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Effect of Spirulina and Canthaxanthin Injection into Hatching Eggs on Hatchability Traits and Subsequent Growth Performance of Chicks

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ABSTRACT

This work aimed to analyze the result of in-ovo injection at the 14th day of hatching period on hatchability, embryonic mortality (%) and subsequent growth performance additionally physiological status for hatched chicks. A 540 hatching Sinai eggs were collected, weighted (52 ± 1 g), distributed into 6 groups and incubated. At the 14th day of incubation, all eggs were candled then total fertile eggs of each group spliced into equal 3 replicates. Experimental groups were organized as follow: negative control (NC) without injection while the other groups were injected with sterile distilled water alone as a positive control (PC), spirulina (SP) with 2.5 and 5.0 mg/egg, canthaxanthin (CX) with 0.025 and 0.050 mg/egg, respectively. Results indicated that the highest value of hatchability (%) of fertile eggs was achieved by injected eggs with 0.050 mg CX/egg followed by in-ovo injection with 2.5 mg SP/egg in comparison with the PC and NC groups. In-ovo injection with 0.050 mg CX/egg resulted in a heavier chick body weight at 8 days after hatch and relative heart weight than other treatments and the control groups. Chicks produced from the eggs injected with 0.050 mg CX / egg recorded a significant improvement in BW at 28 days of age and subsequent BWG than other injected groups and NC during 0-28 days of age. The highest value of the survival ratio percentage was recorded for chicks produced from injected eggs with 2.5 mg SP/ egg. Total protein, globulin and HDL recorded higher ($P \leq 0.01$) values in chick's serum by injected hatching eggs with 0.025 and 0.050 mg CX/egg in comparison with NC group, however, total cholesterol and LDL recorded the lower value. It was concluded that, in-ovo injection Sinai hen's eggs with spirulina and canthaxanthin could be used to improve hatchability and decrease embryonic mortality (%) and enhance subsequent growth performance additionally physiological situation of hatched chicks.

Keywords: in-ovo injection, spirulina, canthaxanthin, hatching traits, growth performance

INTRODUCTION

In-ovo injection has created new opportunities to enhance the health and development of broiler chickens, using the same technology as with vaccination, it's potential to produce developing embryos with exogenous nutrients in a very method developed by Uni and Ferket (2003). Provisioning embryos with exogenous nutrients in ovo might improve hatchability, increase chick weight, and /or the final body weight of broiler through modifying embryo gut morphology (Uni and Ferket, 2003). Day-old chicks are the final product of the hatchery industry and supply the important starting point for broiler production. A high hatchability of marketable chicks within a narrow spread of hatch is the major objective of hatcheries industry, because these chicks have perform well, which interpret their high viability, growth rate and breast meat yield and better feed conversion (Decuypere and Bruggeman, 2007). Carotenoids are particularly paramount to avian species, where pigmentation quality is expressive of a bird's general health (Olson and Owens, 1998). Accumulated carotenoids may elevate the quality of chicken by improving flavor (Josephson, 1978), delay oxidation and pigmenting bodies (An *et al*., 2004). Also, carotenoids have antioxidant abilities, which are effective in protecting tissues from oxidative stress (von Schantz *et al*., 1999; Blount *et al*., 2000).

