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The Action Of Myo-Pro gene On The Morphology Of Skeletal Muscle Of Fayoumi Chicken

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A B S T R A C T

The aim of the present study was to evaluate the changes in skeletal muscle which may occur after in ovo injection of Myo-Pro gene . A total number of 200 Fayoumi fertilized pure breed eggs were obtained from EL-Tkamoly poultry project - EL-Azab – Fayoum and divided into two equal groups. Control group, n= 100 that was injected with saline, and treated group n = 100 was injected with MyoPro gene. To check both possibilities, we examined the *gastrocnemius* muscle fibers number and diameter (cross sectional area). The results revealed that Chicken hatched from Myo-Pro injected eggs showed a significant increased muscle masses. This increase in muscle mass and subsequently meat production is due to hypertrophy rather than hyperplasia. From the obtained results, using Myo-Pro gene causing improvement in skeletal muscle fiber (diameter and number) in Fayoumi chicken.

Keywords: Fayoumi chicken, eggs, Myo-Pro, Skeletal muscle.

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1. INTRODUCTION

During the postnatal development of skeletal muscle, increase in skeletal muscle mass are mainly due to increase in muscle fibre size. This is called hypertrophy, which is opposed to hyperplasia that occurs prenatally. Although the number of muscle fibres largely determines the amount of postnatal muscle growth, the total count of muscle fibres typically remains the same in postnatal muscle growth (Luff and Goldspink 1970, Wegner et al. 2000). Some pervious results suggested that there might be an increase in fiber numbers shortly after birth (Rehfeldt et al. 2000) and this is possibly occur a result of maturation and elongation of the existing myotubes during the initial postnatal muscle growth period (Ontell and Kozeka 1984).

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). Myostatin is the most powerful inhibitor for skeletal muscle development 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 McPherron et al.,(1997). Therefore, inhibition of this gene either naturally as in some cattle breeds (Kambadur et al. 1997, McPherron and Lee 1997, Rodgers and Garikipati 2008) or experimentally will cause sever increase in muscle growth (double-muscling).

The myostatin propeptide (Myo-Pro) 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 Myo-Pro. 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 Myo-Pro suggests that direct administration of recombinant Myo-Pro to animals may also promote muscle growth.
The aim of this study was to use a novel biotechnological approach to increase meat production of local Egyptian chicken breed, Fayoumi, through injection of myostatin inhibitor (MyoPro).

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. The eggs was divided into two equal groups. Group 1(control 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 r 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 through 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)

-3.Birds and housing

Birds were allowed *ad libitum* access to feed 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 showen in table 1

For histological study, samples from gastrocneumis muscle of Fayoumi chickens were fixed overnight in 10% Neutral buffered formalin, dehydrated through a graded series of ethanol, and cleared with xylene. Samples were then embedded in paraffin according to standard procedures and serially sectioned with a microtome at a thickness of 5 µm. Sections were dewaxed in xylene, rehydrated, then stained by Harris's haematoxylin and eosin (H&E) for general histological structure and Masson Trichrome for identification of muscle fibers. Samples were then washed under running water, dehydrated in graded ethanols and cover slipped. Image J software was used as the cross section area of 100

fibers from each muscle was measured directly from electron micrograph. All of the fibers within each randomly selected fasciculus were measured and the mean myofibre cross sectional area and the mean standard error was calculated for each muscle provided that using fixed magnification power and surface area for all examined slides.

II-4.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 analysis system, SPSS and *P* value of 0.05 or less was considered significant.

III- Results

Chicken hatched from Myo-Pro injected eggs showed a significant increased *gastrocnemius* muscle masses as revealed by a significant increase in muscle weight (g), total fiber number, and cross sectional area of the muscle fibers as compared to the control chicken (Table 2 and Fig. 1).

The histological examination of the stained slides showed significant hypertrophy of the muscle fibers in both sex of transgenic chickens lack Myostatin (Myostatin$^{-/-}$) as compared to control chickens. The cytoplasm of muscle fibers in Myostatin$^{-/-}$ chicken contained inclusion structure seem to be aggregation of sarcoplasmic reticulum (Fig.2, black arrows).

