated with leucocytes (Plate1, 3) at 6-15 dpi . Intestine showed necrosed mucosa at 3 dpi (Plate 2, 3).as well as mucosal necrosis , leucocytic infiltration, submucosal congestion and mononuclear cells at 6-15 dpi (Plate 2, 3a). At 3-15 dpi Bursa showed follicular atrophy and interfollicular haemorrhage (Plate 3, 3) ; while thymus showed medullary haemorrhage (Plate 4, 3) at 3-15 dpi

In group 4 at 3-15 dpi liver showed focal area of necrosed hepatocytes infiltrated with mononuclear cells with dissociation of hepatic cells (Plate1,p4). Intestine showed mucosal necrosis inflammation and submucosal congestion (Plate2, 4) at 3-15 dpi. Furtherore, at 3-15 dpi bursa showed necrosed follicles (Plate 3, 4) and thymus showing haemorrhagic cortex and medulla (Plate 4, 4).

Liver of group 5 showed dissociation and disorganization of hepatic parenchyma at 3 dpi (Plate1, 5) followed by focal area of mononuclear cells infiltration at 6-15 dpi (Plate1, 5a). Intestine section showed mucosal necrosis and leucocytic infiltration at 3-15 dpi (Plate 2, 5). Bursa showed necrotic follicles and interfollicular edema at 3 dpi (Plate13, photo5), as well as follicular disintegration at 6-15 days (Plate 3, 5a); while Thymus showed medullary necrosis at 6-15 dpi.

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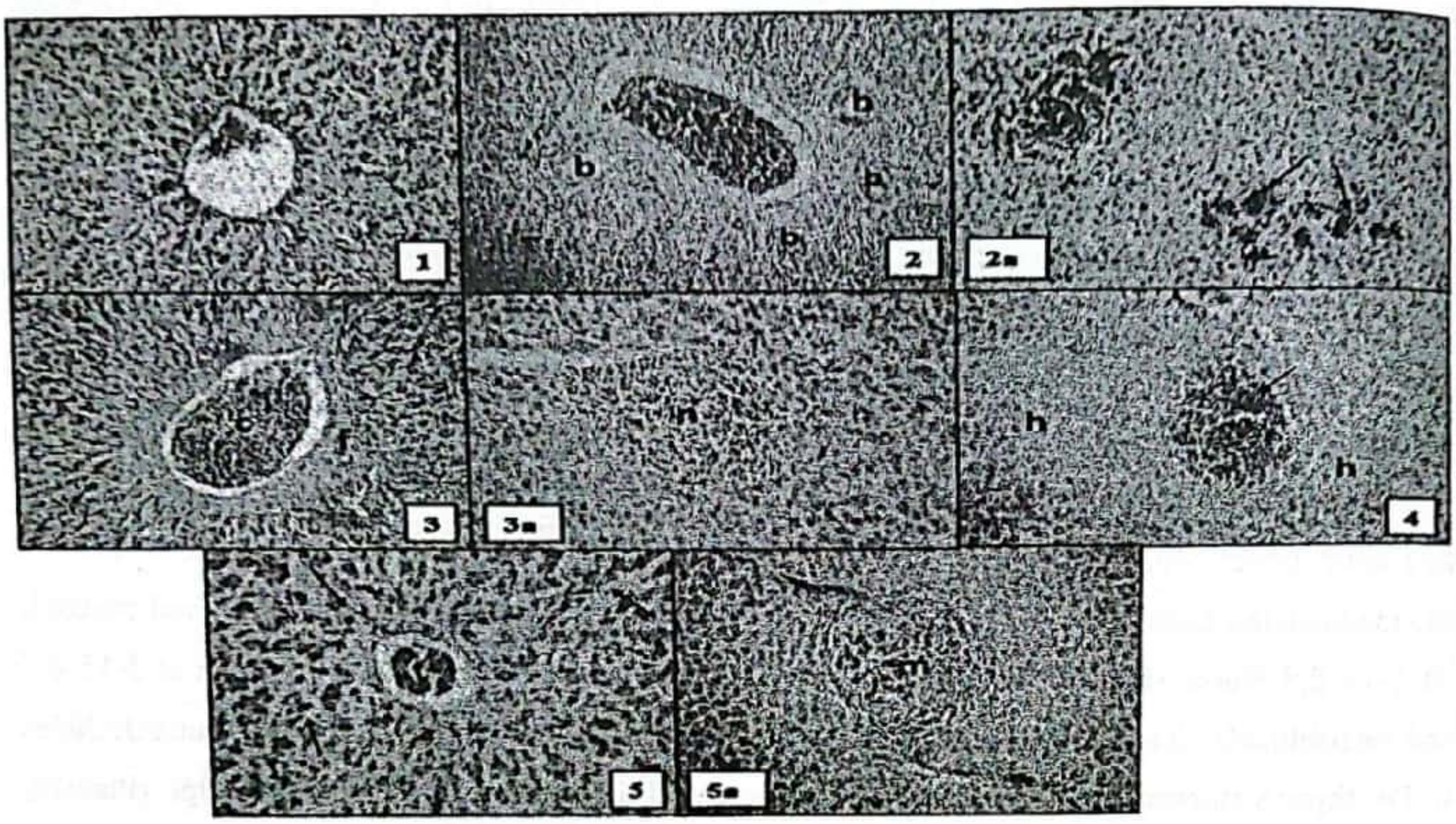