Red-orange carotenoid that belongs to the xanthophyll group is called canthaxanthin, its naturally present in bacteria, algae and some fungi. Canthaxanthin have possesses high antioxidant activity in vitro model (Soffers *et al*., 1999), in animal experiments in vivo (Zhang *et al*., 2011; Rosa *et al*., 2012), due to its ability to scavenge singlet molecular oxygen and extinguish other free radicals (Bohm *et al*., 2012). Canthaxanthin is necessary carotenoid that might efficiently deposited in egg yolk and further (Surai and Speake, 1998). It's one of the most powerful lipid-soluble antioxidants in nature, and it has specified as a potent free radical scavenger (Rengel *et al*., 2000). Canthaxanthin (CX) is a carotenoid which can reduce lipid peroxidation and enhance serum total antioxidant capacity (Rocha *et al.*, 2013). Existence carotenoid content in maternal diet may be influence in these compounds concentration in progeny tissues until 28 days of age (Koutsos *et al.* , 2003). Spirulina has enhance immune function reproduction and increase growth because its rich in nutrients such as vitamins, amino acids, gamma linoleic acid, phycocyanins, tocopherols, chlorophyll and ×-carotenes (Khan *et al.*, 2005). Spirulina platensis have some promising biological activities such as antitumor, antimicrobial, antiviral and anti-inflammatory (Farag, *et al.*, 2016). Spirulina in hens diets had achieved superior productive and reproductive performance (Nikodémusz *et al*., 2010). Therefore, current study aimed to evaluate the in-ovo injection at the 14th days of hatching period on hatchability, embryonic mortality (%) and subsequent growth performance additionally physiological status for hatched chicks reared up to 28 day.

MATERIALS AND METHODS

Current work carried out at El-Serw Research Station, Agricultural Research Center. It conducted to evaluate the in-ovo injection of Sinai hen's eggs with spirulina and canthaxanthin at the 14th day of incubation on hatching traits, subsequent growth and physiological performance for hatched chicks up to 28 days. A 540 hatching eggs for Sinai hens were collected, weighed (52 ± 1 g) and incubated at 37.8 °C and 63% RH in 6 groups. At the 14th day of incubation, the eggs were candled, then the infertile eggs or those containing early dead embryos were removed for each group. All fertile eggs which containing live embryos for each group were distributed to equal 3 replicates , then the groups arranged as, the 1st group employed as a negative control (non-injected, NC), the 2nd injected with 0.3 ml/egg sterile distilled water (positive control, PC) into the air sac, the 3rd and 4th injected with 0.3 ml/egg sterile distilled water contained 2.5 and 5.0 mg spirulina, respectively, while the 5th and 6th groups were injected with 0.3 ml/egg sterile distilled water contained 0.025 and 0.050 mg canthaxanthin, respectively. The injection area was disinfected with ethyl alcohol, the pinhole site was closed with paraffin wax

immediately after injection, then injected eggs transferred to hatcher. At the 21th day of incubation, late dead embryos were recorded, then embryonic mortality percent was calculated a number of dead embryos as a percentage of fertile eggs, whereas, hatchability percent was estimated a number of healthy hatched chicks as a percent of fertile eggs.

All hatched chicks weighted, then distributed to equal 3 replicates per group and grown till 28 days after hatch at the same managerial conditions. Bird body weight (LBW) and feed consumption (FC) were recorded per replicate then averaged and expressed in grams per chick from hatch up to 28 day. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated at the same period. Basal diet composition are shown in Table 1. At 8 days of age, 3 chicks per group were randomly taken and massacred, then blood samples were collected during massacring in heparinized tubes, while another samples in non-heparinized tubes and it centrifuged (3500 rpm) for 20 minutes to separate blood serum. The following biochemical markers were assayed in serum: total cholesterol (Ellefson and Caraway, 1976), triglycerides (Bucolo and David, 1973), total protein (Peters, 1968), which were carry out calorimetrically by utilizing commercial kits. Hematological parameters: differential white blood cells (WBC) counts were carry out in standard avian guidelines introduced (Ritchie *et al*. 1994). Total white blood cells were determined by the method of Campbell (1995), Leucocyte cells (neutrophils and lymphocytes) counted in different microscopic fields in a total of 200 WBC by the same person (Gross and Siegel, 1986).

