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EFFECT OF IN-OVO INJECTION WITH PROBIOTIC ON HATCHING TRAITS AND SUBSEQUENT GROWTH AND PHYSIOLOGICAL RESPONSE OF HATCHED SINAI CHICKS

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ABSTRACT: This study aimed to investigate the effect of in-ovo injection with probiotic on hatchability traits, growth and physiological response of post-hatch Sinai chicks. A total of 600 fertile eggs at the initial of the 18th day of incubation were used and divided into 4 equal treatments (150 eggs per each). The experimental groups of eggs were arranged as follows, the first group as a control negative (un-treated), the second group as a control positive (injected in air sac with 0.3 ml/egg of sterile distilled water), the third and fourth groups injected with 0.3 ml/egg solution of sterile distilled water contained 1.0 and 2.0 g probiotic per one liter, respectively. Hatched chicks were reared up to 12 wks of age, then growth and some physiological parameters were estimated through the experimental period. Results indicated that in- ovo injection eggs by probiotic with 1.0 or 2.0 g / L result in improve hatchability and decrease embryonic mortality percentages. Both body weight gain and feed conversion ratio were significantly improved as a result of in-ovo injection with 1.0 g probiotic/L than the negative control during the overall experimental period after hatch (0-12wks of age). Relative weights of some giblets and organs were increased as a result of in-ovo injection with probiotic as compared with negative control with or without significant effect. Growth and physiological response of hatched Sinai chicks. Therefore, in-ovo injection by probiotic with 1.0 or 2.0 g / liter at 18th day of incubation period could be used to improve hatching and subsequent growth performance of hatched Sinai chickens.

Key Words: Sinaichicks - hatchability - growth and physiological response - probiotic.

INTRODUCTION

Many definitions have been proposed for the term probiotic. Probiotics are pure cultures of one or more live bacteria that exhibit a beneficial effect on the health of the host when they are ingested. Improved epithelial cell integrity, increased immune response, well balanced gut microflora, better utilization and digestion of diet are also additive beneficial effects of dietary probiotics (Jin et al., 1998; Panda et al., 2001). The beneficial modes of action include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function (Salminen et al., 1996), expression of bacteriocins (Mazmanian et al., 2008), enzymatic activity inducing absorption and nutrition (Hooper, 2002), immunomodulatory effects (Salzman et al., 2003), inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa (Gill, 2003). Probiotic prescription is a good alternative for antibiotics for several reasons: suitable function, non-existence of residue in poultry productions, environmental protection and also prohibition of antibiotics usage in Europe union (Dilworth and Day, 1978; Tortuero and Fernandez, 1995). A major problem with the use of bioactive compounds is their efficient administration under fully controlled conditions. In order to be effective, they have to be administered to animal as early in life as possible. Above that uncontrolled variables (i.e. water quality) should be minimized. To eliminate some of these factors that could influence the responses to bioactives, the in ovo injection technology of these bioactives, directly into a chicken embryo, has been defined (Gulewicz et al., 197726). By in ovo injection, pre-/pro -/synbiotics are administered as early in life as possible, and uncontrolled environmental factors are minimized and/or eliminated (Villaluenga et al., 2004. Around embryonic day 18, the chick will have its first meal when it consumes the amniotic fluid before internal pipping (Ferket, 2006). Loddi et al. (2006) inoculated 10^6 CFU of a commercial formulation of Lactobacillus acidophilus and Bifidobacterium bifidum into embryos they obtained an improved intestinal integrity and reduction of Salmonella. Therefore, this study amid to investigate the effect of in-ovo injection some types of probiotic of incubated eggs at the 18^{th} day on hatching traits and post-hatching performance for hatched chickens of local Sinai chicks breed.

