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EFFECT OF IN OVOMETHIONINE, GLUTAMINE, CARNITINE OR BETAINE INJECTION ON HATCHABILITY, GROWTH PERFORMANCE AND PHYSIOLOGICAL STATE.

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ABSTRACT: the present study was carried out to estimate responses of Fayoumi chickens to in ovo injection of some nutrients during late stage of incubation and post-hatch. At 18 day of incubation 360 eggs contain live embryos were chosen and distributed randomly into 6 treatment groups each of which contain 60 eggs divided into 3 replicates (20eggs/replicate). Eggs in group 1 confirm control treatment without injection but eggs in group 2 injected with pure Marek's solution and performed sham treatment. Eggs in groups 3, 4, 5 and6 injected with 0.2 mm^3Marek's solution contain 5mgglutamine/egg,5mg methionine /egg, 16mg L-carnitine/egg and 5mg betaine/egg respectively. Hatching, growth performance and some hematological parameters were measured, moreover response against Newcastle disease vaccination and liver histopathology were estimated.

The following results were obtained:

1- Most parameters didn't recorded significant differences between control and sham treatment which reflect success of injection procedure and time.

2- In ovo glutamine injection decreased hatchability percent but increased hatch weight compared with control treatment. Glutamine injection improved growth performance compared with other treatments except for betaine injection

3- In ovo methionine injection significantly ($P \leq 0.05$) decreased hatchability percent and significantly ($P \leq 0.05$) increased hatch weight compared with control. Methionine didn't provide significant improvement in growth performance.

4- In ovo L-carnitine injection decreased hatchability percent and hatching weight than control treatment and recorded the worst growth performance.

5- In ovo betaine injection improved hatching parameters and achieved the best growth performance and physiological state.

6- Histopathological results show that in ovo L-carnitine injection presented liver damage and support hatching and growth results. In general we recommend using in ovo betaine injection to improve hatchability, growth performance and physiological state of Fayoumi chickens.

Key words: In ovo – methionine – glutamine – carnitine – betaine - and growth.

INTRODUCTION

Technology of in ovo injection illustrates that supplemented egg with nutrients improve physiological state during and post hatching (Kucharska-Gaca, et al., 2017).Nutrients enhance body weight by improve nutrients digestion, intestine absorption and promoting cell metabolism of digestive system (Kucharska-Gaca, et al., 2017). Antibodies constitute most egg protein (Losch et al., 1986) so injection of amino acids or substance that spares amino acids from degradation may preserve antibody within egg from hydrolysis (Bhanja and Mandal, 2005).

Glutamine Classified as conditionally essential amino acids (Smith, 1990) and incorporated in synthesize of several amino acid (Boza et al., 2001).Glutamine in ovo injection stimulates gastrointestinal tract development subsequently, enhanced post hatch growth performance (Salmanzadeh et al., 2016). In poultry methionine is essential amino acid with one methyl group donate it in methyl reactions (Waterland, 2006). Methionine in ovo injection increased chick weight (Coşkun et al. 2014), feather diameter and density (Nazem, et al., 2015).When methionine acts as methyl donor it metabolized to homocysteine that has toxic effect (Xie et al., 2007).

Carnitine presents in cells as the L form but D form is toxic and chicken synthesis less L-carnitine (Zhai et al., 2008). Nouboukpo et al., (2010) reported that in ovo L-carnitine injection resulted in earlier utilization of yolk sac moreover it has antioxidant ability (Zhai et al., 2008).In ovo L- carnitine injection may be useful for chicken embryos because their requirements were higher than L-carnitine content of the egg (Zhai et al., 2008). L-carnitine in ovo injection affects division of myonuclei (skeletal muscle fibers) during embryonic myogenesis (El-Azeem et al., 2014).Betaine a methyl group donor possesses positive and negative charges so it classified as zwitterion. Betaine zwitterion allows water retention in cells, prevents dehydration and preserves optimum performance (Maddahian et al, 2017). Hu et al. (2017) observed that in ovo betaine injection induced protection effect for liver.

Therefore the present experiment was conducted to study effect of injected egg embryos with glutamine, methionine, L-carnitine or betaine on hatchability, growth performance, and physiological state of Fayoumi chicken.

