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POST HATCH FEEDING AND FEED ADDITIVES AS A STRATEGY TO ENHANCE GUT HEALTH OFBROILER CHICKS

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ABSTRACT: An experiment was carried outusing three hundred and sixty (308 Ross) 1-d-old broiler chick. In the pre starter phase (0-7d) birds were randomly divided into three experimental groups 120 chicks each,with 3 replicates (40 birds/ pen) and fed diet (ME; 2950 kcal/kg, CP; 24%). as suggested by the Ross guided 2018. Dietary additives were consisted of: (i) basal diet without supplementation of additives (control); (ii) basal diet supplemented with 0.05% omega3 (iii) basal diet supplemented with 0.5% yeast. Results showed no significant differences among different groups, regarding; in live body weight, body weight gain, feed intake and feed conversion. Data ofvillus height (um), villus width (µm) crypt (um), villus height to crypt depth ratioand number of villus of broiler chicks at 7 day-oldsdid not reveal any significant difference among all studied experimental groups however, the addition of yeast lead to anumerical increase in villus height and villus height to crypt depth ratio. In addition, microbial counts showed increase numbers of Lactobacillus only in yeast feed group. Both omega-3 and yeast increased the viability of chicks compared to the control. In conclusion, early nutrition of yeast at level of 0.5% could have the beneficial effect on productive performance and related traits to avoid frequent administration of traditional antimicrobials.

Key words: broiler, gut health, performance, feed additives, diet, post-hatch

INTRODUCTION

The post hatch period is considered a very criticaland necessary period for the morphological development of the intestine. The goals of poultry industry were to improveperformance, feed efficiency, gut health, and (Yadav and Jha. 2019). Clearly, the first week after hatch is a very important period, which has reflection on the development of the digestive and intestine, which has a relationship and relevance to the enzymatic and absorption activities (Sklan, 2001).

In commercial production, delaying feeding to newly hatched as a result of vaccination, sex determination and transportation increases infection as a result of immunodeficiency Noy and Sklan, 1999). Starvation of newly hatched chicks leads to a decrease in intestinal morphology in a particular villus length and villus surface.In addition,elevate plasma fatty acid breakdown which negatively affects the performance (Noy et al., 2001) .Providing feed and water directly to the newly hatched chicks has proven that early feeding is beneficial (Hornasio et al., 2011)and could improve immunity and consequently chick's health. (Dibner et al, 1998). Also, Van der Heijden et al., (2018) reported that the first four days post hatch, broiler chicken's life represents about 10% of their entire life,indicating crucial care is essential in young birds for future growth and to immune function.

Therefore, many attempts have been undertaken in order to find alternate ways to accelerate gastrointestinal maturation of newly hatched birds which may be necessary to replace antibiotic growth promoter supplementation(Valenzuela-Grijalva et al., 2017).On such way involves the use of fish oil. As demonstrated by Calder, (2006), the dietary supplementation of fish oil (FO), as a source of n-3 polyunsaturated fatty acids, has nutritional benefits as a feed additive. Also, the supplementation of fats or oils (as an omega sources) leads to increase dietary metabolizable energy which improvesgrowth rate and utilization of feed (Shang et al., 2014).Supplementing diets with corn oil or fish oil did not significantly affect BWG, FI, feed efficiency, and immune response(Yang and Guo, 2006).

Anothermethod included the use of Brewer's yeast (*Saccharomyces cerevisiae*) extractssupplementation, have been added to poultry feed for its high nutritional value (Westendorf and Wohlt, 2002). Also, the whole yeast or yeast cell walls have been used to improve performance of chicks (Zhang et al., 2005).However, yeast has the ability to stimulate digestion and aid in maintaining microbial equilibrium in the gut (Pelicia*et al*., 2010).

The present study aimed to investigate the effects of early nutrition and dietary additives , on the productive performance, microbial counts and histological structural and integrity of the intestinein addition to microbial counts of litter.

MATERIAL AND METHODS

The present study was performed in the Poultry Research Center and Laboratories of the Poultry Production Department, and Central Laboratory, Faculty of Agriculture, Alexandria University.

