Pathological studies on caecal coccidiosis and trials for prevention using micro-flora

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Abstract
This work was carried out to study the pathological changes associated with **Eimeria tenella (*E. tenella*)** infection in balady chicks after egg treatment (spraying) using normal intestinal microflora preparation (NIMP) during hatching time. Results showed that NIMP could protect ***E. tenella***-infected chicks against mortalities and severe form of disease. The infected not treated chicks showed severe clinical signs (bloody diarrhea with mortalities). The pathological lesions were massive caecal hemorrhage with thickened wall with lesion score of +3. Caeca of the birds showed denuded mucosa and submucosa with fragments containing developmental stages of **Eimeria**, extravasated erythrocytes and fibrinus shreds from necrotic mucosa. Proliferation of the epithelial lining with submucosal extensive hemorrhages. Caecal core consists of extensive necrosis, erythrocytes, different stages of **Eimeria** and sheet of sloughed mucosa. NIMP treated infected chicks showed milder clinical signs (Brownish diarrhea without any mortalities) they showed slight caecal hemorrhages with mean lesion score of +2.1. Milder findings in caeca in form of desquamated epithelial cells mixed with few erythrocytes, oocysts, schizonts, macro and microgamets. Lamina propria infiltrated with lymphocytes, heterophils, with hyperplasia of intestinal glands. The shedded oocyst numbers were reduced in ***E. tenella* -** infected treated birds. From results, spraying the hatched eggs with NIMP could protect and provide a defensive mean against ***E.tenella*** infection.

***Key words: E. tenella** , balady chicks, egg treatment, Caeca .*

Introduction

Coccidiosis is a common disease problem of poultry, worldwide. It is caused by protozoa of genus **Eimeria** that is highly host and tissue specific. It multiplies in intestinal tract and its developmental stages damage the intestinal mucosa, resulting in interruption of feed digestion processes, nutrient absorption, dehydration and blood loss. Caeacal coccidiosis **(*E. tenella* infection)** affects mainly young chickens causing severe caecal hemorrhage with high mortalities **(McDougald and Reid 1979, Bafundo and Donovan 1988** and **Alam 1989)**. Increasing regulating bans on the use of anticoccidial drugs coupled with high cost of developing vaccines and searching for new drugs to overcome resistance prompted the researchers to develop a novel approach and alternative control strategy **(Schneitz, 2005; Hessen, 2006 and Dalloul and Lillehoj, 2006).**

Normal intestinal microbial eco-balance does provide young chicks with the tools of the first line of defence against entero-pathogens **(Dalloul and Lillehoj 2005).** The phenomenon by which the normal intestinal microflora protect the host against invading pathogens is called competitive exclusion (CE) **(Pivnick and Nurmi, 1982).** Many researchers could prove the protective role of NIMP against bacteria entero-pathogens **(Schneitz, 2005 and Hessen, 2006**), but still little known about its protection role against intestinal protozoal infection.

So the present work was planned to study,

1- The pathological changes of ***E. tenella*** infection in balady chickens.
2- Evaluation of protection ability of NIMP (spraying on hatching eggs) against ***E. tenella*** infection in hatched chicks.

Material and Methods

A - Donor chickens for normal intestinal microflora

Twenty , apparently healthy balady chicks of one day old were obtained from a private hatchery, reared under hygienic conditions up to five months of age, fed *ad-libitum* ration free from antibiotics and vaccinated as routinely applied against MD, AI, ND, IBD and Pox diseases. These birds were periodically tested for Salmonella according to the method described by **Hollister, *et al*., (1999)** and considered as donor for normal intestinal microflora preparation (NIMP). Caecal contents of the donor chicks were subjected to qualitative and quantitative bacteriological examination. NIMP were prepared according to **Snoeyenbos *et al*., (1978**).

B – Embryonated eggs and hatched chicks

Two hundred and forty embryonated chicken eggs of balady breed at the 20th day of incubation were kept in controlled sector in a private hatchery (A and B) each of 120 eggs. Only eggs of sector (A) were sprayed with NIMP at dose of 0.5ml /egg and continued in incubation till hatching, (stimulation for natural intestinal microflora transmission to the progeny). Sixty of the hatched chicks were divided into two equal groups (1 and 3) each of 30 birds. While eggs of sector (B) were kept without treatment and sixty hatched chicks were divided into two equal groups (2 and 4) each of 30 chicks. Birds of groups (1 and 2) were inoculated with ***E. tenella*** sporulated oocyst while chicks of group (4) kept non-treated non inoculated as illustrated in table (1).