Plate (1): liver tissue sections stained with H&E of sacrificed chickens infected with C. *perfringens*

1: 3-15 dpi; Gr 1; Apparently healthy tissue (X 200)

2: 6-9 dpi; Gr 2; Congestion of the portal vessels (v) with formation of newly formed bile ductules (b) (X 200)

2a: 12-15 dpi; Gr 2; Focal haemorrhage (arrows) and focal area of coagulative necrosis infiltrated by leucocytes (L) (X400)

3: 3 dpi; Gr 3; Portal congestion (c) and fibrosis (f) (X 200)

3a: 6-15 dpi; Gr 3; Focal area of necrosed hepatocytes infiltrated with leucocytes (n) (X400)

4: 3-15 dpi; Gr 4 showing focal area of necrosed hepatocytes infiltrated with mononuclear cells (arrows) with dissociation of hepatic cells (h) (X200)

5: 3 dpi; Gr 5; Dissociation and disorganization of hepatic parenchyma (arrows) (X400)

5a:6-15 dpi; Gr 5; Focal area of mononuclear cells infiltration (m) (X200)

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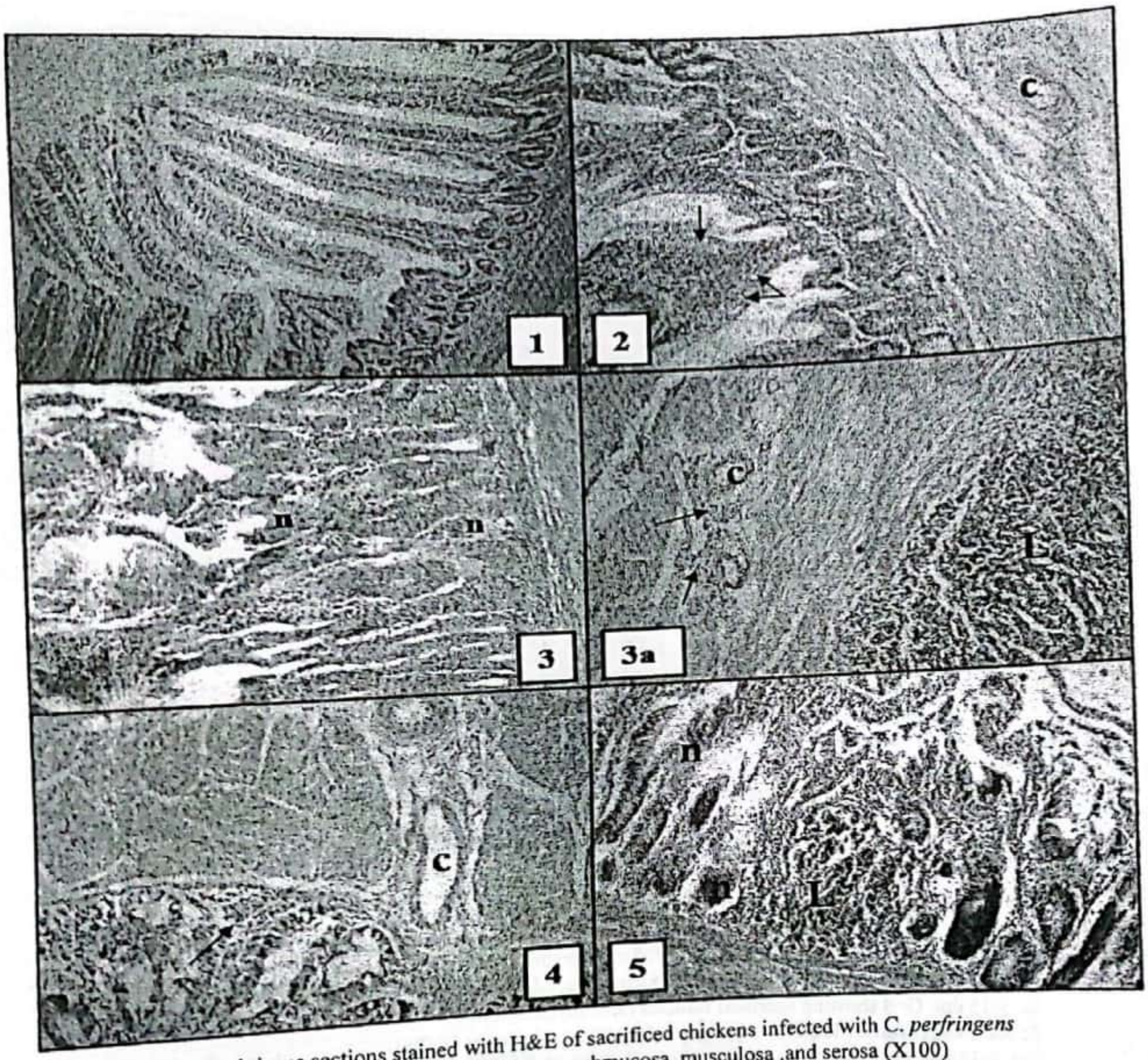

Plate (2): Intestinal tissue sections stained with H&E of sacrificed chickens infected with C. *perfringens*
1: 3-15 dpi; Gr 1 apparently normal mucosa , submucosa, musculosa ,and serosa (X100)
2: 3-15 dpi; Gr 2 vacuolated enterocytes (arrows), and submucosal congestion (C) (X 200).
3: at 3 dpi; Gr 3 necrosed mucosa (n) (X 200). 3a: 6-15 dpi; Gr 3 mucosal necrosis and leucocytic infiltration (L) and submucosal congestion (c) and mononuclear cells (arrows) (X 200).
4: 3-15 dpi; Gr 4 mucosal necrosis inflammation (arrows) and submucosal congestion (c) (X 200)
5: 3-15 dpi; Gr 5 mucosal necrosis (n) and leucocytic infiltration (L) (X 200).