Three hundred and sixty 308 Ross 1-d-old broiler chicks body weight were procured from a commercial hatchery and transported directly to the Poultry Research Center. Chicks were wing banded at the start of the experiment and randomly divided into three experimental groups 120 chicks each,with 3 replicates (40 birds/ pen) and housed in deep litter pens from the first day to day seven. Chicks were raised under continuous lighting and brooding temperature at 32ºC. The basal diet used in the experiment was mash feed and was prepared in the local feed mill. Diet of the pre-starter period (ME 2950

kcal/kg and CP, 24%) were formulated to meet or exceed requirements suggested by the Ross guided (2018). Ingredients and calculated analysis of the pre-starter diet used in experiment are shown in Table (1). Either of tested additives was administrated through the pre-starter diet as follows:

T1: basal diet without supplementation, control

T2: basal diet supplemented with 0.05% fish oil omega3

T3: basal diet supplemented with 0.5% yeast

Chemical and physical characteristics of Omega3 used in the experiment

Chemical Name	EicosapentaenoicAcid , and Docosahexaenoic Acid
Chemical Family	Omega-3 Fatty Acids
Chemical species	Fish Oil capsule shell

Chemical and physical characteristics of Yeast used in the experiment

Chemical Name	Ascosporidae)
Chemical Family	Yeast
Chemical species	*Saccharomyces cerevisiae*

Data collection: -

Birds body weight were recorded, feed consumptions (FC) were determined daily. Also, body weight gain (BWG) and feed conversion ratio (FCR) were calculated. Livability was daily monitored by recording and collecting the number of dead birds.

Bacteriological Evaluation:

Small intestine morphology:

Different sections of the small intestine were obtained for morphometric analysis. The procedure was described by Thanh et al. (2009). Segments of about 5 cm in length were removed from the small intestine (duodenum, jejunum and ileum) at the following locations: (i) the middle part of the duodenal loop, (ii) midway between the endpoint of the duodenal loop and Meckel's diverticulum (jejunum) and (iii) midway between the Meckel's diverticulum and the ileocaecal junction. Removed segments were flushed with a 10% neutral buffered formalin solution and were then used for morphometric analysis. The morphometric variables included villus height (from the tip of the villi to the villus-crypt junction) and crypt depth (depth of the invagination between adjacent villi). The villus height and crypt depth were measured and recorded using an image analyzer. Only villi sectioned from their base to the top with a single epithelial layer at their tip, and crypts with a visible lumen along their entire depth, were considered for quantitative evaluation (Bancroft et al. (2013).

Microbiological analyses:

At the end ofthe tail, 3 birds were taken for each treatment around mean average of the treatment and euthanized by severing the jugular vein. The carcasses were subsequently opened, and the entire GI tract was removed aseptically. The GI tract was then divided into sections that were ligated with light twine before being separated. The ceca were collected and sealed in sterile bags filled with 50 mL of ice-cold cryoprotective broth (i.e., preproduced sterile brain heart infusion broth containing 20% vol/vol glycerol) suitable to maintain the viability of intestinal bacteria and were immediately stored at -80° until all subsequent analyses. For all analytical procedures, deep frozen ceca per bird were thawed for 20 min. and removed from storage bags. Cecal digesta content were then aseptically emptied in a new sterile bag and were immediately diluted.

Fecal samples were serially diluted 10-fold with peptone water (0.1%, pH 7.0). Four 20-µl drops (was serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently) of each dilution were plated in duplicate on desired media (Kleessen et al., 1997). Total bacterial counts were enumerated using

plate count agar and plates were incubated at 32°C for 24h (Jorgensen et al., 2015). Selective media were used to enumerate the two bacterial populations (coliforms and lactobacilli). MacConkey agar was used for counting coliforms and LAMVAB agar was used for counting lactobacilli. Plates were incubated aerobically 37°C for up to 3 days. Yeasts were enumerated on sabouraud dextrose agar medium (Jorgensen et al., 2015). Plates were incubated aerobically 25°C for up to 5 days. Cell counts were expressed as log colony forming-units (CFU) per gram (wet weight) of feces.

Faecal pH determination:

One gram of fresh fecal sample was mixed homogeneously with 9 ml of deionised distilled water in a sterile tube. The pH was measured using a Mettler-Toledo pH meter with a glass electrode (Mettler-Toledo, England). The meter was calibrated using a buffer solution (Merck, KGaA, Darmstadt, Germany) at pH 4.