C - Challenge *E. tenella* sporulated oocyst

Sporulated oocysts of ***E. tenella*** were kindly supplied by Dr. Hyun Lillehoj, Animal parasitic diseases laboratory, Agriculture Research Service, Beltsville, USA. The sporulated oocysts were counted by using McMaster technique, ***(*Permin and Hansen, 1998)** to be used for experimental infection at dose of 1×10^4 sporulated oocyst/bird via the crop **(Miyamoto *et al*., 2002).**

Table (1). Illustrate the experimental design

Group No.	No of birds	Treatment	Infection		
			E. tenella		
			Age	Route	Dose
Group 1	30	NIMP*	14th day	Oral	$10^4\times1$
Group 2	30	-	14th day	Oral	$10^4\times1$
Group 3	30	NIMP*	-	-	-
Group 4	30	-	-	-	-

* NIMP were sprayed over embryonated eggs at dose of (0.5ml of NIMP/ embryonated chicken egg), which contains (4×10^6, 2×10^7, 1×10^8, 5×10^4 and 7×10^3 CFU/gm) Lactobacillus, total aerobic, total anaerobic, coliform and Enterococci count respectively **(Barnes *et al*., 1972** and **Barnes *et al*., 1980).**

All birds were reared in separate, fed on balanced ration free from anticoccidial drugs they were vaccinated against ND, AI and IBD as routinely adapted along the experimental period (6 weeks).

D - Criteria for evaluation

- Clinical signs and mortality rate.
- Postmortem and scoring of caecal lesions.
- Histopathological changes
- Performance parameters (feed intake, weight gain and FCR)
- Counting the shedded ***E. tenella*** oocysts.

E - Samples collection

Caecal samples were collected and examined daily by counting the shedded oocyst using MacMaster technique according to **Johnson and Reid (1970)**. Ten birds were sacrificed a week post infection (PI) for caecal lesion scoring. Four stages of scores (+1 to +4) were considered according to the criteria of **Johnson and Reid (1970)**. Samples of caecum were collected in 10% formalin, prepared and

stained by H&E for histopatholgical examinations. Caecal content were prepared and pH was measured as described by Hinton *et al*., (1990).

Results

Normal intestinal microflora preparation could protect infected birds against mortalities and severe form of the disease through the following parameters

Clinical signs

Birds of NIMP treated which followed by ***E. tenella*** infection (group 1) showed general signs of illness (decreased the appetite, depression, some birds showed bloody tinged dropping, others showed bright brownish colored diarrhea without any mortalities). ***E. tenella*** infected non treated group (2) (control + ve) showed anorexia, sleepiness, ruffled feathers, huddling together, most of the chicks showed severe bloody diarrhea starting at the 5th till 7th day P.I. with 9.09% mortality rate along the experimental period.

PM and Scoring of caecal lesions

Most of chicks of (group 1) showed petechiae on serosal surface of caecum with mild thickening of its wall, its contents were slightly tinged with blood at 7th day P.I. in (5/10). Two chicks showed a few scattered petechiae on caecum without wall thickening and its contents were tinged with slight blood. Some birds (3/10) showed caecal bleeding and bloody caecal core with mean lesion score of (+2.1) (Figures 1, 2).

Chicks of group (2) showed severe bleeding, thickened caecal wall, caecum is distended with blood (Sausage like appearance), at the 7th day P.I. with mean lesion score of (+3). Figures (3, 4). At the 14th day P.I., a contracted and shortened caecum with hard and shrunken caecal core noticed in some dead birds. Severely congested caecal mucosa and thickening of caecal wall with caecal core (Fig. 5).

On the other hand, chicks of group (1) at 14th day P.I. showed absence of caecal core and petichea, mild to moderate reduction in the size (Fig. 6) if compared to the control group (4) (Fig. 7). The mentioned scores were summarized in table (2).

Histopathological findings

Caeca of treated infected chicks group (1) at 7th day P.I. showed desquamated epithelial cells mixed with few erythrocytes and oocysts, the intestinal glands and epithelium contain various developmental stages (schizonts, macrogamets and microgamets). Lamina propria and interglandular tissue infiltrated with lymphocytes, heterophils, with hyperplasia of intestinal glands. (Figures 8 and 9).