MATERIALS AND METHODS

At 18th day of incubation 360 eggs contain live embryos and produced from Fayoumi chickens flock at 40 weeks age were chosen. Eggs distributed randomly into sex treatment groups where each group contains 60 eggs distributed into 3 replicates with 20 eggs per each.

Injection and hatching procedures:

The mixtures were prepared by dissolving experimental substances in commercial Marek's solution according to **(**Zhai et al., 2008) immediately before injection. Each egg within each treatment injected with 0.2 mL according to (Madej et al., 2015) except eggs in the 1st group remain without injection and conformed control treatment. The 2nd group injected with 0.2 mL pure vaccine diluents and performed sham treatment. Each egg in group 3, 4, 5, and 6 injected with0.2 mL vaccine diluents contain 5 mg L-glutamine (Gln), 5 mg DL-methionine (Met), 16 mg L-carnitine (L-c) and 5 mg betaine (Bet) respectively. After injection each replicate put in plastic mesh net then transfer to chick master hatchery. After hatching chicks per each replicate counted and hatchability percent calculated then weighed and hatch weight

were recorded. Chicks within each replicate per each treatment were wing-banded and vaccinated against IB and Marek's diseases then transfer to brooding house.

Growth performance:

Chicks in each replicate brooded separately under the same conditions. Starter and grower diets were formulated to satisfy strain requirement (Table, 1) during starter (1day – 4weeks of age) and grower periods (4weeks- 8weeks of age).Chicks consumed their diets according to strain guidelines where strain requirements were 735 gm for starter, 1050gm for grower and 1785 gm, for overall periods. Chicks weighed individually at 4 and 8 weeks of age. Body weight recorded and body weight gain calculated from subtraction final weight minus primary weight of each period. Feed conversion calculated where feed requirements divided by body weight gain. All chicks challenged at day 6 with Hitchner B_1, at 11 days of age with inactivated oil vaccine and at 15, 25, 35 and 50 days of age with LaSota strain against Newcastle disease virus (NDV).

Physiological measurements:

Three birds from each treatment chosen and slaughtered at 8 week of age. One blood sample from each bird collected in test tube without anticoagulant and sent to Reference Laboratory for Veterinary Quality Control on Poultry Production, Egypt to estimate antibody titers against Newcastle disease. Manual of Diagnostic Tests Vaccines for Terrestrial Animals (OIE, 2012) were used. Another one blood sample from each bird collected in heparinized test tube to estimate erythrocyte count, packed cell volume (PCV) and hemoglobin concentration (Hb) according to Clark et al. (2009).

After bleeding and carcass evisceration one sample from liver was taken from each bird and put in 10% neutral buffer formalin (PH 7.0) to study histopathology state. On completion of the experimental period, animals were euthenized by cervical dislocation. Tissue specimens from liver were taken from all groups and preserved in 10% neutral buffer formalin (PH 7.0). The specimens were processed by convention method and cutting at 4.5µm was performed to obtain paraffin sections stained by H&E for histopathological examinations (Bancroft and Gamble, 2013).

Statistical analysis:

Statistical compare means (One-Way ANOVA) of SPSS (2007) were used to test the significant. Mean of variables that showed significant differences were compared at F-test ($P \leq 0.05$) using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSIONS

Hatchability performance:

In ovo Met injection caused significant decrease ($P \leq 0.05$) in hatchability percent compared with control and Bet injection where other treatments differ insignificantly (Table, 2). In contrast in ovo Met injection recorded significantly ($P \leq 0.05$) the highest hatch weight followed by Bet. On the other hand L-c did not provide any improvement in hatch weight compared with control treatments. The best value of hatchability percent obtained when eggs injected with Bet. There was no significant difference in hatchability percent between control and sham treatment.

Decreasing hatchability percent and increasing hatch weight with regard to Met injection agree with (Coşkun et al., 2014).Decreasing hatchability percent may be due to extra Met addition may be

toxic for poultry (Zeng et al., 2015) when Met metabolite to homocysteine (Xie et al., 2007). Moreover, manufactured not naturally Met that spared from building tissues converted to toxic components as methyl-propionate (Kalbandeet al., 2009). Increasing hatch weight may be due to part of Met used for building tissue (Coşkun et al., 2014).