Table 1. Basal diet composition and calculated analysis

%	Starter diet
Corn	64.00
Soybean meal (44%)	32.10
Wheat brain	00.00
Gluten meal (60%)	0.00
Dicalcium phosphate	1.80
Ground limestone	1.40
Vit.&Min. premix*	0.30
Sodium Chloride	0.30
DL-Methionine	0.10
Total	100.00
Cal. Analysis	
CP, %	19.11
ME , Kcal/Kg	2863
EE, %	2.91
CF, %	3.82
Ca., %	1.06
Av.phos., %)	0.47
Sodium	0.12

***Each 1 kg of diet including Vit. A 10000 I.U; D3 2000 I.U; E 15 mg; K3 1 µg; B1 1mg;. B2 5mg;. B12 10 µg; B6 1.5 mg; Niac 30 mg; Pantothc acid 10 mg; folic 1mg; Biot 50 mg; chol chlo 300 mg; zinc 50 mg; copper4mg; iodine 0.3 mg; iron 30 mg; Se.1mg; Man 60 mg; Cob 0.1mg and carrier $CaCO_3$ up to 1 kg**

After slaughter and complete bleeding, the birds were dressed and the weight of carcass and some other components (liver, gizzard, heart, bursa of Fabricius and spleen) were measured. Relative organ weights were calculated as percentage of body weight as follows: [(Organ weight/Body weight) × 100].

Statistical analysis:

Obtained data were statistically analyzed by using one-way analysis according to the GLM of SAS (2004). Significant differences tested by Duncan's Multiple Range (Duncan, 1955). All data collected were analyzed by one-way analysis of variance.

RESULTS AND DISCUSSION

Hatching traits:

Data of spirulina (SP) and canthaxanthin (CX) in-ovo injection into Sinai fertile eggs at the 14th day of incubation on hatchability and embryonic mortality percentages are presented in Table 2. A significant differences in hatchability and embryonic mortality (%) of fertile eggs were observed among the different experimental groups. The highest value of hatchability of set or fertile eggs was achieved by injected eggs with 0.050 mg CX/egg followed by in-ovo injection with 2.5 mg SP/egg in comparison with the positive (PC) and negative (NC) control groups. Embryonic mortality (%) significantly decreased by injected eggs with 2.5 mg SP and 0.05 mg CX/egg in comparison with PC and NC groups. The improvement in hatchability may be due to canthaxanhtin is a β-carotene which is pro-vitamin A (Surai *et al*., 2001), and is also classified as an oxycarotenoid (Zhang *et al*., 2011). During embryonic development, poultry embryos exposed to oxidative stress, so antioxidant enzyme synthesized (Surai, 2012). Canthaxanhtin is necessary carotenoid that could be efficiently distributed into the chick embryonic tissues and prevent oxidative stress because it's including vitamin E, tocopherols and flavonoids. These results are in agreement with those obtained by Jose-maria (2011) who showed that in-ovo injection with canthaxanthin at day 14 improves hatchability of broiler breeder eggs and biological function than the control group. Canthaxanhtin is potentially and effectively aid in reducing oxidation reactions in chick embryos tissues during incubation period (Surai *et al*., 2012).

Subsequent growth performance of chicks:

Data of Table 3 shows all studied growth performance parameters of chicks from hatch up to 28 days of age which significantly affected except for feed conversion ratio by in-ovo injection with spirulina (SP) and canthaxanthin (CX) at the 14th day of incubation. Hatched chicks LBW was improved by 2.18 by in-ovo injected eggs with 0.025 mg CX / egg than the negative control (non-injected), however it higher by 1.98% than the positive control group at hatch. Chicks produced from the eggs injected with 0.050 mg CX / egg recorded a significant improvement in BW at 28 days of age and subsequent BWG in comparison with other in-ovo injected groups and NC at the period for 0-4 weeks of age. Chickens FBW and BWG was significantly improved by 36.1 and 33.7 % of the group injected with 0.050 mg CX /egg as compared with the negative control, respectively. Daily feed consumption per chicks hatched form in-ovo injected eggs with SP or CX was comparable as compared with those hatched from NC group. Although, feed conversion (FC) was not significantly affected due to in-ovo injection treatment but it improved by SP and CX injection materials compared with the negative control. In-ovo injection by SP and CX significantly ($P \leq 0.05$) elevated the survival percentage compared with NC group. The highest value of the survival ratio percentage was recorded for chicks produced from injected eggs with 2.5 mg SP/ egg, this may be due to spirulina have some promising biological activities like antitumor, antimicrobial, antiviral and anti-inflammatory (Farag *et al.*, 2015).