Caeca of infected not treated chicks group (2) at 7th day P.I. showed caecal core consist of extensive necrosis, erythrocytes, different stages of Eimeria and sheet of sloughed mucosa. Denuded mucosa and submucosa with mucosal and submucosal fragments containing different developmental stages of Eimeria inside the intestinal lumen, sheets of desquamated epithelial, extravasated erythrocytes and fibrinus shreds from necrotic mucosa. In addition to the intestinal glands showed proliferation of the epithelial lining with extensive hemorrhage in submucosa. Muscular coat show partial hyalinization and odema with numerous round cells infiltration (Figures 10, 11 and 12). While caeca of chicks at 14th day P.I showed massive number of asexual developmental stages of **Emieria** in intestinal glands and lumen (Figure 13).

Performance parameters

Birds of group (1) showed no significant difference in performance parameters if compared to those of group (2) control +ve. While birds of group (3 and 4) showed a significant higher performance parameters than those of group (2). (Table 3).

Ph value of caecal contents

Birds of group (1) showed mild decrease in Ph values if compared to those of group (2) control +ve. (Table 4).

Counting of the shedded oocysts

Birds of group (1) showed decrease in the average number of the shedded oocysts (1.82 (10^5×if compared to birds of group (2) (2.02 (10^5×control +ve. (Table 5).

Table (2). Lesion scores of *E. tenella* infected groups

Group number	Number of birds showed lesion score				Mean lesion scores
	Grade 1 Very mild changes	Grade 2 Mild changes	Grade 3 Moderate chang-es	Grade 4 Severe changes	
Group 1	2	5	3	-	2.1
Group 2	-	2	6	2	3
Group 3	-	-	-	-	-
Group 4	-	-	-	-	-

Table (3). Performance parameters of NIMP treated groups infected with *E. tenella*

Group number	Perfor-mance parameters	Age / week						Total over 6 weeks
		1st	2nd	3rd	4th	5th	6th	
Group 1	B.Wt /g	52.72± 0.42a	103.4± 0.22a	133.8± 0.14b	173.7±0.0 8b	216.7±0.0 8d	254± 0.08d	254±0.08 d
	FI/g	43.1	120.1	105.7	146.4	177.1	179	771.4
	FCR	2.18	2.37	3.48	3.67	4.12	4.79	3.48
Group 2	B.Wt /g	51.5± 0.45a	98.4± 0.71a	122± 0.16b	157.1± 0.10b	198.2± 0.18d	226± 0.30d	226±0.30 d
	FI/g	42.07	112.56	96	135.3	148	152	685.93
	FCR	2.28	2.40	4.06	3.85	3.60	5.46	3.52
Group 3	B.Wt /g	53.5 ± 0.90a	103 ± 2.33 a	177.6± 8.31a	255.9± 0.48a	321.7± 0.34a	383 ± 0.44a	383 ± 0.44a
	FI/g	43.05	116.8	193.9	240	249	254.6	1097.35
	FCR	2.10	2.36	2.60	3.06	3.78	4.15	3.13
Group 4	B.Wt /g	51.9 ± 1.45a	97.2 ± 2.88a	160.9± 2.78a	230.9± 0.74bc	283.4± 0.36bc	335 ± 0.27bc	335 ± 0.27bc
	FI/g	42.52	108.4	170	223.3	202.4	221.6	968.22
	FCR	2.25	2.39	2.67	3.19	3.85	4.29	3.20

Table (4). Changes on the caecal Ph value of NIMP treated groups and infected with *E. tenella*

Group Number	**Age / days**					
	1st	**7th**	**14th**	**21st**	**28th**	**42nd**
Group 1	6.33 ± 0.07 a	6.42 ± 0.30a	6.87 ± 0.11a	6.57 ± 0.18a	6.61 ± 0.7 a	6.53 ± 0.09 a
Group 2	6.52 ± 0.08 a	6.29 ± 0.24a	6.78 ± 0.13a	6.58 ± 0.08a	6.78 ± 0.27 a	6.88 ± 0.07 a
Group 3	6.33 ± 0.07 a	6.46 ± 0.30a	6.87 ± 0.11a	6.60 ± 0.24a	6.48 ± 0.19 a	6.51 ± 0.10 a
Group 4	6.52 ± 0.08 a	6.58 ± 0.24a	6.78 ± 0.13a	6.70 ± 0.20a	6.98 ± 0.03 a	6.72 ± 0.09 a

Table (5). Average count of the shedded *E. tenella* oocysts of NIMP treated groups

Group number	Count of the shedded oocysts /gm faeces (Days post challenge)						Average × 10^5
	5th	6th	7th	8th	9th	10th	
Group 1	0.63×10^5	1.70×10^5	2.07×10^5	2.27×10^5	2.15×10^5	2.13×10^5	1.82±0.12 b
Group 2	0.81×10^5	1.84×10^5	2.27×10^5	2.48×10^5	2.37×10^5	2.35×10^5	2.02 ±0.07 a
Group 3	-	-	-	-	-	-	-
Group 4	-	-	-	-	-	-	-

Means within the same column carrying different superscripts are significant at ($P \leq 0.05$).