Improvement in hatchability percent and hatch weight with Bet injection compared with control agree with (Gholami et al., 2015) who reported that Bet in ovo injection improved hatching weight and hatchability percent. This improvement may be due to Bet would not adversely affect hatchability (Kadam et al., 2013) and act as a methyl donor thus it prevent Met that present naturally in egg from degradation to harmful substances and spare Met for growth (Zhan et al.,2006). Youssef et al., (2016) confirm this supposition where they injected eggs using the same Met level with Bet they obtained higher hatchability percent than control group.

Regarding in ovo injection procedure the insignificant difference between control and sham treatment in hatchability percent show that the procedure may be conduct in correct manner with regard to injection time (18 day), using diluents solution (Marek's solution) and injection pattern.

Growth performance:

Concerning body weight the results show that treatment that received Bet in ovo injection recorded the highest values during starter, grower and overall periods (Table, 3). Body weight values during different periods for Bet in ovo injection were significantly higher ($P \leq 0.05$) than sham treatment. In contrast L-c in ovo injection recorded the lowest values during starter, grower and overall periods that significantly decreased ($P \leq 0.05$) than Bet and Gln in ovo injection. Body weight gain during starter and overall periods get the same trend of body weight but didn't record significant differences during grower period. In general all growth performance showed no significant differences between control and sham treatment.

Betaine results agree with (Hu et al., 2015) who reported that Bet injection improved body weight, moreover Hu et al.(2017)reported that improvement in body weight was significantly higher ($P < 0.05$) for Bet injection. This enhancement in post hatch body weight may be due Bet in ovo injection advanced body function of chicken embryo (Gholami et al., 2015). Moreover Bet has ability to prevent dehydration (Maddahian et al., 2017) and maintain water retention through preserving osmolytic protective activity (Saeed et al., 2017). Furthermore it includes in methylation reactions and prevents Met from methylation to form harmful metabolizable products (Hu et al., 2015).Another Bet function was converting homocysteine to Met (Hu et al., 2015) so prevent detoxification of homocysteine accumulation(Saeed et al., 2017).

Regarding effect of in ovo L-c injection on post hatch responses, the results showed that growth performance recorded the lowest values for this treatment. The results of hatching measurements illustrated that in ovo L-c injection reduced hatch weight so this may be effect post hatch growth performance.

Feed conversion was significantly affected by in ovo injection where, Bet injection recorded the best values (Table, 3). The results agree with Hu et al., (2015) who reported that Bet injection improved feed conversion. This may be due to Bet

possess a positive effect on nutrient digestibility (Saeed et al., 2017).

Regarding L-c in ovo injection, generally the undesirable results of hatching parameters and growth performance may be due to L-c needed during early stages of embryonic development. L-c may be play very important role in fat metabolism during the first stage where it implicated in transporting fatty acids through mitochondrial membrane. We injected L-c at 18 day of age after consuming yolk sac fat, hence it may be undesirable during this late period especially for Fayoumi that possesses small yolk amount in their eggs compared with egg yolk produced from broiler breeder hens.

Physiological and histological states

Hematological measurements:

The results of sham treatment (Table, 4) illustrated that there were no harmful effect of in ovo injection procedure and solution on hematological parameters. In ovo Bet injection significantly increased ($P \leq 0.05$) erythrocytes number and hematocrit compared with other treatments. The results in agree with Youssef et al., (2016) who reported that in ovo injection of Bet significantly increased erythrocyte count moreover, Park and Kim,(2017)reported that Bet supplementation led to significant improvement in hematological indicators. This may be due to Bet participate in hematocrit maintenance and increasing erythrocyte number (Gudev et al., 2011).

Regarding improvement of some hematological parameters by in ovo Met injection compared with control group. The result agree with Adeniran et al., (2017) who reported that Met confirm higher hematological parameters such erythrocyte count, hematocrit and packed cell volume, hemoglobin concentration.

Immunity:

There were no significant effects of in ovo nutrient injection on anti body titer response against Newcastle disease virus vaccination. Numerical Met and Bet improvement may be due to Met was necessary for response of some antibody components and required T helper cell function (Tsiagbe et al.,1987) and Bet has positive effects on immune responses (Maddahian et al., 2017).