Statistical Analysis:

Data were analyzed by the Computer Program, SPSS (2016), using the model one-way ANOVA. The significant differences among treatments means were separated by Duncan's Multiple Range-Test (Duncan, 1955) when significant P values was obtained. Treatment effects were considered significant at $P \leq 0.05$. The statistical model used was as follow:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = The observation of the statistical measured.

μ = The overall mean.

T_i = The effect of treatment. .(1-3)

e_{ij} = The experimental random error.

RESULTS

Growth performance parameters:

Data presented in Table (2) showed theproductive performance traits as affected byvarious additives (omega-3 and yeast). Obtained data indicated that there were no significant differences among the different experimental groups of broiler chicks fed on pre-starter basal diet supplemented with yeast or omega3 compared with the un-supplemented group, in live body weight, body weight gain, feed intake and feed conversion ratio of broiler from (0-7) days.

Mortality Percent:

Comparing the results of mortality rates among the three diet types included control omage3 and yeast during pre-starter diet as shown in Table (2), it is obvious that there is a tendency to increase($P \leq 0.01$)the mortality percentage with the control diet (At a percentage of 2.5%). On the other hand, the groups of birds that received yeast or omage3 supplemented diets recorded no mortality during this phase

Microbiological count of gastrointestinal:

The different dietary additives effect on viable counts of different bacterial groups in gastrointestinal tracts of one-week-old chicks is shown in Table (3). In general, add yeast had no significant effect on the viable counts of coliforms, yeasts and Salmonella as well as total bacteria in gastrointestinal contents. While the viable counts of Lactobacillus increased significantly ($P<0.05$) in gastrointestinal contents obtained from yeast-administrated chicks compared with the control group. On the other hand, omega3 administration appeared to have no significant effect on the viable counts of different microbial groups.

Microbiological content of litter

The effect of different dietary additives on viable counts of different bacterial groups in litter of one week–old broiler chicks is shown in Table (4). Generally, administration of omega3 and yeast had no significant effect on the viable total bacterial counts, and viable counts of coliforms, Lactobacillus, yeasts and Salmonella as well as total bacteria in litter contents.

Morphology intestine properties:

Data presented in Table (5) and figure (6) showed the morphology of small intestine including villus height (um), villus width (µm) crypt (um), villus height to crypt depth ratioand number of villus of broiler chicks fed different dietary additives at age 7 day-old. Analysis of variance of the present data did not reveal any significant difference among all studied experimental groups at the end of the pre-starter phase of the experiment.

DISCUSSION

Preventing growth losses by positively affecting intestinal health as a result of adding some feed additives in early nutrition stage lead to increased marketing weight and disease resistance (Hollemans et al., 2018**)**.Several investigations have recorded negative effects of post-hatch fasting period on the intestine function and growthperformance in the long run **(**Adeleye et al., 2018).Starve the chicks as a result of vaccination, sex determination, transportation increases the risk of infection because immunodeficiency, which increases the cost of production. (Van den Brand et al., 2010).So, theearly nutrition promotesgastrointestinal tractmaturation with pre-starter diet, early absorption of the yolk sac, improved growthperformance and the health status of the birds later in life **(**Sirsat, et al., 2017).

The measured performance of birds in all studied nutrition treatments was within the breed performance standard guide (Ross308). Meanwhile, no significant differences were detected with tested dietary treatments and that of the control in terms of live weight, gain, feed intake and feed conversion ratio. These results are similar to with finding of Noh et al., (1994) who showed no significant effect on body weight gain of birds fed inclusion level of yeast culture (0.10%). Also, Shang et al. (2004) investigated that growth performance of poultry are improved by supplementation of fatty acids or their sources, the supplementation of fats and oils (as an omega source) in limited amounts leads to better utilization of feed and energy, with subsequent improvement in traits performance. In line with the above-mentioned result, a beneficial effect was obtained by adding either of omega 3 or yeast to the formulated pre-starter diet compared to un-supplemented diet. It may be means that the studied feed additive changed the condition of gut health as reported by Fuller, (2001).The term "gut health" is a very complex topic that including, gut physiology, microbiology, and immunology .It can be used that the early access to certain supplements post hatch, such as yeast and omega-3insignificant improvement the development of intestinal morphologycompetency of the chicks, thus enhanced the absorptive potential and the immune response of chicks under phase feeding program used in this study. prebiotics produced from yeast cells and cell walls are used due to the positive effect on gut health and modification of microorganisms. Contents of this supplement manna-oligosaccharides and Beta-D-glycan bind to the receptor mannose specific type-1 fimbriae and prevent the colonization of pathogenic bacteria,with an increase in fecal bacteria, which is associated with intestinal health(Rodriguez-Estrada et al., 2013).