Table 2. Hatching traits as affected with spirulina (SP) and canthaxanthin (CX) in-ovo injection into Sinai fertile eggs at the 14th day of incubation.

Items, %	Experimental treatments						SEM	Sig.
	Control		SP, g		CX, mg			
	NC	PC	2.5	5.0	0.025	0.050		
Hatchability of set egg	85.00^{c}	87.5^{bc}	90.0^{ab}	87.5^{bc}	87.5^{bc}	92.5^{a}	0.63	**
Hatchability of fertile eggs	91.89^{bc}	92.1^{b}	94.73^{a}	89.74^{c}	92.1^{b}	94.87^{a}	0.49	**
Embryonic mortality	8.11^{b}	7.90^{b}	5.27^{c}	10.26^{a}	7.90^{b}	5.13^{c}	0.49	**

a-c: means in the same row having different superscripts are significantly different; SEM; standard error mean; NC & PC, negative and positive control **: (P≤ 0.01; NS, non-significant

Table 3. Growth performance traits as affected with in-ovo injection with spirulina (SP) and canthaxanthin (CX) into Sinai eggs at the 14th day of incubation for hatched chicks up to 28 days of age

Items	Experimental treatments						SEM	Sig.
	Control		SP, mg		CX, mg			
	NC	PC	2.5	5.0	0.025	0.050		
body weight at hatch , g	32.5^{ab}	32.7^{ab}	31^{b}	33.1^{ab}	34.68^{a}	34.6^{a}	0.41	**
body weight at 28 d, g	191.6^{d}	210^{c}	214^{b}	183.4^{e}	210.3^{c}	227.7^{a}	3.56	**
body weight gain, g	159.1^{d}	177.4^{c}	183^{b}	150.3^{e}	175.6^{c}	192.8^{a}	3.48	**
daily feed consumption, g/chick	13.75^{a}	10.35^{b}	11.2^{ab}	11.96^{ab}	12.89^{ab}	11.92^{ab}	0.38	**
Feed conversion ratio	2.4	1.6	1.7	2.2	2.0	1.7	0.15	NS
Survival rate, %	88.1^{cd}	91^{bc}	97^{a}	94^{ab}	85^{d}	91.33^{bc}	1.01	**

a-d: means in the same row having different superscripts are significantly different; SEM; standard error mean; NC & PC, negative and positive control ; **: P≤ 0.01; NS, non-significant;

Carotenoids such as canthaxanhtin is antioxidants and immune-stimulating agents immediately after hatching, adding carotenoids in maternal diet might improve broiler breeder progeny performance (Fenoglio *et al*., 2002), also, carotenoids have effective for protecting tissues from oxidative stress by their antioxidant abilities (Blount *et al*., 2000). Improving FCR for chicks produced from in-ovo injection eggs with spirulina may be due to spirulina may elevate lactobacillus population and enhance the absorbability, as well as prevent chick's diarrhea, and improve nutrient digestion (Gruzauskas *et al*., 2004). These findings are in agreement with those obtained by Ali *et al*. (2016) who found canthaxanhtin addition numerically increased live body weight compared to control group for broiler chickens at 35 days of age. Increasing canthaxanhtin concentration in body tissues might enhance antioxidant status and result in better health conditions and therefore enhanced production performance of the birds (Robert *et al*., 2007) . Dietary inclusion of spirulina addition improved FCR as compared the control (Kharde *et al*., 2012; Shanmugapriya *et al*., 2015)

Some organs relative weights of hatched chicks:

Results of Table 4 shows in-ovo injection effect with spirulina (SP) and canthaxanthin (CX) into Sinai fertile eggs on some organs weights of chicks at 8 days after hatch. Live BW of chicks at 8 days of age and relative weights of all studied organs were (P≤0.01) affected by in-ovo injection except for spleen weight (%). In-ovo injection with 0.050 mg CX/egg resulted in a heavier chick BW at 8 days after hatch and relative heart weight as compared with other treatments and the control groups, however, chicks liver weight recorded a higher percent by in-ovo injection with 5.0 mg SP/egg. Relative gizzard weight significantly increased by in-ovo injection with 2.5 mg SP/egg and 0.025 or 0.050 mg CX/egg as compared with NC group.