MATERIALS AND METHODS

This experiment was carried out at EL-Serw Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. This study was conducted to investigate the effect of in-ovo injection eggs by probiotic (some types of useful bacteria such as Lactobacillus lactis 2.5x 10^8 CFU/g and Bacillus subtilis 1.8 x 10^9 CFU /g) on hatching traits and subsequent some growth performance parameters as well as relative weights of some organs of hatched Sinai chickens. Hatching eggs were taken from Sinai hens at 50-wks old, then incubated at 37.8 °C and 63% RH and candled at the 7^{th} day of incubation to remove the infertile eggs. At the initial of the 18^{th} day of incubation period, a total of 600 fertile eggs were taken, weighted (52.0 ± 1.0 g) and divided into equal four experimental groups (each of three replicates). The experimental groups of

eggs were arranged as follows, the first group as a negative control (un-treated), the second group as a positive control (injected in air sac with 0.3 ml/egg of sterile distilled water), the third and fourth groups were injected with 0.3 ml/egg of sterile distilled water solution contained 1.0 and 2.0 g probiotic (Lactobacillus lactis 2.5x 10^8 CFU/g and Bacillus subtilis 1.8 x 10^9 CFU /g) per one liter (10^7 bacteria/egg), respectively. The injection whole area was disinfected with an ethyl alcohol; the pinhole site was sealed with sterile paraffin wax immediately after injection, then all experimental groups of eggs were transferred to the hatcher after the injection treatment. All hatched chicks from each treatment were weighted and divided into three replicates then reared up to 12 weeks of age under similar hygienic and managerial conditions. During rearing period, chickens in all treatments groups did not take any antibiotics. Composition and calculated analysis of the basal starter and grower diets are shown in Table 1.

The probiotic used in the current study was produced by pic-Bio, Inc Company – Japan and purchased from El-Youser Company for medicine trade- Cairo. It is a Saltose Ex which is a thermo stable probiotic where each 1 kg contains lactic acid bacteria (Lactobacillus lactis) 2.5x 10^8 CFU, Bacillus subtilis 1.8x 10^9 CFU and calcium carbonate up to 1 gram as carrier.

Data collection and estimated parameters:

1- Hatching traits: hatched chicks, un-hatched eggs, dead chicks and embryos were counted and recorded at the end of hatching process, then hatchability and embryonic mortality were estimated as well as hatched chick's weight.

2- During rearing period: Live body weight (LBW) and feed consumption (FC) were recorded for each replicate per each treatment then were averaged and expressed in grams per chick/ 4 wks throughout the experimental periods as 0-4, 4-8,8-12 wks of age and the overall experimental period (0-12 wks of age). Body weight gain (BWG) and feed conversion ratio (FCR) were calculated during the same periods.

3- After slaughter and complete bleeding, the birds were dressed and the carcass and some organs (liver, gizzard, heart, spleen, and pancreas) were weighed as well as the lengths of some small intestine parts (duodenum, jejunum and ileum) were also measured cm / 100 g of the carcass weight. Dressing percentage = [(Dressed carcass weight/Live body weight) × 100). Relative organ weights were calculated as percentages of carcass weight = [(Organ weight/carcass weight) × 100].The digestive enzymes were carried out in on samples of small intestine contents (3birds/ treatment).The microbial examination was carried out in on samples of cecum contents (3 birds/ treatments) according to **Mackie and Mc Carteny (1953) , APHA (1960) and Difco Mannual(1977**).

4- **Statistical analysis:** Data obtained were statistically analyzed using the General Liner Model of SPSS, (2008). The following model was used :$Yij = \mu + Ti + eij$ where: Yij = an observation, μ = overall mean, Ti = effect of treatment (i=1,2,3 and 4) and eij = experimental random error. Significant differences among means were tested by Duncan's Multiple Range Test Duncan (1955) at 5% level of significance.

RESULTS AND DISCUSSION

Hatching traits:

The effects of in-ovo injection with probiotic on the hatchability and embryonic mortality results are given in Table 2. Hatchability of fertile eggs and embryonic mortality were differ significantly ($P \leq .01$) among the experimental groups as a result of in- ovo probiotic injection. The eggs injected with 2.0 g probiotic/L recorded the higher hatchability percentage, while the lowest percentage produced from the positive control group Hatchability (%) was improved by about 7.06 % and 9.47% for eggs injected with 2.0 and 1.0 g probiotic/L, respectively as compared to negative control . The lowest embryonic mortality percent was recorded for the group injected with 2.0 g probiotic /liter treatment, while the highest value was recorded for the positive control group. These results are agreed with Pilarski et al. (2005) who revealed that RFO (raffinose family oligosaccharides) injection into the air cell during embryogenesis decreased embryonic mortality by 50% than un-treated group.