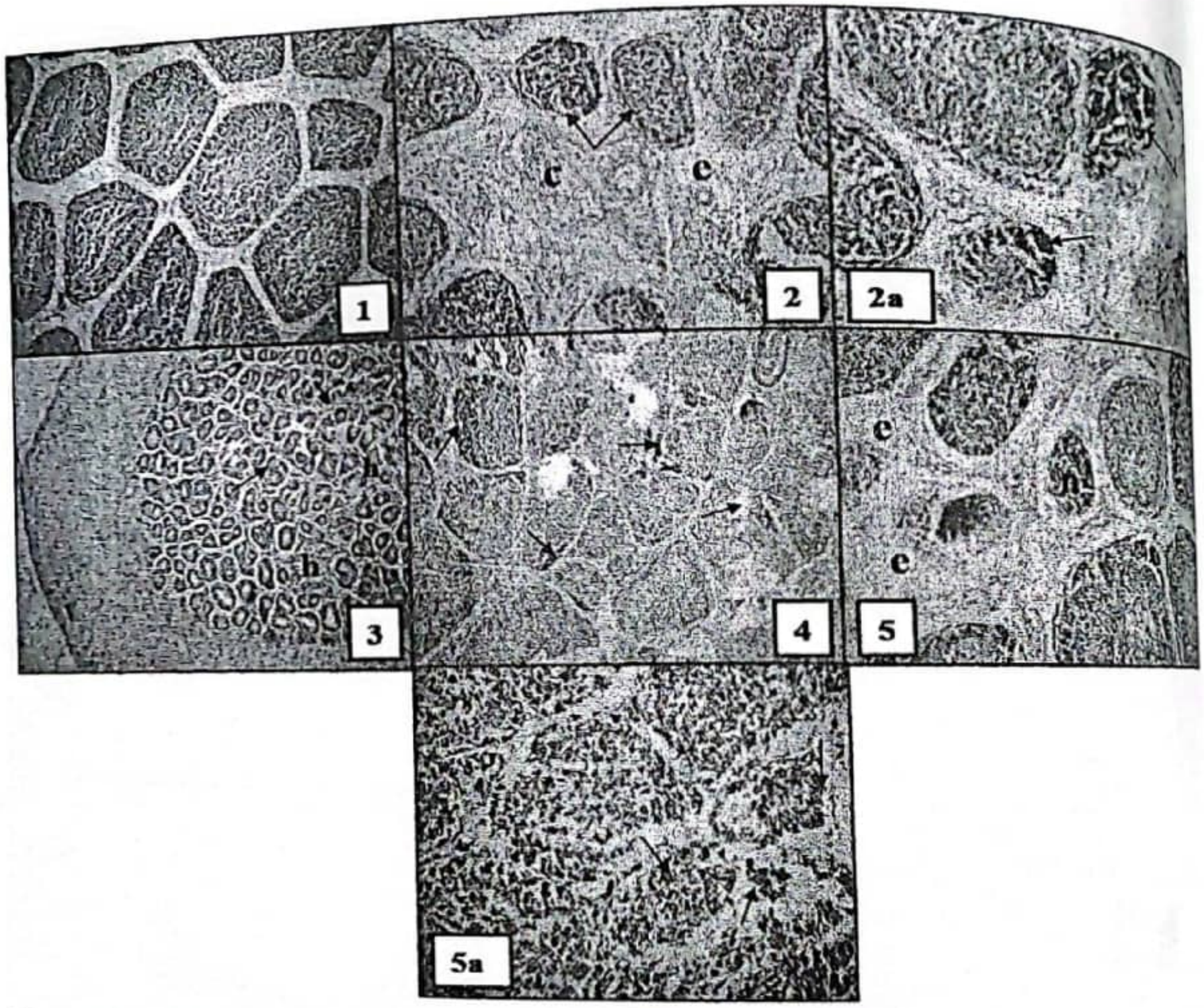

Plate (3): Bursal tissue sections stained wit H&E of sacrificed chickens infected with C. *perfringens*

1: 3-15 dpi; Gr 1 apparently healthy follicles (X 200)

2: 6-9 dpi; Gr 2 atrophy of follicles (arrows) and interfollicular oedema (e) and congestion (c) (X 200)

2a: 12-15 dpi; Gr 2 necrosis of lymphoid follicles (arrows) (X 400)

3: 3-15 dpi; Gr 3 follicular atrophy (arrows) and interfollicular haemorrhage (h) (X 200)

4: 3-15 dpi; Gr 4 showing necrosed follicles (arrows) (X 100)

5: 3dpi; Gr 5 necrotic follicles (n) and interfollicular oedema (e) (X 200)

5a: 6-15 dpi; Gr 5 follicular disintegration (arrows) (X 400).