Histopathology:

There were no histopathological alterations detected in the liver sections of the control, sham treatment and Bet injection, where normal hepatic structure without any pathological alterations was observed (Fig, 1A). Vacuolar degenerative change in the hepatocytes associated with congestion in central vein was recorded in Gln injection (Fig, 1B). Microscopical examination of liver tissue of Met injection revealed minimum tissue reactions in the form of focal area of hepatocellular necrosis with mononuclear inflammatory cells infiltrations (Fig, 1C). Individual hepatocellular necrosis and apoptosis observed in liver sections of L-c injection (Fig, 1D).

Histopathology results confirmed the previous results of hatching parameters and growth performance. Harmless effects of Bet on liver may be due to Bet in ovo injection protects chickens from fatty liver via epigenetic modifications (Hu et al., 2017) and modulate lipid metabolism thus prevents liver fat accumulation (Deminice et al., 2015). Moreover Bet may be beneficial factor for liver fibrosis decline (Tsai et al., 2015).

CONCLUSION

In conclusion it is beneficial to injected egg with Bet at 18 day of incubation where it improved hatchability, growth performance and physiological states. Undesirable results of Met and L-c may be due to their injection during last stage of incubation may be inappropriate time for metabolism so more experiments needed in this respect.

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Table (1): Composition and calculated analysis of starter and grower diets.

Ingredients	**Starter 1day-4wk**	**Grower 5-8 wk**
Yellow corn	62.70	64.10
Soybean meal (44% CP)	20.80	9.40
Corn gluten (60% CP)	8.20	6.43
Wheat bran	3.80	16.57
Di-calcium phosphate	2.10	1.34
Lime stone	1.68	1.44
Salt	0.39	0.39
Premix*	0.30	0.30
DL- methionine	0.03	0.03
Total	100	100
Calculated analysis		
CP	20.00	16.04
ME.	2918.73	2811.58
Ca	1.00	0.91
Av.P	0.45	0.38
Lys.	1.00	0.82
Met.	0.38	0.31
TSAA	0.76	0.63
Na	0.17	0.17

*Supplied per kg of diet: Vit. A, 10000 IU; Vit. D3, 2000 IU; Vit. E, 10 mg; Vit K3, 1 mg; Vit. B1, 1 mg; Vit. B2, 5 mg; B6, 1.5 mg; B12, 10 mcg; Nicotinic acid 30 mg; Folic acid 1mg; Pantothenic acid 10 mg and Biotine 50 mcg; Choline 250 mg; Copper 4 mg; Iron 30 mg; Manganese 60 mg; Zinc 50 mg; Iodine 0.3 mg ; Cobalt 0.1 mg and Selenium 0.1 mg.

Table (2): Effect of in ovo Met, Gln, L-c and Bet injection on hatching performance.

Items / Treatments	Hatchability percent	Hatch weight
Control	88.33[a]	28.89[cd]
Sham	85.00[ab]	28.72[d]
Gln (5 mg/egg)	86.67[ab]	29.13[c]
Met (5 mg/egg)	78.33[b]	30.33[a]
L-c (16 mg/egg)	85.00[ab]	28.72[d]
Bet (5 mg/egg)	93.33[a]	29.62[b]
SEM±	1.49	0.14
Probability	0.041	0.001

[a–c] Means within the same column with different superscripts are significantly differ ($P \leq 0.05$)
Gln:L-glutamine Met: DL-methionine L-c: L-carnitine Bet: betaine

Table (3): Effect of in ovo Met, Gln, L-c and Bet injection on growth performance of Fayoumi chicken.

Items / Treatments	Body weight		Body weight gain			Feed conversion		
	Starter period 4 wks	Grower period 8 wks	1day-4wks	5wks-8wks	1day-8wks	1day-4wks	5 wks - 8wks	1day-8wks
Control	235.00^{ab}	474.00^{bc}	206.11^{ab}	239.00	445.11^{bc}	3.82^{bc}	5.66^{a}	4.32^{ab}
Sham	221.63^{bc}	475.00^{bc}	192.91^{bc}	253.38	446.28^{bc}	4.19^{ab}	5.82^{a}	4.64^{a}
Gln (5 mg/egg)	251.34^{a}	505.47^{ab}	222.17^{a}	254.13	476.30^{ab}	3.49^{c}	4.52^{ab}	3.88^{b}
Met (5 mg/egg)	240.46^{ab}	493.86^{abc}	210.14^{ab}	253.40	463.54^{abc}	3.68^{bc}	4.45^{ab}	3.99^{ab}
L-c (16 mg/egg)	204.00^{c}	442.41^{c}	175.28^{c}	238.41	413.70^{c}	4.51^{a}	4.88^{ab}	4.63^{a}
Bet (5 mg/egg)	258.61^{a}	537.73^{a}	228.99^{a}	279.12	508.11^{a}	3.54^{c}	3.95^{b}	3.66^{b}
SEM±	4.01	7.97	4.01	5.76	7.96	0.09	0.20	0.09
Probability	0.001	0.016	0.001	NS	0.018	0.005	0.046	0.008