These results may be due to in-ovo injection enhance the development or health status of broiler chickens by providing the embryos with exogenous nutrients which may increase chick weight at hatch , or final BW of broiler by modifying embryo gut morphology (Uni and Ferket, 2003). Also, canthaxanthin may transferred to the evolving embryos and circulated in many organs and tissues (Karadas *et al*., 2005), which it might help safeguard the developing embryos from against oxidative damage, particularly during the sensitive periods of hatching and early post-hatch life (Robert *et al*., 2007).

Table 4. Effect of in-ovo injection with spirulina (SP) and canthaxanthin (CX) into Sinai fertile eggs on some organs weights (%) of chicks at 8 days after hatch.

Items	Experimental treatments						SEM	Sig.
	Control		SP, mg		CX, mg			
	NC	PC	2.5	5.0	0.025	0.050		
LBW, g	59.91^{ab}	62.85^{a}	52.6^{b}	58.85^{ab}	57.88^{ab}	64.25^{a}	1.267	**
Bursa %	0.40^{a}	0.42^{ab}	0.28^{b}	0.35^{ab}	0.35^{ab}	0.34^{ab}	0.016	**
Liver %	3.77^{b}	4.11^{ab}	4.81^{ab}	5.23^{a}	4.28^{ab}	4.82^{ab}	0.186	**
Gizzard %	6.5^{c}	6.77^{bc}	8.21^{b}	7.72^{bc}	8.47^{a}	8.09^{b}	.242	**
Heart %	0.83^{ab}	0.85^{ab}	0.79^{b}	0.70^{b}	0.83ab	1.02^{a}	0.312	**
Spleen %	0.184	0.177	0.197	0.099	0.147	0.160	0.171	NS

a-c: means in the same row having different superscripts are significantly different; SEM; standard error mean; NC & PC, negative and positive control ; **: P≤ 0.01; NS, non-significant; LBW. Live body weight

Blood hematology parameters of hatched chicks:

In-ovo injection eggs with spirulina (SP) and canthaxanthin (CX) had significant effect for all studied hematological measurements except RBCs count and monocytes (%) of hatched chick at 8 day of age (Table 5). The obtained results showed that a significant increase in blood Hb and its concentration for chicks produced from injected eggs with 5.0 mg SP/egg as compared with PC and NC groups. The lowest leucocytes count was recorded for chicks produced from injected eggs with 0.025 mg CX/egg as compared with the negative and positive control groups. However, a significant increase (P≤0.01) in lymphocytes (%) by the injection with 5.0 mg SP/egg and 0.025 or 0.050 mg CX/egg, while neutrophils (%) was significantly decreased as compared with the NC and PC groups.

Table 5. Effect of in-ovo injection with spirulina (SP) and canthaxanthin (CX) into Sinai fertile eggs on some hematological measurements of chicks at 8 day after hatch

Items	Experimental treatments						SEM	Sig
	Control		SP, mg		CX, mg			
	NC	PC	2.5	5.0	0.025	0.050		
Hb, mg/dl	10.1^{b}	8.5^{c}	10.1^{b}	11.6^{a}	10.7^{b}	10.7^{b}	0.242	**
Hb concentration %	63.0^{b}	53.0^{c}	63.0^{b}	72.0^{a}	66.0^{ab}	66.0^{ab}	1.551	**
HCT, %	31.0^{ab}	26.0^{b}	31.0^{ab}	36.0^{a}	33.0^{a}	33.0^{a}	0.939	**
RBCs x 10^{6}/ul	3.1	2.9	3.3	4.0	3.6	3.6	0.201	NS
Total Leucocytes , x 10^{3}/ ul	128.0^{b}	162.0^{a}	128.0^{b}	105^{d}	81.0^{e}	117.0^{c}	6.023	**
Neutrophil,%	65.0^{b}	73.0^{a}	66.0^{b}	55.0^{c}	52.0^{c}	56.0^{c}	1.842	**
Lymphocytes,%	21.0^{c}	13.0^{d}	22.0^{c}	36^{ab}	38.0^{a}	31.0^{b}	2.236	**
Monocytes,%	8.0	7.0	8.0	6.0	7.0	8.0	0.642	NS
Eosinophil,%	4.0^{ab}	5.0^{a}	3.0^{ab}	2.0^{b}	2.0^{b}	3.0^{ab}	0.381	**