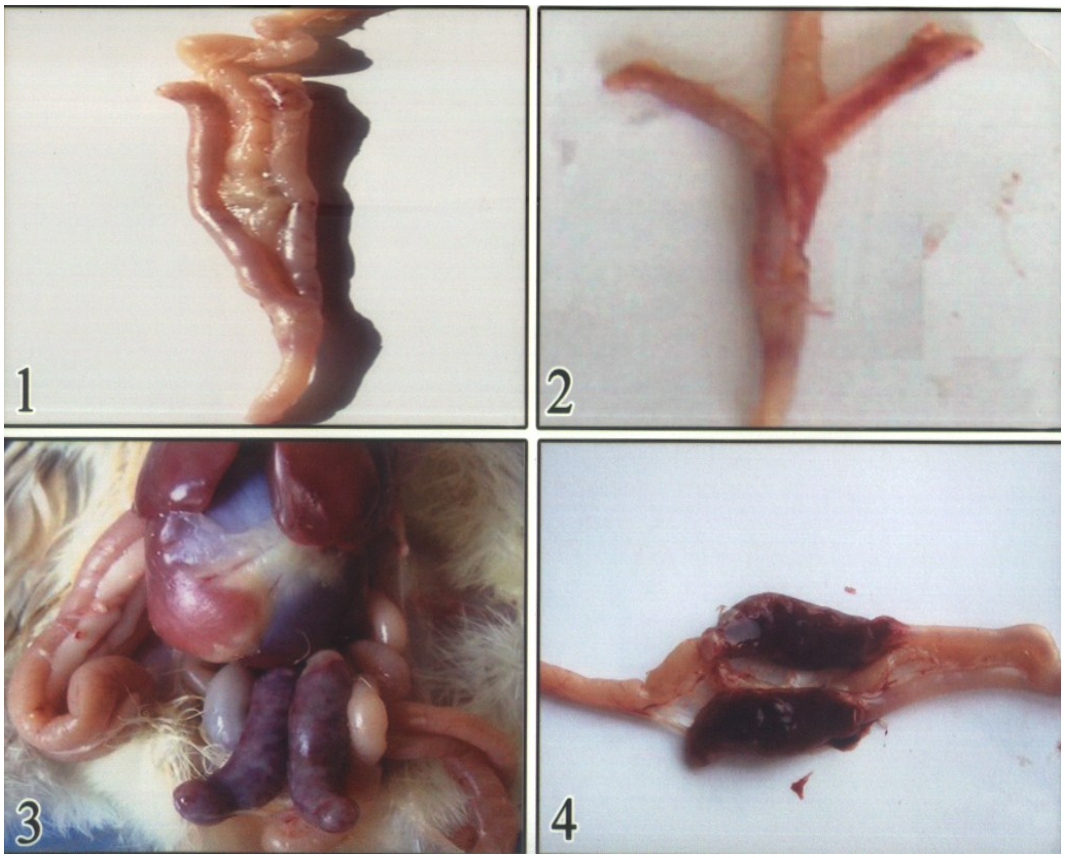

Fig. (1). Unopened caecum at 7th day P.I. group (1) showing slight petechiae on serosal surface.
Fig. (2). Caecum at 7th day P.I. group (1) content tinged with slight blood, were scored (+1).
Fig. (3). Unopened caecum at 7th day P.I. group (2) showing distention with blood (Sausage like appearance).
Fig. (4). Caecum at 7th day P.I. group (2) showing massive bleeding.