$^{a–c}$ Means within the same column with different superscripts are significantly differ ($P \leq 0.05$)
NS: not significant
Gln: L-glutamine Met: DL-methionine L-c: L-carnitine Bet: betaine

Table (4): Effect of in ovo Met, Gln, L-c and Bet injection on hematological parameters and antibody titer against NDV at 8 wks of Fayoumi chicken.

Items / Treatments	RBCs ($X10^6$/mm)	PCV (%)	Hb (g/dl)	Antibody titer against NDV
Control	1.22^{c}	33.00^{b}	7.89	7.00
Sham	2.13^{b}	35.50^{b}	7.36	7.00
Gln (5 mg/egg)	2.38^{b}	35.33^{b}	7.89	8.17
Met (5 mg/egg)	2.46^{b}	35.67^{b}	7.99	8.33
L-c (16 mg/egg)	2.59^{b}	33.50^{b}	7.58	8.33
Bet (5 mg/egg)	3.25^{a}	40.50^{a}	9.18	8.33
SEM±	0.16	0.79	0.21	0.22
Probability	0.001	0.042	NS	NS

$^{a-c}$ Means within the same column with different superscripts are significantly differ ($P \leq 0.05$). NS: not significant

Gln: L-glutamine Met: DL-methionine L-c: L-carnitine Bet: betaine

RBCs: Red blood cell count, Hb: hemoglobin concentration, PCV: packed cell volume %. NDV: Newcastle disease virus