a-c: means in the same row having different superscripts are significantly different; SEM: standard error mean; NC & PC, negative and positive control ; NS: non-significant; **: P≤0.01 ; Hb, hemoglobin; HCT, Hematocrit; RBC, red blood cells;

These results may be due to spirulina enhance immune function and increase growth because its affluent in some nutrients like vitamins, amino acids, gamma linoleic acid, phycocyanins, tocopherols, chlorophyll and B-carotenes (Khan *et al.*, 2005) which plays a major role as antioxidants materials and prevent oxidative stress. Also, Bendich and Shapiro (1986) stated that ×-carotene and canthaxanhtin could enhance splenic T and B lymphocyte proliferation. Shanmugapriya *et al*. (2014) showed insignificant increase in packed Cell volum (PCV) by spirulina supplementation, while it significantly improved the blood picture parameters.

Blood serum constituents of hatched chicks:

All studied blood serum constituents were significantly affected by in-ovo injection with spirulina (SP) and canthaxanthin (CX) into eggs (Table 6). The highest values (P≤0.01) of total protein, globulin and HDL were noticed for chicks produced from injected eggs with 0.025 and 0.050 mg CX/egg in comparison with NC group, however, lowest total cholesterol and LDL values were noticed for same groups. These findings are agreement with those obtained by Mobarez *et al.* (2018) who stated that supplementing SP to layers diet significantly decreased blood cholesterol, total lipids and low density lipoprotein (LDL), while significantly elevated values of high density lipoprotein (HDL), total antioxidant capacity (TAOC) . Park *et al.* (2015) noticed that decreased blood cholesterol observed in Spirulina-supplemented groups in comparison with the control. Abd El-Hady *et al*. (2018) showed SP supplementation resulted in a significant decrease in serum total lipid, triglyceride and total cholesterol levels of broiler chicks, also, serum high density lipoprotein (HDL) concentration significantly increased. Plasma total lipids, triglycerides and cholesterol significantly decreased by spirulina supplementation to the diets with 0.2 or 0.3 g/kg (Mariey *et al*., 2014). These findings are disagreement with Rocha *et al.* (2013) who found that canthaxanthin (CX) is a carotenoid which can reduce lipid peroxidation and enhance serum total antioxidant capacity.

Table 6. Effect of in-ovo injection with spirulina (SP) and canthaxanthin (CX) into Sinai fertile eggs on some serum constituents for chick at 8 day after hatch

Measurements	Experimental treatments						SEM	Sig
	Control		SP, mg		CX, mg			
	NC	PC	2.5	5.0	0.025	0.050		
Total Protein, g/dl	2.82^{d}	3.03^{c}	3.11^{b}	2.77^{d}	4.50^{a}	4.51^{a}	0.18	**
Albumin (A), g/dl	1.05^{b}	1.04^{b}	1.03^{b}	1.31^{a}	1.05^{b}	0.84^{c}	0.37	**
Globulin (G), g/dl	1.77^{d}	1.98^{c}	2.08^{c}	1.46^{e}	3.45^{b}	3.67^{a}	0.20	**
A/G ratio	0.59^{b}	0.53^{b}	0.49^{bc}	0.93^{a}	0.30^{cd}	0.22^{d}	0.05	**
Cholesterol, mg/dl	179.5^{c}	173.9^{d}	217^{a}	182.33^{b}	134.7^{e}	118.5^{f}	7.88	**
Triglycerides, mg/dl	50.3^{e}	56.26^{d}	69^{b}	74.9^{a}	65^{c}	35.6^{f}	3.17	**
HDL , mg/dl	36^{b}	42.3^{a}	33^{c}	34^{bc}	42.5^{a}	44^{a}	1.11	**
LDL , mg/dl	133.4^{b}	119.4^{b}	170.2^{a}	112.67^{b}	76.63^{c}	67.76^{c}	8.70	**
TAC , mmol/l	0.82^{a}	0.55^{d}	0.79^{a}	0.72^{b}	0.63^{c}	0.78^{a}	0.02	**

