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Effect of in Ovo Injection of MyoPro Gene on Growth Parameter in Fayoumi Chicken

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ABSTRACT

The current study aimed at evaluation the effects of *in ovo* injection of the myostatin inhibitor, myostatin pro-peptide (MyoPro) gene on growth performance of Fayoumi chicken. A total number of 200 Fayoumi fertilized pure breed eggs were obtained from EL-Tkamoly poultry project - EL-Azab – Fayoum. Eggs were divided into two equal groups. Group 1(control group, n=100) was injected with saline, and group 2 (treated group, n=100) injected with Myo-pro gene. Growth parameters were recorded (body weight, body weight gain, feed consumption per group was measured for calculating feed conversion ratio). The obtained results revealed that injection of myo-pro to Fayoumi eggs resulted in highly significant increase in body weight and weight gain during all experimental period when compared with control group. Feed conversion ratio of Myo-pro injected group was significantly lower than control group. However, food intake showed no significant change between the two groups. From the obtained results, injection of Myo-Pro gene causing marked increase in body weight gain and improvement in feed conversion ratio without change in food intake in fayoumi chicken.

Keywords: Fayoumi chicken, in Ovo-adminstration, MyoPro, body weight.

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1. INTRODUCTION

Recently, animal meat is the main source of protein in Egypt. Chickens are source of cheap and high quality animal proteins. Different local and foreign chicken breeds are reared in farms (on large scale) and at homes (small scale) all over the country. The only two pure native breeds are Fayoumi and Dandarawi. Fayoumi is the most important Egyptian native chicken breed which recently spreads to many countries worldwide EL-Hossari (1996). Pure Fayoumi is known for wildness in that it is free scavenging flighty, aggressive, escape artist, quick feathering and precocious. This native breed has the carcass characteristics and flavor desired by Egyptian consumers and adapted to adverse environmental conditions including many infectious diseases as compared to foreign strains (broilers) (Tixier-Boichard 2009). However, because their growth rate and meat production are very poor when compared to broilers, breeding of local chickens is rare in farms and so their meat production is insufficient for our needs.

With advent of molecular genetics, it becomes possible to significantly improve the meat production of animals through using of biotechnological approach to transfer valuable

genes or to block myogenesis inhibitor to produce chickens with high production (superior). Myostatin, a member of the transforming growth factor ß (TGF-ß) superfamily, is a negative regulator of skeletal muscle growth in mammals, and loss or inhibition of myostatin signaling dramatically increases muscle mass (McPherron et al., 1997).

The myostatin pro-peptide (MyoPro) is known to bind and inhibit myostatin both in vitro and in vivo. In two independent studies, (Yang et al. 2001) and (Lee and McPherron 2001) created transgenic mice that overexpressed the MyoPro. This resulted in an increase of body weight (17-30%) and muscle mass (22-44%) due to increase in muscle fibre diameter and number. The dramatic increase of muscularity displayed in the transgenic mice over-expressing MyoPro suggests that direct administration of recombinant MyoPro to animals may also promote muscle growth. Indeed, injection of recombinant MyoPro into neonates or adult mice resulted in significant weight increase of individual muscles within short time (Wolfman et al., 2003).

In advanced countries, gene transfer succeeded to increase meat production and genetic improvement of chickens. However, in Egypt we still use the traditional methods (growth promoters and phenotype selection) mainly due to lack of facilities and experiences. These traditional methods failed to solve the problem of meet shortage and also failed to genetically improve our valuable local strains of chickens.

The current study aimed at evaluation the effects of *in ovo* injection of the myostatin inhibitor, myostatin pro-pepetide (MyoPro) gene on growth performance of Fayoumi chicken

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II. MATERIALS AND METHODS

II-1.Experimental design

A total number of 200 of pure breed chicken fertilized eggs of the native Egyptian breed, Fayoumi_obtained from EL-Tkamoly poultry project - EL-Azab in Fayoum Governorate. eggs divided into two equal groups. Group 1control group injected with saline, and group 2 (treated group) was injected with MyoPro gene which was obtained from Prof. Ketan Patel, Reading University, Reading, UK.