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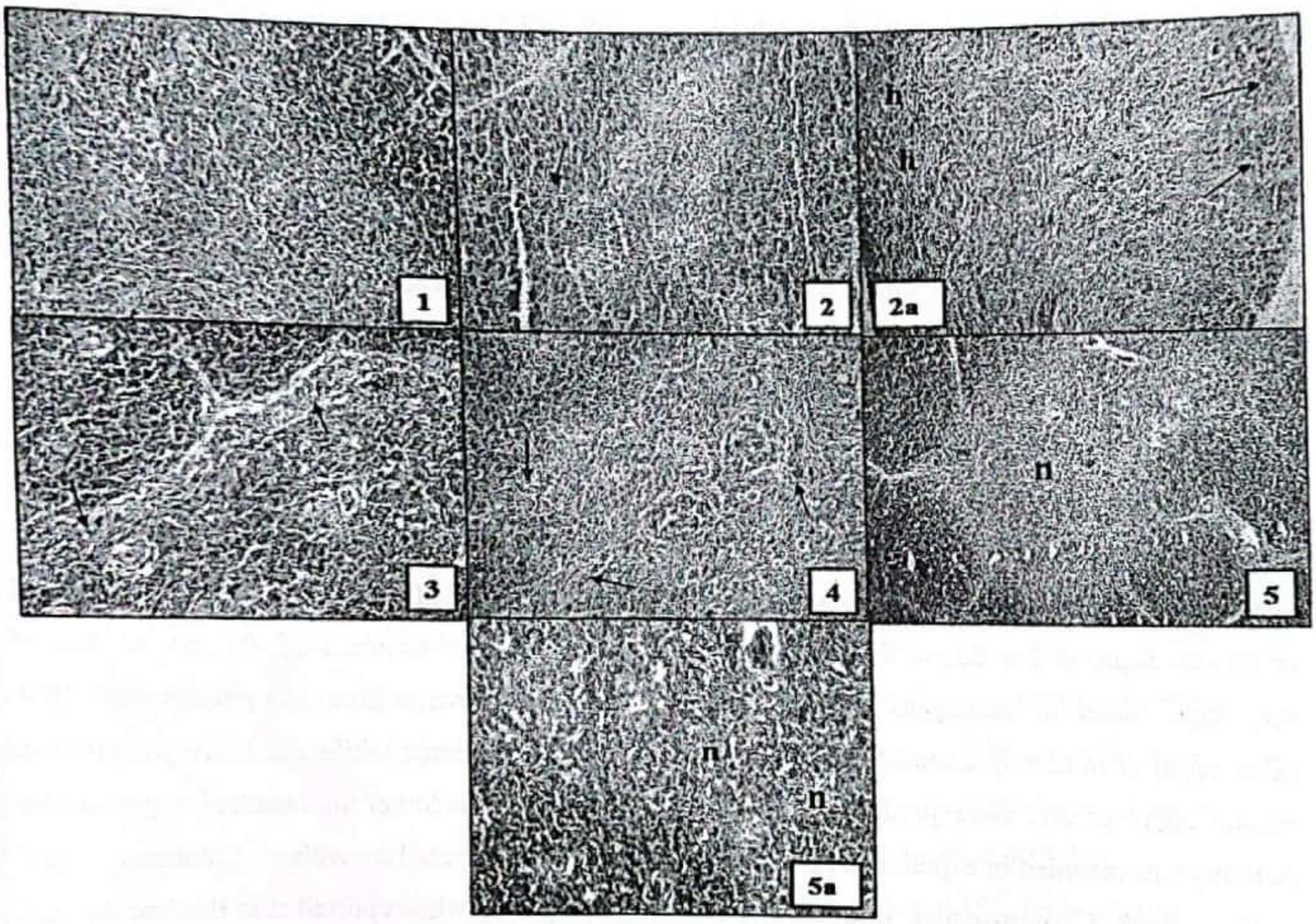

Plate (4): Thymus tissue sections stained with H& E of sacrificed chickens infected with C. *perfringens*
1: 3-15 dpi; Gr 1 apparently normal cortex and medulla (X 200)
2: 6-9 dpi; Gr 2 diffuse cortical and hemorrhagic haemorrhage (arrows) (X 200)
2a: 12-15 dpi; Gr 2 cortical atrophy (arrows), and medullary haemorrhage (h) (X 100)
3: 3-15 dpi; Gr 3 medullary haemorrhage (arrows) (X 200)
4: 3-15 dpi; Gr 4 hemorrhagic cortex and medullar (arrows). (X 200)
5: 6-15 dpi; Gr 5 medullary necrosis (n) (X 100)
5a: 6-15 dpi; Gr 5 higher magnification of the medullary necrosis (n) (X 400).