Growth performance parameters:-

In-ovo injection by probiotic at the 18th day of incubation period resulted in a significant improvement in live body weight (LBW) for Sinai chickens at hatch and different experimental ages except of 8 wks of age (Table 3). Chickens LBW were significantly improved by 4.99 and 3.48% for the groups injected with 1.0 and 2.0 g probiotics / L, respectively than the negative control (non- injected), however the group injected with 1.0 g probiotics was significantly higher LBW by 3.19% than the positive control group at hatch. Chickens produced from eggs injected with 1.0 g probiotic/L had the heaviest LBW at different studied ages with or without significant effects than the negative control, whereas, the other in-ovo injection groups resulted in a significant lower LBW than the negative control at 4 and 12 wks of age.

Chickens body weight gain (BWG) was significantly affected due to in-ovo injection with probiotic during incubation period (Table 3). Chickens produced from the eggs injected with 1.0 g probiotic /L recorded a significant improvement in BWG than other in-ovo injection groups at the period of 0-4 of age, however, they were comparable with the negative control. Chickens BWG was significantly improved by 0.90, 16.33 and 7.31% of the group injected with 1.0 g probiotic/L as compared with the negative and positive control and in-ovo injection with 2.0 g probiotic/L during the overall experimental period (0-12 wks of age), respectively. Therefore improvement in body weight gain of the birds in this study may be attributed to better digestibility of crude protein, which may have contributed in better growth of the birds. Probiotic bacteria may also produce antimicrobial substances such as volatile fatty acids, bacterocins, and low pH that limit the growth and/or survival of pathogenic microbes (Hume, 2011)

Feed consumption (FC) of chicks was significantly affected due to in-ovo injection during incubation period (Table 3). It was significantly decreased for treated groups as compares to negative control (un-treated). Also, we noticed that chickens produced from eggs injected by 0.3 ml sterile distilled water contained 2 g probiotic / liter had significantly consumed lower amount of feed than those of the negative control(-) during all experimental periods, it was significantly lower by 10.29% than negative control during the overall experimental period.

However, these chickens had consumed more amount of feed than those in the positive control group during all experimental periods except the period of 0-4 wks of age which were consumed a lower amount by 5.96%.Feed conversion ratio (FCR) for hatched chicks was significantly affected as a result of in-ovo injection eggs by probiotics during incubation period at the first 4 weeks of age only, but it not significantly affected during the last 8 experimental weeks (Table 3). The best improvement of FCR was achieved during period of 4-8 wks of age by injected 0.3 ml sterile distilled water contained 2.0 g probiotics /liter as compared with control (–) or (+). Generally, FCR was significantly improved for hatched chicks as a result of in-ovo injection eggs with probiotic during the overall rearing period (0-12 wks of age). It was significantly improved by 3.48 and 4.5 % for chicks produced from eggs injected with 1.0 and 2.0 g probiotic /L, respectively than the negative control. These results are in agreement with the findings of Ramesh et al. (2000) who reported the use of Lactobacillus acidophilus based a probiotic to broiler chick's diet resulted in a better FCR. Also, Kabir et al. (2004) found that supplementing probiotic to chick's diet improved body weight gain and feed conversion ratios.

Intestinal microbial count:

Means of total coliform and total lactobacillus bacteria count in intestinal tract of chicks produced from eggs injected with different levels of probiotic during incubation period are given in Table 4. We noticed that chicks produced from eggs injected with 1.0 g probiotic/L had the lowest count in total coliform bacteria count compared with the other treatments at 12 wks of age, which it decreased by 72.13% compared to the negative control group. However, chicks injected with 2.0 g probiotic /L had the highest count in total lactobacillus count and ratio of total lactobacillus / total coliform % compared with those of other treatments. Ratio of total lactobacillus / total coliform % was increased for all treated compared to the negative control group. This effect may be due to Lactobacilli bacteria are able to produce lactic acid from carbohydrate and are resistant to acidity as a result, while acid is fatal to other bacteria e.g. Escherichia coli (Gippert et al., 1992). In general, the reduction of pathogenic microbial species in the intestine could be due to a direct action of the probiotic or the indirect result of the stimulation of the beneficial bacteria (Nicodemus et al., 2004). Changes in the physical microenvironment inhibit pathogen growth in two ways. First ,probiotic organisms compete with pathogens for nutrients thus preventing them from acquiring energy to grow and function in the gut environment (Cummings and Macfarlane, 1997).Second, probiotics produce a variety of organic acid end products, such as volatile fatty acids as a part of their metabolism of nutrients in the gut digest (Gibson, 1999).