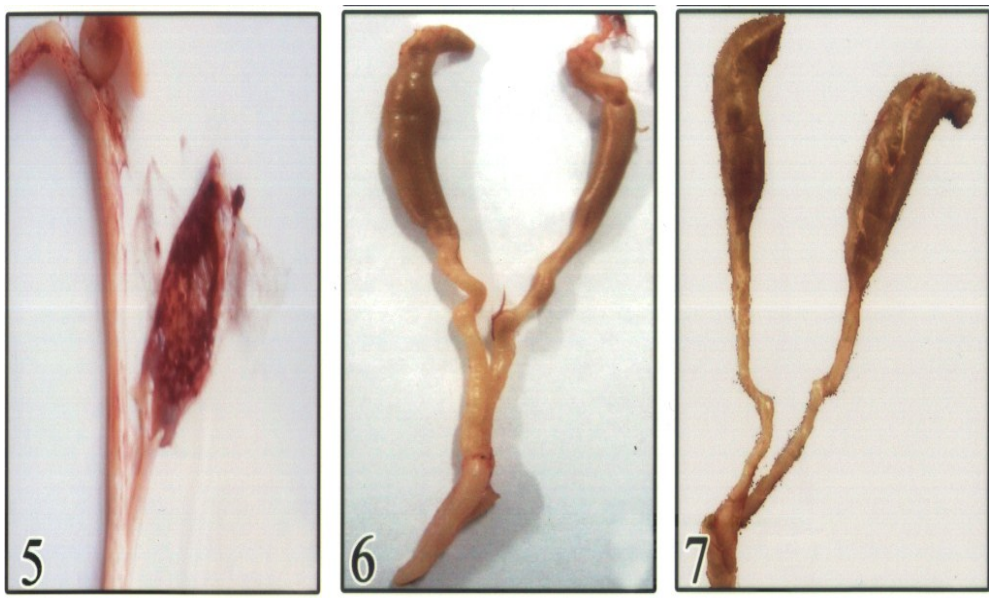

Fig. (5). Caecum at 14th day P.I. group (2) showing severely congested mucosa and thickened caecal wall, were scored (+ 4).
Fig. (6). Caecum at 14th days P.I. group (1) showing no petichae on serosal surface with mild reduction in the size.
Fig. (7). Normal caecum at 14th days P.I. group (4).