DISCUSSION

Understanding the disease progression of NE has been very difficult due to its complexity and because several predisposing factors such as dietary components, immunosuppression, mechanical irritation of the gut, and sudden gut microflora changes appear to contribute to this syndrome (Smith, 1965; Elwinger *et al.*, 1992; Ficken and Wages 1997).

For induction of NE in 31 day old male layer chickens the subjected model of McReynolds *et al.* (2004) was conducted as all groups were given C. *perfringens* in the presence or absence of commercial infectious bursal vaccine as immunosuppressant, with or

without commercial coccidia vaccine. Natural outbreaks of NE have been reported in 2 to 5 weeks old chickens (Bains, 1968 and Berinier *et al.*,1974a) and at age 4-7 weeks old (Cygan and Nawak 1974).

There were no marked clinical signs or mortality detected during this experiment, this finding agrees with Cowen *et al.* (1987) who reported only a small incidence of NE in some chickens challenged with C. *perfringens* but failed to induce signs of NE in others .also Pedersen *et al.* (2003) failed to induce mortality or clinical signs of NE despite inoculation of very high doses of pathogens .In addition Olkowanski *et al.*(2006) demonstrated that no clinical signs of NE were produced and no mortality was recorded in experiment involving challenge with C. *perfringens* in short term exposure using a single and high dose. Also Pedersen *et al.* (2008) who carried out an experiment to establish an infection and disease model for C. *perfringens* using coccidia vaccine at 10 times the prescribed dosage, and found that no mortality was detected in any of the groups, however, chickens developed the subclinical form of NE.

Total BWG in group 2, it can be seen that the over dose of coccidia vaccine with the infection by C. *perfringens* culture produced significant decrease lower than infected control positive group, the result agreed with Pedersen *et al.* (2008) who reported that chickens in the groups receiving both coccidia vaccine and C. *perfringens* developed the subclinical form of NE which demonstrated by low growth rate and than groups receiving either coccidia vaccine or C. *perfringens* alone. in group 3 receiving aflatoxin and C. *perfringens* culture , it was the highest in treated groups and lower than control negative group , it can be noticed that aflatoxin dose in the group 3 was act as stimulant ,this agreed with Diaz *et al.* (2008) who reported that low levels of aflatoxin consumption in feed may affect as stimulant of the growth rate of chickens known as hormesis phenomenon (low dose stimulation); while the body weight gain of group 3 was lower than control negative, this result disagreed with Calabrase and Blain.(2005) who reported that the low dose of aflatoxin stimulation is typically maximal at 30% to 60% greater than control .it can be explained by the fact that the infection by C. *perfringens* culture was influenced the growth rate of the group and was the cause of the decrease. In addition the group 4 receiving both aflatoxin and coccidia vaccine and infected with C. *perfringens* culture revealed a low rate than the group which received only aflatoxin and than infected control positive group. This result is in accordance with that reported by Ruff (1978) who demonstrated that chickens which received aflatoxin diet and inoculated

with sporulated oocysts of E. *acervulina* gained significantly less weight than chicks received either aflatoxin or coccidia alone. Also Wyatt *et al.* (1975) reported that the depression weight was more severe in chickens that received both aflatoxin and coccidia than that received either aflatoxin or coccidia alone.

In addition, all treated groups showed an increase in total FCR compared to the control negative group. This result agreed with (Hofshagen and Kaldhusdal, 1992; Kaldhusdal *et al.*, 2001 and Hofacre *et al.*, 2003).