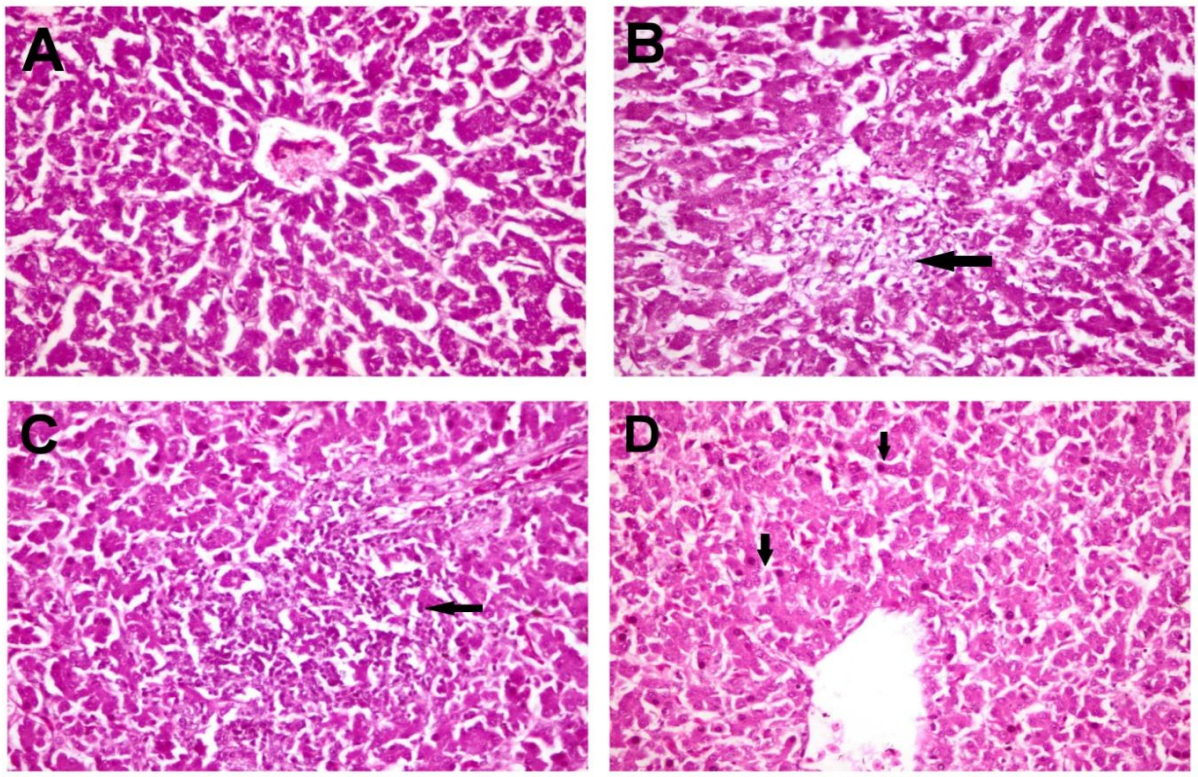

Figure (1): Effect of in ovo DL-metionine, L-glutamine, L-carnitine and betaine injection on liver histopathological state.
A: Control, sham and betaine treatments showing normal histological structures (H&E X 20).
B: L-Glutamine treatment showing hepatocellular vacuolar degenerations.
C: DL-Methionine treatment showing focal area of hepatocellular necrosis (arrow) infiltrated with mononuclear inflammatory cells (H&E X 20).
D: L- Carnitine treatment showing mild individual hepatocellular necrosis and apoptosis (arrow) (H&E X 40).

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الملخص العربي

تأثيرحقن بيض التفريخ بالمثيونين، الجلوتامين ، كارنيتين أو البيتايين على أداء التفريخ، النمو والحالة الفسيولوجية.

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أجريت هذه الدراسة لتقدير استجابات حقن بيض تفريخ الدجاج الفيومي أثناء وبعد التفريخ لبعض المغذيات. في اليوم الـ 18 من التفريخ تم اختيار 360 بيضة تفريخ تحتوي على جنين حي وزعت عشوائياً لـ 6 مجاميع معاملة، تحتوي كل واحدة منها على 60 بيضة مقسمة على 3 مكررات يحتوي كل مكرر على 20 بيضة. تم استخدام بيض المجموعة الاولى كمعاملة مقارنة بدون حقن في حين تم حقن البيض في المجموعة الثانية بمحلول بدون مغذيات. وتم اعتبارها مجموعة حقن زائفة. تم حقن البيض في المجموعات 3و 4 و5 و 6 بمحلول (0.2مم^3) يحتوي على 5مجم جلوتامين/بيضة ، 5مجم مثيونين/بيضة ، 16مجم لـكارنيتين/بيضة ، 5مجم بيتايين/بيضة على الترتيب. . تم تقدير بعض مقاييس التفريخ والنمو والدم بالاضافة لقياس الاستجابة المناعية للتحصين ضد مرض النيوكاسل و هستوبثولوجي الكبد.

هذا وقد تم الحصول على النتائج التالية:-

1- لم تسجل فروق معنوية بين مجموعة المقارنة والمجموعة الزائفة في معظم القياسات، مما يوضح نجاح عملية حقن البيض فيما يخص أسلوب ووقت اجرائها.
2- أدى حقن البيض بالجلوتامين لخفض نسبة التفريخ مع زيادة وزن الكتكوت المفرخ مقارنة بمجموعة المقارنة. وقد تحسن أداء النمو بعد بحقن الجلوتامين مقارنة بباقي المجموعات فيما عدا مجموعة حقن البيتايين.
3- أدى حقن البيض بالمثيونين لخفض نسبة التفريخ وزيادة معنوية على مستوى 5٪ في الوزن عند التفرخ. ولم يحسن حقن البيض بالمثيونين من الأداء في مقاييس النمو.
4- أدى حقن البيض با لـكارنيتين الى خفض نسبة التفريخ ووزن التفريخ مقارنة بمجموعة المقارنة وأعطى أسوء أداء في مقاييس النمو.
5- حسن حقن البيض بالبيتايين وزن التفريخ ونسبة التفريخ. كما أعطى أفضل أداء للنمو والمقاييس الفسيولوجية.
6- أوضحت نتائج الهستوبثولوجي حدوث أضرار في الكبد وأكدت نتائج التفريخ والنمو في ما يتعلق بحقن الكارنيتين. وعموماً يمكن التوصية بحقن البيتايين لبيض التفريخ لتحسيناداء التفريخ والنمو والحالة الفسيولوجية.