Development of the GIT traits is an important aspect of decreasing mortality rate, especially during the early post hatching period which agree with our work through the first week of age. In this respect, Lactobacillus counts was significantly increased as feeding diet of yeast compared with those of omega or un-supplemented, whereas no difference was concerned with the counts of the other studied bacteria enumeration compared with those of the un-supplemented diet. These results are in agreement with

findings of Kilonzo-Nthenga et al., (2008) who found that several harmful pathogenic bacteria have been shown to exhibit a binding specific for the sugar mannose in yeast wall act as a decoy for the attachment of pathogens. Although no difference was found in histological structure of intestine, yeast supplementation had a beneficial effect in this respect where an increase was seen in the intestinal mucosa. Such development can be considered as indicators of nutrients absorption capacity and consequently decreased feed

IN CONCLUSION,

under the condition of this experiment, the obtained results indicate that incorporating yeast at level of 0.5% into pre-starter diet could havethe beneficial effect on productive performance and related traits to avoid frequent administration of traditional antimicrobials.

Table (1): Ingredients and calculated analysis of pre-starter diet used through the pre-starter period of (1-7) days.

Ingredients	**[1]pre-starte(1to 7 d)**
Yellow corn	54.00
Soybean meal, (46% CP)	35.50
Corn gluten meal (60%)	5.00
Soy oil	1.00
Limestone	1.70
Salt (Nacl)	0.38
[4]Vitamin premix	0.30
Mono calcium phosphate	1.50
DL-methionine,	0.27
L-lysine HCL	0.25
Choline chloride	0.10
Total	100
ME, (kcal/kg)	2950
Crude protein, (%)	24.00
Fat, (%)	3.60
Fiber, (%)	2.20
Calcium, (%)	1.00
Available Phosphorus, (%)	0.50
Digestible lysine, (%)	1.6
Digestible Methionine, (%)	0.71
Cysteine (%)	0.33
Arginine (%)	1.33

[1]Pre-Starter diets provided during d 0 to 7; with Omega3 (fish oil) 0.5% or yeast 0.5%
Vitamin premix provides per diet: Vit. A; 12000000 IU, Vit. E: 400000 mg, Vit. Bl: 2000 mg. Vit. B2: 160000 mg, Vit.B6: 5000 mg, Vit, B12: 12 mg, Niacin: 45000 mg, Pantothenic acid: 12000 mg, Vit. K: 3000 mg, Vit. D3; 3000000 IU, Biotin: 70mg and Folic acid: 2000mg.

Table (2): Live body weight, weight gain, feed consumption, feed conversion ratio and mortality rate of broiler chicken fed pre starter phase based diet supplemented with omega-3 or yeast from 0 to 7day of age.

Treatments	Initial Live weight/ g	Final Live weight/ g	Weight gain g	Feed consumption g	Feed conversion ratio	Mortality rate%
PSD1	47.9	177.3	129.4	162.6	1.3	2.5[a]
PSD2	47.8	173.4	125.6	163.2	1.3	0.00[b]
PSD3	48.0	176.5	128.5	165.2	1.3	0.00[b]
SEM	0.65	0.51	0.57	0.70	0.30	0.41
P value	0.105	1.33	1.37	1.17	0.02	0.0001
Sig	NS	NS	NS	NS	NS	**

[a and b]Means in the same columns for each treatment having different letter(s) are significantly different ($p<0.05$)
PSD1=Pre-starter diet without additives supplementation
PSD2 = Pre-starter diet supplemented with omega3
PSD3=Pre-starter diet supplemented with yeast
SEM=Standard error mean
NS= Non significant

Table (3): Gastrointestinal microbial (log10 cfu⁄g) of broiler chicken fed pre starter phase-based diet supplemented with omega-3 or yeast from 0 to 7day of age

Treatments	Lactobacillus	Coliform	Total bacterial counts	Yeasts	Salmonella
PSD1	5.2[b]	6.07	6.4	5.35	4.61
PSD2	5.3[b]	6.15	6.2	4.96	5.01
PSD3	5.9[a]	6.16	6.3	5.46	4.93
SEM	0.11	0.019	0.25	0.11	.165
P Value	0.001	0.113	0.71	0.176	0.64
Sig	**	NS	NS	NS	NS