PM examination of sacrificed chickens in control group showed no lesion, While in group2 liver necrosis with serosal intestinal haemorrhage, necrotic foci and intestinal thickness with haemorrhage, distended ceci and haemorrhage in intestinal serosa at 6^{th} ,12^{th} ,15^{th} dpi ,respectively, were observed. This result agreed with Gholamiandehkordi *et al.*, (2007) who demonstrated that combined inoculation of C. *perfringens* and an overdose of live coccidial vaccine and E. *maxima* resulted in typical necrotic lesions. Pedersen *et al.*, (2008) recorded that chickens in the groups receiving both coccidial vaccine and C. *perfringens* developed the subclinical form of NE, demonstrated by focal necrosis in the small intestine. In addition, Williams (2002) mentioned that live attenuated coccidial vaccine strains can induce a certain degree of mucosal damage.

Chickens of group 3, which were given aflatoxin and infected with C. *perfringens* culture showed liver necrosis, haemorrhages in muscles, nephritis and necrotic foci in intestinal wall at the 3^{rd} dpi ; necrotic foci, haemorrhages and thickness in intestinal mucosa at 6^{th} and 12^{th} dpi ; distended ceci was seen at $12^{th}$and 15^{th} dpi. Sacrificed birds at the 15^{th} dpi showed also nephritis.

Chickens of group 4, which were given coccidia vaccine and aflatoxin and infected with C. *perfringens* culture showed liver necrosis with haemorrhages in intestinal wall at 3^{rd} dpi; massive haemorrhage and necrotic foci in intestinal mucosa at 12^{th} dpi; while the most additional lesion at 15^{th} dpi was enlarged ocher discoloration, haemorrhages in the capsular surface of liver. As reported by Carnaghan, *et al.* (1966), who demonstrated that aflatoxicosis in chickens causes yellow, ocher discoloration of the liver, with multifocal haemorrhage and a reticulated pattern on the capsular surface. Also Shareef (2010) demonstrated that chickens suffring from coccidiosis and aflatoxicosis presented greasy, yellow, ocher discoloration of liver with scattered areas of subcapsular haemorrhage and caseous necrotic materials in the cecum. Chickens of group 5 infected with C. *perfringens* culture showed haemorrhages in

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intestinal wall at 3rd dpi, massive hemorrhage and necrotic foci in intestinal mucosa at 6th dpi; liver necrosis with hemorrhages and necrosis in intestinal wall at 9th and 12th dpi were detected. Similar findings were reported by (Kaldhusdal and Hofshagen, 1992; Park *et al.*, 1994; Das *et al.*, 1997b; Olkowski *et al.*, 2006).

Collectively, pathological lesions were detected in all infected groups in form of thickened mucosa, heamorrhages and or necrotic foci in the middle part of intestine with friable wall and distension with gas. These findings are in agreement with those of Narin and Bamford (1967), Bains (1968), Helmboldt and Bryant (1971), Collins et al (1975) and Tsai and Thug (1981). The detected cecal lesion was also reported by (Long, *et al.* 1974). The detected thickened mucosa with necrosis at the 3rd dpi was also reported by AI-Sheikhly and Truscott (1977a). Liver lesions including swollen, discolored livers with necrotic foci (Eleazer and Harrell, 1976) in addition to Kidney lesions (Onderka, *et al.* 1990) have been reported in association with clinical and subclinical NE infections by Lovland and Kaldhusdal (1999 and 2001).

The score lesions of principal pathological lesions of birds (Table 9) declared that treated groups showed higher score lesions in both intestinal and liver of affected birds than control negative group (Al-Sheikhly and Al-Saieg, 1980; Baba *et al.*, (1997) and Das *et al.*, 1997a, b; McReynolds *et al.*, 2004).

In the liver there was a focal area of necrosed hepatocytes infiltrated with leucocytes in group 2 and 3 and dissociation of the hepatic cells with focal area of mononuclear cells infiltration in group 4 and 5.

In the intestine there was vacuolated enterocytes, mucosal necrosis inflammation and submucosal congestion in group 2 and 4 and in group 3 and 5 there was mucosal necrosis, leucocytic infiltration and mononuclear cells.