II-2.Preparation of gene and gene injection as described by (Morgan and Fekete, 1996):

Ligation of the MyoPro to shuttle vector (Slax12NCO) to give ClaI- ClaI flanking region then Insertion of ClaI- MyoPro – ClaI by standard subcloning techniques into retro viral vectors derived from SR-A strain of Rous sarcoma virus (RCAS) at the virus' single ClaI site. Inject the MyoPro gene with virus into eggs at the age of 3-5 day after incubation. The needle was inserted in the PSM under the labelled ectodermal layer. If the tip of the needle was in the sub-embryonic space, the inoculum diffused rapidly. The needle tip was slowly withdrawn until it was in the presomatic mesoderm (PSM). At this point, the flow from the needle became slow. While the needle tip was slowly withdrawn, about 0.1µl viral stock was slowly injected. To assure the distribution of RCAS-MyoPro throught all precursor of skeletal muscles. The injection was done in the developing chick embryo at the region will form the muscle, which called somites, using a very small needle fitted on a micromanipulator (micropipettes that an outer diameter of less than 10-15 microns about 0.1µl stock was slowly injected)

II-3 Birds and housing

Birds were allowed *ad libitum* access to food and fresh water. A commercial balanced broiler starter ration (from Elkhawas company) containing 22 % crude protein and metabolizable energy of about 3000 (K. cal/kg) was used for feeding of the young birds. While growing chicks (from 4 weeks of age) were fed diet containing 20 % crude protein and 3015 kcal/Kg metabolizable energy, Ca 2.25 % and Available Phosphorus 0.44 %. As shown in Table 1.

II-4.Growth parameters

The chicks were weighed individually at the start of experiment, then every week for recording the live body weights(W0-W6) then every 2 weeks from W6-W12, food intake was measured every 3 weeks during W0-W6 then every 2 weeks from (W6-W12), and body weight gains (differences between each two successive weights).

The feed conversion rate was calculated by dividing the food intake on average body gain in body weight.

II-5. Statistical analysis

Results are expressed as mean ± standard error (SE). Differences between means in different groups were tested for significance using a t test, using the statistical package for social science (SPSS) and *P* value of 0.05 or less was considered significant.

III.Results

Collectively, all transgenic (myostatin$^{-/-}$) chicks at different time points (from hatching to 12 w) had significant ($P<0.05$) higher body weight (Table 2, 3). and bogy weight gain than the control group (Table 4, 5).

Food intake of the injected group (myostatin$^{-/-}$) did not significantly change in the amount was noticed and wild type chicken (Table 6) so, The feed conversion ratio was significantly decreased ($P<0.05$) in Myostatin$^{-/-}$ as compared to control chicken (Table 7).

Table 1: Composition of the ration was as the following:

	Ration	
Ingredients	*Starter*	*Growing*
Maize (%)	54	62
Soya bean meal, 44 % CP (%)	33	22
Concentrate (%)	10	10
Wheat bran (%)	3	-
Limestone (%)	-	5.7
Sodium chloride (%)	-	0.3
Calculated nutrient content		
Metabolizable energy (kcal/Kg)	3000	3015
Crude protein (%)	22	20
Methionine (%)	0.46	0.648
Cysteine (%)	0.325	0.257
Methionine + Cysteine (%)	0.211	0.211
Crude fiber (%)	3.60	3.41
Crude fat (%)	6.40	5.91
Linoleic acid (%)	1.37	1.45
Calcium (%)	0.84	2.25
Available phosphorus (%)	0.49	0.44

Concentrates providing the following per kilogram of diet: crude protein 520g; vitamin A 120000 IU; vitamin E 100 mg; vitamin K_3 21 mg; vitamin $B_1$10 mg; vitamin B_2 40 mg; vitamin B_6 15 mg; pantothenic acid 100 mg; vitamin B_{12} 0.1 mg; Fe 0.3 mg; Mn 600 mg; Cu 50 mg; Co 2 mg; Se1 mg and Zn 450 mg.

Table2: Effect of in ovo injection of MyoPro on live body weight (g) of male Fayoumi chicken (means ± standard errors).