Intestinal enzyme activity:

A significant differences were observed among the experimental groups in intestinal lipase enzyme activity for chicks as a result of in-ovo injection eggs with probiotic at the 18^{th} day incubation period (Table 4). Chicks produced from eggs injected with 1.0 g probiotic /L recorded the lowest lipase value as compared with the other experimental treatments. Un-treated group (control -) gave the highest level of lipase enzyme activity compared with other treated

groups. Probiotics are used to help maintain a healthy microbial balance within the intestine to promote gut integrity and prevent enteric disease. This is accomplished through three main mechanisms: competitive exclusion, bacterial antagonism, and stimulation of the immune system (Ohimain and Ofongo, 2012). Competitive exclusion is the idea that probiotic strains have the ability to maintain normal intestinal microflora and inhibit establishment of pathogenic bacteria through competition for space, attachment sites, and available nutrients

Relative weights of some organs and parts of intestinal tract:

Results of Table 5 declare that most experimental measurements of relative weights of some organs and parts of intestinal of chickens at 12 weeks of age as a result of in-ovo injection eggs with probiotic during incubation period. The carcass weight and the relative weights of dressing, spleen and Jejunium were significantly affected due experimental treatment. Larger spleens have also been observed in studies where probiotics, have an effect on the systemic immune system (Sadeghi et al., 2015 and Ahmadi, 2011). There are conflicting reports, however, suggesting that probiotics do not affect immune organ weights (Al-Barwary, 2012; Naseem et al., 2012). Dressing weight percentage of chicks produced from in-ovo injection eggs was decreased with or without significant effects than the control, also, total parts which ready to cock (carcass weight+ giblets weight) were decreased due to treatment. Chicks produced from in-ovo injection eggs by 2.0 g probiotic/ L gave the lowest value of relative dressing and ready to cock weights as compared with the other treatments at 12 weeks of age. Relative weight of spleen was insignificantly higher of chicks produced from in-ovo injection eggs by 1.0 or 2.0 g probiotic / L , while relative Jejunium weight was significantly elevated than the un-treated group (control -).

CONCLUSION

Standing on our results, the in-ovo injection eggs by probiotic with 1.0 up to 2.0 g /L at the 18th day of incubation period seems to improve hatchability and decrease embryonic mortality percentages, as well as improve subsequent growth and physiological response of hatched Sinai chicks.

Table (1): Composition and calculated analysis of the basal diet

Ingredients %	Starter	Grower
Yellow Corn	64.00	71.25
Soybean meal (44 %)	32.10	18.50
Wheat bran	0.00	6.00
Di-calcium phosphate	1.80	1.35
Limestone	1.40	2.00
Vit. & Min. premix[1]	0.30	0.30
NaCl	0.30	0.30
DL. Methionine	0.10	0.30
Total	100	100
Calculated Analysis [2]		
Crude protein %	19.11	14.57
ME (Kcal / kg)	2863	2750
Crude fat%	2.91	3.00
Crude fiber %	3.82	3.65
Calcium (%)	1.06	1.14
Av. phosphorus (%)	0.47	0.40
Lysine %	1.10	0.82
Methionine %	0.43	0.33
Methio + Cyst %	0.75	0.58

1- Each 3 kg of the Vit and Min. premix manufactured by Agri-Vit Company, Egypt contains: Vitamin A 10 MIU, Vit. D 2 MIU, Vit E 10 g, Vit. K 2 g, Thiamin 1 g, Riboflavin 5 g, Pyridoxine 1.5 g, Niacin 30 g, Vit. B12 10 mg, Pantothenic acid 10 g, Folic acid 1.5 g, Biotin 50 mg, Choline chloride 250 g, Manganese 60 g, Zinc 50 g, Iron 30 g, Copper 10 g, Iodine 1g, Selenium 0. 10 g, Cobalt 0.10 g. and carrier CaCO3 to 3000 g.
2- According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001)

Table (2): Effect of in-ovo injection with probiotic at the 18th day of incubation on hatching traits of local Sinai hen's eggs

Parameters	Control (-ve)	Sterile distilled water contained probiotic (g /L)			SEM	Sig.
		0.0	1.0	2.0		
Hatchability of fertile eggs	82.78^{ab}	81.45^{b}	88.63^{ab}	90.62^{a}	1.34	**
Embryonic mortality EM	17.12^{ab}	18.54^{a}	11.36^{ab}	9.37^{b}	1.34	**

a,b,c,..: means in the same column within each item with different superscripts are significantly different ($P \leq 0.05$). ** = $P \leq 0.01$

Table (3): Effect of in-ovo injection eggs with probiotics at the 18th day of incubation period on subsequent growth performance traits of hatched Sinai chickens at different ages.