a-f: means in the same row having different superscripts are significantly different; SEM: standard error mean; NC & PC, negative and positive control; NS: non-significant; **: P≤0.01

CONCLUSION

From obtained results, we concluded that in-ovo injection Sinai hen's eggs with Spirulina and canthaxanthin could be used to improve hatching traits and subsequent growth performance as well as physiological status of hatched chicks.

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تأثير حقن بيض التفريخ بالسبرولينا وكانزانسين على صفات الفقس والنمو اللاحق للكتاكيت

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الهدف هو دراسة تأثير حقن بيض التفريخ بالسبرولينا والكانزانسين في اليوم الرابع عشر من فترة التفريخ على صفات الفقس و النمو اللاحق والحالة الفسيولوجية للكتاكيت والمرباة لعمر 28 يوما. تـمَّ وضع عدد 540 بيضة سينا (52.1 ± 1.0 جم) فى ستة مجموعات متساوية، تم تقُسّيم البيض المخصب لكل مجموعة الى ثلاث مكررات ، تم توزيع المجموعات كالتالى : الأولى كنترول سالب (بدون حقن)، الثانية حقنت ب 0.3 ملى/بيضة ماء مقطر(كنترول موجب)، أما الثالثة والرابعة حقنت ب 0.3 ملى ماء مقطر تحتوى على 2.5 و 5.0 ملجم سبرولينا على التولى بينما المجموعة الخامسة والسادسة حقنت ب 0.3 ملى ماء مقطر تحتوى على 0.025 و 0.050 ملجم وكانزانسين على التولى . وأشارت النتائج إلى أن أعلى نسبة للفقس وأقل نفوق جنينى كانت للبيض المحقون بـ 0.05 ملجم / بيضة كانزانسين يليها المحقون ب 2.5 ملجم / بيضة سبرولينا بالمقارنة بمجموعتى الكنترول الموجب والسالب . كما سجل أعلى وزن للكتاكيت عند عمر 8أيام لتلك الناتجة من البيض المحقون ب 0.05 ملجم / بيضة كانزانسين وكذلك وزن القلب بالمقارنة بالكنترول كما سجلت الكتاكيت الناتجة من البيض المحقون ب 0.05 ملجم / بيضة كانزانسين تحسنا في وزن الجسم عند عمر 28 يوما ومعدل الزيادة فى الوزن للمجموعات المحقونة خلال الفترة من الفقس حتى 28 يوم من العمر بالمقارنة بالكنترول. كما سجل أعلى معدل للحيوية للكتاكيت الناتجة من حقن البيض بـ 2.5 ملجم / بيضة سبرولينا. لوحظ ارتفاع قيمة البروتين الكلي والجلوبيولين و HDL في سيرم الكتاكيت الناتجة من حقن البيض بـ 0.025 ، 0.05 ملجم / بيضة كانزانسين بالمقارنة بالكنترول السالب كما انخفضت قيمة الكولسترول و LDL لنفس المجموعات؛ لذلك يمكن التوصية بـحقن بيض التفريخ لدجاج السينا بالسبرولينا (2.50 ملجم/بيضة) أو الكانزانسين (0.05 ملجم /بيضة) فى اليوم الرابع عشر من فترة التفريخ لتحسين صفات الفقس وأداء النمو والحالة الفسيولوجية للكتاكيت الناتجة.