Group Weeks	Control Males	Myostatin$^{-/-}$ males
W0	21.25^{b} ±0.95	40.5^{a} ±1.25
W1	66^{b} ±0.57	140.33^{a} ±1.88
W2	116.6^{b} ±0.87	244.33^{a}± 2.33
W3	173.81^{b} ±1.74	345.65^{a} ±15.5
W4	226.42^{b} ±0.86	474.33^{a} ± 2.33
W5	286^{b} ±0.57	604.33^{a} ±2.33
W6	376.25^{b} ±1.58	744.5^{a} ±17.3
W8	551.5^{b} ±1.74	1005.4^{a} ±15.8
W10	664. 9^{b} ±1.42	1263.8^{a} ±19.1
W12	889.4^{b} ±2.4	1650.7^{a} ±34.2

Means with different letters at the same row differ significantly at ($P<0.05$).

Table3: Effect of in ovo injection of MyoPro on live body weight (g) of female Fayoumi chicken (means ± standard errors).

Group	Control females	Myostatin$^{-/-}$ Females
weeks		
W0	20.72^{b} ±1.05	38.5^{a} ±1.15
W1	60.56^{b} ±0.86	115.62^{a} ±1.45
W2	103.33^{b} ±1.76	195.33^{a} ± 1.45
W3	144.7^{b} ±11.15	292.84^{a} ±12.36
W4	203.76^{b} ±2.33	422.33^{a} ± 1.45
W5	263.67^{b} ± 2.33	557.33^{a} ± 1.67
W6	320.6^{b} ±13.2	682.4^{a} ±15.6
W8	500.7^{b} ±11.2	928.5^{a} ±12.3
W10	608. 2^{b} ±14.8	1179.3^{a} ±16.3
W12	714.5^{b} ±29.8	1425.8^{a} ±31.5

Means with different letters at the same row differ significantly at ($P<0.05$).

Table 4 : Effect of Myostatin Inhibition On Body Weight gain (g) of male Fayoumi chicken (means ± standard errors)

Group		
Weeks	Control males	Myostatin$^{-/-}$ males
Week 1	44.58^{b}±0.51	99.83 ^{a} ±1.09
Week 2	50.6 ^{b} ±0.30	104 ^{a} ±1.52^{a}
Week3	55 ^{b} ±0.1.9	103.55 ^{a} ±1.7
W0-W3	44.58 ^{b} ±0.51	152.56 ^{a}±5.1
Week 4	54.8 ^{b} 1±1.82	126.45 ^{a} ±3.2
Week 5	59.58 ^{b} ±0.29	130 ^{a} ±2.88
Week 6	81.03 ^{b} ±0.65	140.83 ^{a}±1.9
W3-W 6	202.44 ^{b} ±6.2	398.85^{a}±11.2
Week 8	175.25 ^{b} ±5.8	260.9 ^{a} ±8.5
Week 10	113.4 ^{b} ±4.9	258.4 ^{a} ±9.0
Week 12	224.5 ^{b} ±8.2	386.9 ^{a} ±12.4

Means with different letters at the same row differ significantly at ($P<0.05$).

Table 5 : Effect of Myostatin Inhibition On Body Weight gain (g) of female Fayoumi chicken (means ± standard errors)

Group	Femles	
Weeks	Control females	Myostatin$^{-/-}$ Females
Week 1	39.99 ^{b} ±1.08	76.5 ^{a} ±1.36
Week 2	42.76 ^{b} ±0.906	80.5 ^{a} ±1.36
Week3	41.9 ^{b} ±1.4	97.5 ^{a} ±1.36
W0-W3	123.98 ^{b} ±5.3	254.44 ^{a} ±8.7
Week 4	58.43 ^{b} ±2.5	130.72 ^{a} ±2.29
Week 5	60 ^{b} ±4.04	135 ^{a} ±2.03
Week 6	56.866 ^{b} ±2.5	125.13 ^{a} ±1.27
W3-W 6	175.9 ^{b} ±6.2	389.56 ^{a} ±10.5
Week 8	180.1 ^{b} ±4.9	246.1 ^{a} ±7.4
Week 10	107.5 ^{b} ±3.6	250.8 ^{a} ±9.1
Week 12	106.3 ^{b} ±7.5	246.5 ^{a} ±11.5

Means with different letters at the same row differ significantly at ($P<0.05$).