[a and b]Means in the same columns for each treatment having different letter(s) are significantly different ($p<0.05$)
PSD1 = Super Pre-starter diet without additives supplementation
PSD2 = Super Pre-starter diet supplemented with omega3
PSD3= Super Pre-starter diet supplemented with yeast
SEM=Standard error mean
NS= Non significant

Table (4): Litter microbial (log10 cfu/g) of broiler chicken fed pre starter phase-based diet supplemented with omega-3 or yeast from 0 to 7day of age

Treatments	Lactobacillus	Coliform	Total bacterial counts	Yeasts	Salmonella
PSD1	6.38	6.5097	7.01	6.64	6.34
PSD2	6.96	6.1327	6.84	6.62	6.67
PSD3	6.47	6.5603	6.64	6.52	6.58
SEM	0.12	0.19	0.16	0.14	0.09
P Value	0.067	0.691	0.73	0.949	0.364
Sig	NS	NS	NS	NS	NS

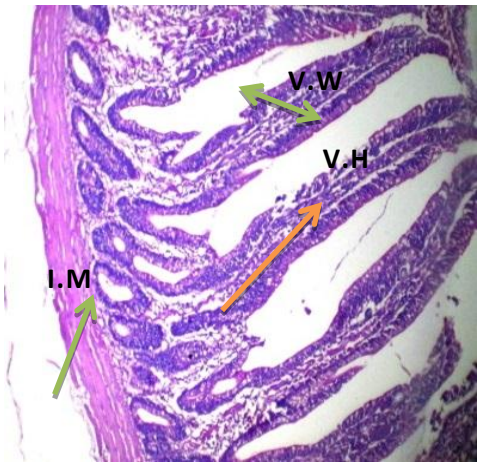
PSD1 = Pre-starter diet without additives supplementation
PSD2 = Pre-starter diet supplemented with omega3
PSD3= Pre-starter diet supplemented with yeast
SEM=Standard error mean
NS= Non significant

Table (5): Duodenum morphology of broiler chicken fed pre starter phase based diet supplemented with omega3or yeast from 0 to 7day of age

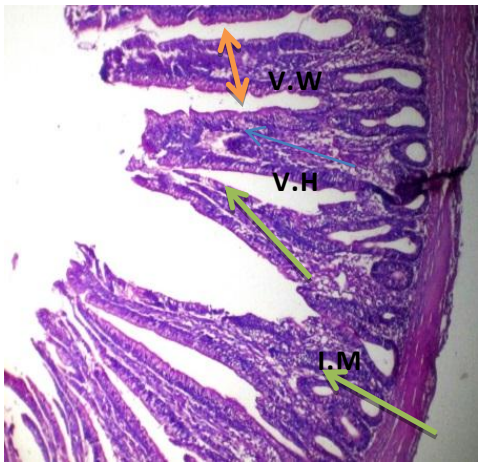
Treatments	Villus height (um)	Villus Width(µ)	Crypt depth (um)	Villus height to crypt depth ratio	Number of Villus
PSD1	1068.33	154.33	173.33	6.37	25.83
PSD2	900.00	197.67	160.00	5.94	28.17
PSD3	1111.67	182.67	123.33	9.16	29.17
SEM	57.41	13.17	14.02	0.705	1.66
P value	0.320	0.45	0.37	0.12	0.76
Sig	NS	NS	NS	NS	NS

PSD1 = Pre-starter diet without additives supplementation
PSD2 = Pre-starter diet supplemented with omega3
PSD3= Pre-starter diet supplemented with yeast
SEM=Standard error mean
NS= Non significant

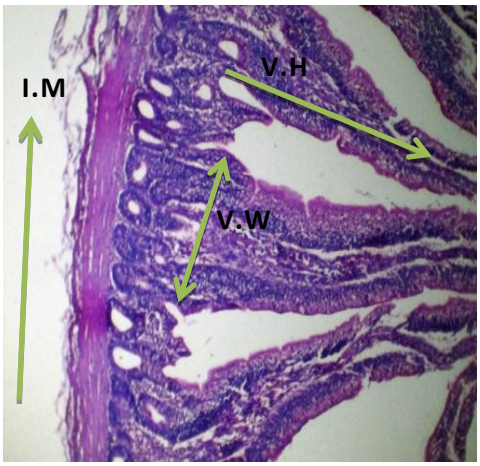
Figure (1): Microscope photo of intestinal Villus from duodenum



Intestine (*SPS D1*): Height Villus (thin arrows) with deep crypt (thick arrows) and thin intestinal wall (W). H&E. X100



Intestine (*SPS D2*): height Villus (thin arrows) with deep crypt (thick arrows) and thin intestinal wall (W). H&E. X100.