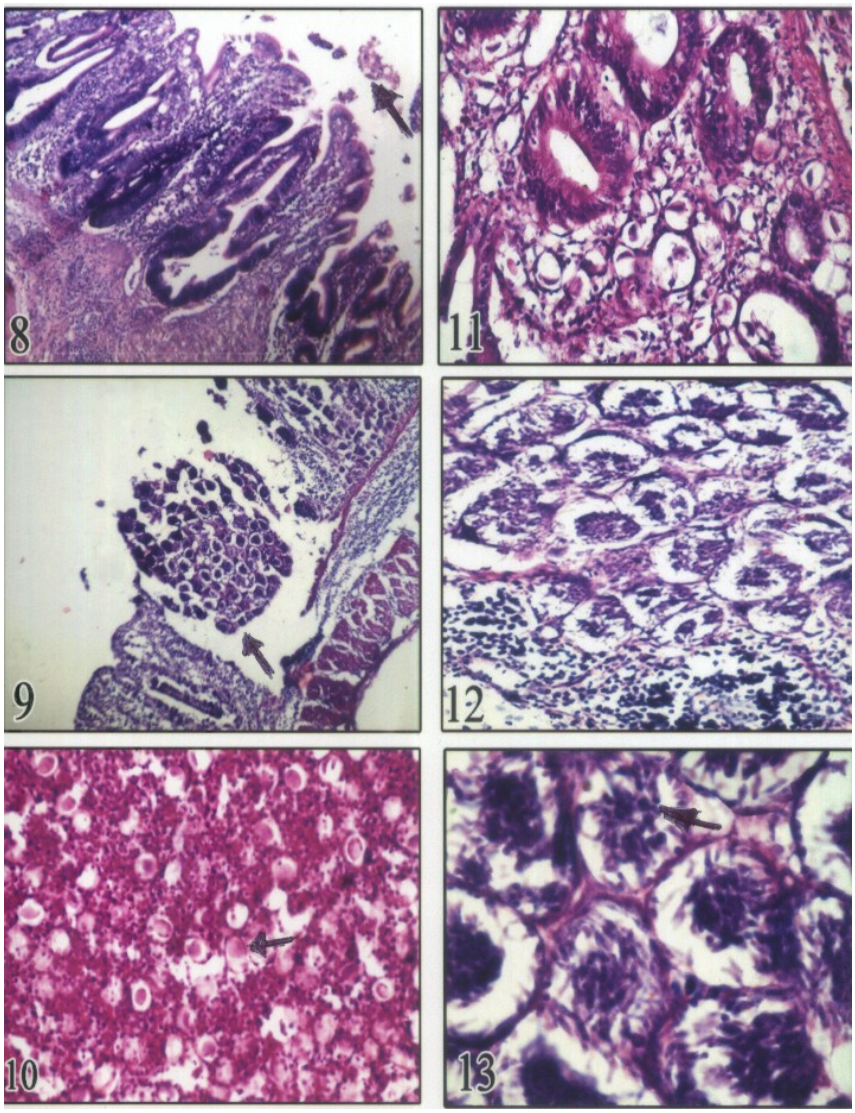

Fig. (8). Caecum at 7th day P.I group (1) showing desquamated epithelium, various developmental stages of **Eimeria**, hyperplasia of intestinal glands and mild damage to submucosa (H&E X300).

Fig. (9). Caecum at 7th day P.I group (1) showing denuded mucosa and submucosa with various developmental stages of **Eimeria** (H&E X200).

Fig. (10). Caecal core at 7th day P.I. group (2) showing extensive necrosis, different stages of **Eimeria** and sheet of sloughed mucosa (H&E X1200).

Fig. (11). Caecum, 7th day P.I. group (2) showing gland necrosis with developmental stages of **Eimeria** mainly oocysts (H&E X1200).

Fig. (12). Caecum at 7th day P.I. group (2) showing odema with round cells infiltration and developmental stages of **Eimeria** (H&E X1200).

Fig. (13). Caecum at 14th day P.I. group (2) showing massive number of asexual developmental stages of **Eimeria** in intestinal glands and lumen (H&E X1500).

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دراسات باثولوجيه على عدوى كوكسيديا الاعورين فى الكتاكيت ومحاولة الوقايه باستخدام الميكروفلورا

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الملخص العربى

اجرى هذا البحث لدراسة التغيرات الباثولوجيه لعدوى كوكسيديا الاعورين فى الكتاكيت البلدى بعد معالجتها اثناء تفريخها بالميكروفلورا الطبيعيه عن طريق الرش على البيض فى اليوم العشرين من التحضين. تم تقييم تأثير الميكروفلورا المعوية الطبيعية فى الوقاية من الإصابة بالأيمريا تينيلا (كوكسيديا الاعورين) فى الكتاكيت البلدى. حيث تم حجز و عزل عدد 240 بيضه تفريخ فى درج منفرد داخل المفرخ وتم رش عدد 120 بيضه منهم بالميكروفلورا المعوية الطبيعية (محتويات الأعورين المخففة) فى اليوم الأخير من التفريخ (اليوم العشرون من التحضين) وتم اختيار (60) من الكتاكيت الفاقسة وتقسيمها الى مجموعتين (1 ، 3) وذلك لتقييم دورها فى حماية النتاج من الاصابه بطفيل الأيمريا تينيلا. بينما باقى البيض المعزول (120 بيضه) تركت دون رش للمجموعتين (2 و 4). تم عمل عدوى صناعية باستخدام طفيل الأيميريا تينيلا للمجموعات (1, 2) فى عمر 14 يوم بينما تركت المجموعتان (3, 4) كضوابط. تم التقييم بمتابعة الأعراض ونسبة النفوق والصفة التشريحية ومعدلات الوزن واستهلاك العلف والتحويل الغذائى وتغيرات الأس الهيدروجينى (Ph) و عمل الفحص الباثولوجى وكذلك عد حويصلات الأيميريا تينيلا حيث أظهرت الدراسة الاتى :

1 - انخفاض الأعراض وانعدام النفوق للمجموعة المعالجة بالميكروفلورا المعوية الطبيعية بالمقارنة بالمجموعة الضابطة المعدية بحويصلات الأيميريا تينيلا حيث كانت نسبة النفوق (9,09%) مع تحسن الصفة التشريحية والفحص الباثولوجى كما لوحظ عدم حدوث فرق معنوى فى الوزن واستهلاك العلف والتحويل الغذائى للمجموعة المعالجة بالميكروفلورا اذا قورنت بالمجموعة الضابطة.

2 – كما تبين انخفاض في عدد حويصلات الأيميريا تينيلا فى زرق المجموعة المعالجة بالميكروفلورا المعوية إذا قورنت بالمجموعة الضابطه.

3 – نستخلص من الدراسه ان رش الميكروفلورا الطبيعية عل بيض التفريخ اثناء الفقس يقلل من الاعراض والصفات التشريحيه ويمنع النفوق بسبب عدوى كوكسيديا الاعورين وكذلك يحسن التحويل الغذائى.