Table 6: Effect of Myostatin inhibition on feed intake (g)/ one chick of Fayoumi chicken (means ± standard errors).

Group Weeks	Myostatin$^{-/-}$	Control
W0-W3	298.2±8.2	262.5±7.9
W3-W6	508.2±10.5	449.4±9.4
W6-W8	551.6±11.3	501.0±11.5
W8-W10	655.2±13.5	625.8±12.8
W10-W12	889.0±15.8	858.9±14.3

Table 7 : Effect of Myostatin inhibition on feed conversion ratio of Fayoumi chicken (means ± standard errors).

Group Weeks	Myostatin$^{-/-}$	Control
W0-W3	0.98 ^{b} ±0.05	1.72 ^{a} ±0.17
W3-W6	1.27 ^{b} ±0.09	2.22 ^{a} ±0.29
W6-W8	2.11 ^{b} ±0.18	3.42 ^{a} ±0.32
W8-W10	2.53 ^{b} ±0.24	4.56 ^{a} ±0.51
W10-W12	2.31 ^{b} ±0.20	3.83 ^{a} ±0.45

Means with different letters at the same row differ significantly at ($P<0.05$).

IV. DISCUSSION

The present study showed that in ovo injection of myo-pro gene caused highly significant increase in body weight and body weight gain. Similar results were recorded by Jianag et al., 2017 in mice that AAV-MPRO/FC gene (Adeno-associated virus-mediated expression of myostatin propeptide) transfer could make hyperglycemia and increase body mass. Several studies were done by myostatin inhibitors in cattle. These showed muscle doubling (Kambadur et al., 1997 , McPherron &Lee, 1997, Rodgers &Garikipati, 2008) and showed muscular hyperplasia (Wegner et al., 2000)

There is no previous studies using Myo pro in chicken so the present work considered the first trail in chicken However, Lee et al., (2017) using Cas9 as myostatin inhibitor in chicken tissue The observed marked .culture increase in body weight and body weight gain in Myo-pro injected group may be attributed to myostatin which is the most powerful inhibitor for skeletal muscle development (McPherron et al., 1997) which is expressed in skeletal muscle (throughout life, from the early stages to late adulthood) and to some extent in fat tissue, heart and mammary tissues. Therefore, of this gene inhibition experimentally will cause severe increase in muscle growth (double muscling) in some cattle. To the best of our knowledge this is the first study to demonstrate increase of Fayoumi chicken live body weight and body weight gain after injection of MyoPro gene as a specific inhibitor for myostatin activity .

Increased body weight may be due to increase of weight skeletal muscles collectively called meat) because muscles make up a large proportion of the body weight approximately 40%) (Steven, 1991). Moreover, myostatin inhibition has the stimulatory role on muscle growth and development (McPherron et al., 1997). The latter author observed increase in diameter and number of muscle fiber after inhibition of myostatin. Earlier studies have attributed the increase in body weight to muscle hypertrophy in knockout mice exhibit approximately 11% more muscle fibers with 43% larger fiber cross sectional area (Amthor et al. 2009).

Suppression of myogenesis by preventing proliferation and differentiation of muscle precursors and myoblasts through inhibition of Pax3, MyoD, and MyoG (Amthor et al., 2002, Joulia et al., 2003, Hayashi et al., 2008 and Muroya et al., 2009. Binding regions for muscle growth related transcription factors MyoD were identified in the promoter of myostatin gene (Salerno et al. 2004).

The marked increase in body weight and body weight gain that observed -after myo pro gene injection in this study may be attributed to change in some anabolic hormones such as growth hormone and IGF-1 as reported by Liu et al. (2003) who showed increase in growth hormone level after myostatin inhibition.

The present work showed that myostatin-/- group cause improvement in feed conversion ratio without significant change in food intake.

There is no pervious study on the effect of myo-pro gene injection on food intake and feed conversion ratio. The marked improvement in feed conversion ratio may be attributed to increase in anabolic hormonal changes .

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V. CONCLUSION

Injection of Myo-Pro gene causing marked increase in body weight and body weight gain with improvement in feed conversion ratio without change in feed intake of Fayoumi chicken. .

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