Intestine (SPS D3): Height Villus (thin arrows) with deep crypt (thick arrows) and thin intestinal wall (W). H&E. X100.

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الملخص العربى

استراتيجية استخدام الإضافات الغذائية والتغذية المبكرة ما بعد الفقس فى تحسين صحة أمعاء كتاكيت اللحم

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قسم الألبان – كلية الزراعة- جامعة الاسكندرية**

أجريت هذه الدراسة في مركز بحوث الدواجن قسم إنتاج الدواجن – كلية الزراعة – جامعة الاسكندرية وذلكفى الفترة العمرية من يوم إلى ٧ أيام لدراسة تأثير تحفيز صحة القناه الهضمية لإنتاج مستداملكتاكيت اللحم من خلال إضافة الاوميجا-٣ والخميرة.

تم إجراء الدراسة باستخدام ٣٦٠كتكوت لاحم (روس)عمر يوم و تم توزيع الكتاكيت عشوائيًا إلى ثلاث مجموعات تجريبية ١٢٠ كتكوت لكل منها، مع ٣ مكررات (٤٠ طائر /مكرر) حتي عمر ٧ أيام وتم تغذية الطيور علي علف باديءالطاقة كانت ٢٩٥٠ كيلو كالوري / كجم، والبروتين ٢٤ ٪ .تتكون المعاملات الغذائية لمرحلة ما قبل البادي من: (١) العلف الأساسي دون إضافة (كنترول) ؛ (ii) العلف الاساسي مضاف اليه ٠.٠٥٪ أوميجا ٣ ؛ (iii) العلف الأساسي مضاف الية ٠.٥٪ من الخميرة. وتم تقدير الأداء وهستولوجيا وميكروبيولوجيا الأمعاء والفرشة مع نهاية المرحلة.

ويمكن ايجاز أهم النتائج المتحصل عليها في النقاط التالية:

١- لم يكن هناك فرق معنوي بين وزن الجسم الحي ، الزيادة المكتسبة فى الوزن ،كمية العلف المستهلك ومعدل التحويل الغذائي بين المجموعات التجريبية المختلفة للكتاكيت في مرحلة ما قبل البادئ والمضاف الي عليقتهاالاوميجا ٣ و الخميرة.

٢- الطيور التي تم تغذيتها علي علف يحتوي على الخميرة أوالاوميجا٣ لم تسجل أي نفوق خلال هذه المرحلة بينما نسبة النفوق في الكنترول كان ٢.٥٪

٣- لم يكن لاضافة الخميرة تأثير معنوي على اعداد بكترياالكوليفورم والخمائر والسالمونيلا وكذلك البكتيريا الكلية في محتويات الأمعاء بينما زادت أعداد اللاكتوباسيلس بشكل معنوي مقارنة بمجموعة الكنترول ومجموعة الاوميجا٣

٤- التركيب البنائى للأمعاء لوحظ فيه زيادةفي طولوعرض وعمق ونسبة الارتفاع إلى عمق الخملات وعدد الخملات بالاضافات موضوع الدراسةبالرغم من عدم وجود فرق معنوي بين المجموعات التجريبية المختلفة.

٥- بشكل عام ، لم يكن لاستخدام الاوميجا٣ تأثير معنوي على اعداد البكتريا الكلية ، واعداد بكتيريا الكوليفورم وبكتيريا اللاكتوباسلس والخمائر والسالمونيلا في محتويات الفرشة

الخلاصة:- بناءً على النتائج الحالية والتى أوضحت إستجابة الكتاكيت لتلك الإضافات فى مرحلة ما بعد الفقس ولمدة ٧ أيام وما سيؤثر ذلك على الأداء الكلى فى عمر التسويق ويجب إجراء مزيد من التجارب على الأوميجا٣ والخميرة في مختلف الاعمار .