The histopathological changes observed in the liver and intestine were almost similar to those described by Kwatra and Chaudhury (1976), who revealed that the lamina propria was infiltrated with small and large mononuclear cells. Al-Sheikhly and Truscott (1977b) found mononuclear cells infiltration in the lamina propria of most of the villi and small areas of focal coagulative necrosis in the liver. Broussard *et al.* (1986) reported extensive necrosis of the villi and infiltration of the lamina propria with mononuclear cells. Park *et al.* (1994) in their histopathological study on 12 naturally occuring cases of necrotic enteritis in chickens showed severe necrosis of the intestinal mucosa.

Atrophy of follicles and interfollicular oedema and haemorrhage as well as necrosis of bursal lymphoid follicles with disintegrated

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follicles were found in the treated challenged groups, while the thymus showed a cortical trophy with diffuse haemorrhage and medullary haemorrhage in group (2, 3 and 4) and medullary necrosis in group 5.

The histopathological changes with the detected lesions in the form of focal areas of intestinal mucosal necrosis, hepatic necrosis and impaired performance (poor BWG and FCR) without clinical signs indicated the induction of mild form of the disease as described by Kaldhusdal and Hofshagen (1992) and Lovland and Kaldhusdal (1999 and 2001). The more clear lesions in groups previously given coccidial vaccine cleared the possible role of coccida vaccine in induction of subclinical NE (Van Immerseel *et al.*, 2004 and Pedersen *et al.*, 2008).

It is proposed that, in addition to toxin production, intra-species growth-inhibition might be a virulence trait (the C. *perfringens* clinical outbreak strains inhibited the growth of other C. *perfringens* strains) that contributes to the ability of certain C. *perfringens* strains to cause NE in broilers (Timbermont *et al.*, 2009). C. *perfringens* NE toxin B, Net B, was recently proposed as a new key virulence factor for the development of NE in broilers (Abildgaard *et al.* 2010). Also, It was cleared that aflatoxin as feed intoxication factor in poultry feed can be incriminated as a predispose factor for NE due to their effect, as aflatoxicosis had been reported to be strongly associated with increased susceptibility to infectious disease (Pier 1973).

Experimentally subclinical NE was induced in presence of aflatoxin and/or coccidian vaccine as predisposing factor by single dose of C. *perfringens* broth culture.

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Experimental Induction of Subclinical Necrotic Enteritis using C. Perfringens Field Isolate in Male Layer Chickens

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فى محاولة لدراسة مقدرة معزول ممثلة للنوع (أ)على احداث المرض معمليا وتجريبيا فى وجود السموم الفطرية او/و لقاح الكوكسيديا فى ديوك البياض عمر 31 يوم والسابق تحصينها بلقاح التهاب غد فبريشى المعدى المستضعف باعطاء الطيور كل جرعة واحدة من الميكروب النامى على منبت الشربة عن طريق الفم. أدت العدوى الصناعي الى احداث الاصابة تحت الاكلينكية ذات الاعراض الطفيفة المتمثلة فى نقص الوزن ومعدل التحويل الغذائى دون حدوث نفوق ؛ بينما اظهر الفحص المرضى لدجاج ذبج بعد ثالث يوم ثم كل 3 ايام من العدوى ظهور افات تشريحية ومجهرية نسيجية فى الامعاء والكبد بينما كانت الافات المجهري اوضح فى اليوم الثالث من العدوى ؛ كما تم التعرف على افات نسيجية فى كل من غدة فبريشى والغدة الثايموثية. تفاوتت الافات تبعا لنوعية العامل المساعد حيث كان اشدها فى المجموعة التى عوملت بالسم الفطرى ولقاح الكوكسيديا ثم المعاملة بلقاح الكوكسيديا ثم السم الفطرى بينما كان اقلها فى المجموعة الابطة المعدي دون سابق معاملة. امكن احداث الحالة تحت الاكلينكية من المرض بالعدوى الصناعية فى وجود السم الفطرى و/او لقاح الكوكسيديا.

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