Age (wks)	Control (-)	sterile distilled water contained probiotic (g /L)			SEM	Sig.
		0.0	1.0	2.0		
Live body weight (g/ chick)						
At hatch	33.86^{c}	34.45^{bc}	35.55^{a}	35.04^{ab}	0.2	**
4	147.51^{a}	135.34^{b}	151.77^{a}	137.12^{b}	2.23	**
8	366.11	342.55	374.76	369.53	6.96	NS
12	636.10^{b}	556.82^{d}	643.23^{a}	601.36^{c}	10.33	**
Body weight gain (g/ chick / 28 day)						
0-4	113.64^{a}	100.88^{b}	116.21^{a}	102.08^{b}	2.22	**
4-8	218.59	207.21	222.98	232.41	6.53	NS
8-12	269.99^{a}	214.27^{b}	268.47^{a}	231.76^{ab}	9.34	**
0-12	602.23^{b}	522.36^{d}	607.68^{a}	566.26^{c}	10.29	**
Feed consumption (g/chick/day)						
0-4	16.94^{a}	15.10^{b}	14.91^{b}	14.20^{b}	0.34	**
4-8	39.23^{a}	33.27^{c}	39.77^{a}	36.94^{b}	0.79	**
8-12	49.00^{a}	39.81^{d}	47.23^{b}	43.20^{c}	1.11	**
0-12	35.06^{a}	29.33^{d}	34.05^{b}	31.45^{c}	2.03	**
Feed conversion ratio (g. feed/ g. BWG)						
0-4	4.02^{ab}	4.19^{a}	3.71^{c}	3.91^{bc}	0.06	**
4-8	5.27	4.5	4.90	4.49	0.18	NS
8-12	5.14	5.21	4.98	5.25	0.11	NS
0-12	4.88^{a}	4.72^{b}	4.71^{b}	4.66^{b}	0.27	**

a,b,c,.. : means in the same row with different superscripts are significantly different ($P \leq 0.05$).
NS = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$

Table (4): Effect of in-ovo injection eggs with probiotics at the 18th day of incubation period on total Coliform and Lactobacillus bacteria count in cecum microbill of chicks and lipase enzyme at 12 weeks of age

Parameters	Control (-ve)	sterile distilled water contained probiotic (g /L)			SEM	Sig.
		0.0	1.0	2.0		
T. coliform (Cfu/g)	$9.15x10^{5b}$	$9.45x10^{5b}$	$2.55x10^{5c}$	$11.25x10^{5a}$	1.00	**
T. Lactob. (Cfu/g)	$2.15x10^{5c}$	$4.35x10^{5b}$	$1.25x10^{5d}$	$7.25x10^{5a}$	0.7	**
T. lacto./ T. coliform%	23.5^{c}	46.03^{b}	49.02^{b}	64.44^{a}	4.47	**
Lipase (U/L)	78.75^{a}	52.50^{b}	30.50^{d}	45.75^{c}	5.32	**

a,b,c,.. : means in the same row with different superscripts are significantly different ($P \leq 0.05$) ; * = $P \leq 0.05$; ** = $P \leq 0.01$

Table (5): Effect of in-ovo injection eggs with probiotics at the 18th day of incubation period on subsequence relative weights and length of some organs and parts of intestinal of chickens at 12 weeks of age.

Parameters		Control (-ve)	sterile distilled water contained probiotic (g /L)			SEM	Sig.
			0.0	1.0	2.0		
Carcass wt. (g)		564.7^{a}	457.6^{b}	500.2^{ab}	492.5^{ab}	16.97	**
Relative weights, %							
Dressing		61.64^{a}	55.34^{b}	57.26^{ab}	53.79^{b}	1.05	**
Heart		0.79	0.70	0.73	0.75	0.02	NS
Liver		4.46	4.62	4.95	4.96	0.11	NS
Gizzard		4.18	4.96	4.95	5.12	0.19	NS
T. giblets #		9.43	10.28	10.63	10.83	0.27	NS
Spleen		3.41^{b}	5.05^{a}	3.88^{ab}	3.57^{b}	0.25	**
Pancreas		0.39	0.59	0.52	0.48	0.04	NS
Cecum		1.76	1.7	1.83	1.54	0.09	NS
Cm/100gm Carcass Weight	Cecum	5.72	6.98	7.12	6.32	0.25	NS
	Dudinum	5.07	5.66	5.67	5.90	0.18	NS
	Jejunium	10.14^{b}	12.01^{ab}	14.45^{a}	13.64^{a}	0.59	**
	Illeum	11.25	12.83	12.78	12.10	0.36	NS

a,b,c,.. : means in the same row with different superscripts are significantly different ($P \leq 0.05$). NS = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$. ; T. Giblets =Liver+Gizzared+Heart

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الملخص العربى

تأثير حقن بيض التفريخ بالبروبيوتك على صفات التفريخ والنمو والأداء الفسيولوجى اللاحق لكتاكيت السينا

ياسر صديق رزق ، ملاك منصور بشاره و ايمن عبده موافى
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أجريت هذه التجربة لدراسة تأثير حقن البروبيوتك فى بيض التفريخ على صفات التفريخ والنمو والاداء الفسيولوجى اللاحق للكتاكيت الفاقسه لسلالة دجاج السينا المحلى . حيث استخدم 600 بيضة مخصبة فى اليوم الثامن عشر من فترة التفريخ وتم تقسيمها الى أربع معاملات (150بيضة للواحدة) فى ثلاث مكررات متساوية لكل منها وتم ترتيب المعاملات الأربع على النحو التالى : الأولى كمجموعة مقارنة سالبة (غير معاملة) ، الثانية كمجموعة مقارنة موجبة (تم حقن البيض بها ب 3، ملى ماء مقطر فى الغرفه الهوائيه) بينما المعاملة الثالثة والرابعة تم حقنهم ب 0.3 ملى محلول ماء مقطر يحتوى على 1.0 و 2.0 جم بروبيوتيك لكل لتر على التوالى وتم تربية الكتاكيت الناتجه لمدة 3 شهور وتم تقدير صفات النمو والآوزان النسبية للذبيحة والغدد عند 12 أسبوع من العمر.
وكانت النتائج كالاتى :
لوحظ تحسنا معنويا فى نسبة الفقس وإنخفاضا معنويا فى نسبة النفوق الجنينى بالحقن بمعدل 2.0 جم بروبيوتيك لكل لتر بينما كان التحسن غير معنويا باستخدام 1.0 جم بروبيوتيك لكل لتر بالمقارنة بمجموعة المقارنة السالبة.
كما لوحظ تحسنا معنويا فى وزن الجسم ومعدل الزيادة الوزنيه بحقن بيض التفريخ بالبروبيوتك بمعدل 1.0 جم/لتر ماء مقطر مقارنة بمجموعة المقارنة السالبة كما لوحظ انخفاض معدل استهلاك العليقه معنويا لتلك المعاملة . لوحظ تحسن معنوى فى معامل التحويل الغذائى بحقن بيض التفريخ بالبروبيوتك خلال فترة التربية بعد الفقس مقارنة بالكنترول. لوحظ وجود زيادة معنوية فى عدد بكتريا لاكتوبسلس وكذلك نسبة لاكتوبسلس الى العدد الكلى للبكتريا بحقن بيض التفريخ بمعدل 2 جم / لتر ولكن لوحظ انخفاض معنوى فى تركيز انزيم الليز بحقن البيض بمحلول يحتوى 1 جم بروبيوتيك/ لتر . كما لوحظ أن حقن بيض التفريخ بالبروبيوتك ادى الى حدوث زياده معنوية فى وزن الطحال وكذلك الصائم وزيادة غير معنوية فى بعض الغدد واجزاء الذبيحة مقارنة بمجموعة المقارنة السالبة .
لذلك تشير هذه الدراسة إلى إمكانية حقن بيض التفريخ بمعدل 0.3 ملى لكل بيضة فى الغرفة الهوائية من محلول يحتوى على البروبيوتيك بمعدل 1.0 أو 2.0 جم / لتر عند اليوم الثامن عشر من التفريخ لتحسين صفات التفريخ ومعدل النمو والاداءالفسيولوجى لكتاكيت السينا المحلى بعد الفقس.