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

Ingredients	***Ration***	
	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

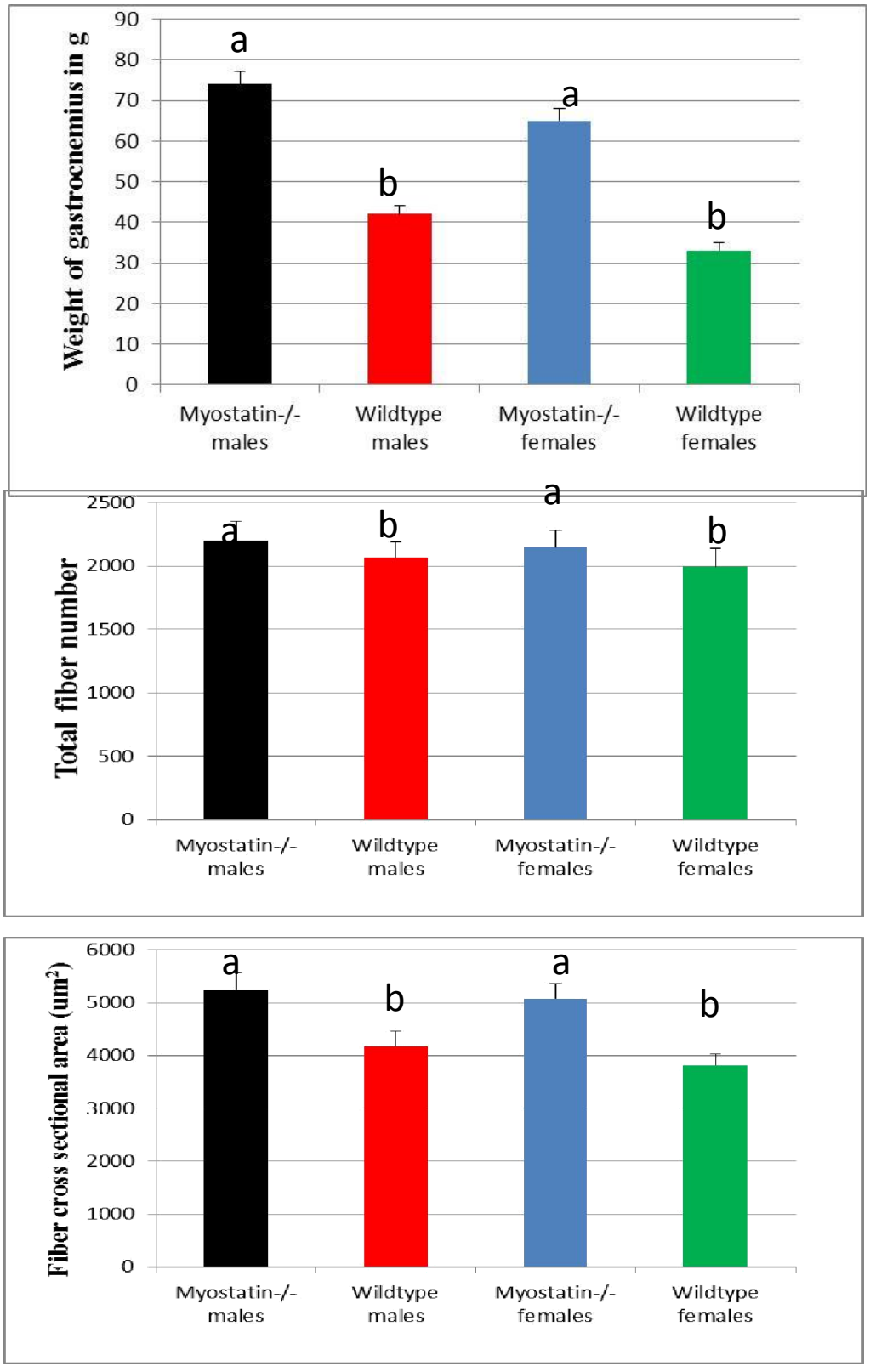
Concentrates providing the following per kilogram of diet: crude protein 520g; vitamin A 120000 IU; vitamin E 100 mg; vitamin K_3 21 mg; vitamin $B_1$10 mg; vitamin B_2 40 mg; vitamin B_6 15 mg; pantothenic acid 100 mg; vitamin B_{12} 0.1 mg; Fe 0.3 mg; Mn 600 mg; Cu 50 mg; Co 2 mg; Se1 mg and Zn 450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

Table 2: Effect of Myostatin inhibition (Myo-pro) on muscles mass and fiber diameters.

Groups	Myostatin-/- males	Control Males	Myostatin-/- females	Control Females
Muscle parameter				
Gastrocnems muscle weight (g)	74 [a] ±3.25	42 [b] ±2.15	65 [a] ±3.05	33 [b] ±1.95
[*]**Total fiber number in gastrocnemius muscle**	2200 [a] ±1.54	2068 [b]±1.24	2149 [a] ±1.31	1992 [b]±1.46
[*]**Fiber cross sectional area (um^2)**	5240 [a] ±3.18	4167 [b]±2.87	5079 [a] ±2.94	3812 [b]±2.28

Different superscript upper case letters in the same row are significantly different at P<0.05.

Fig1. Effect of Myostatin inhibition on muscles mass and fiber diameters.



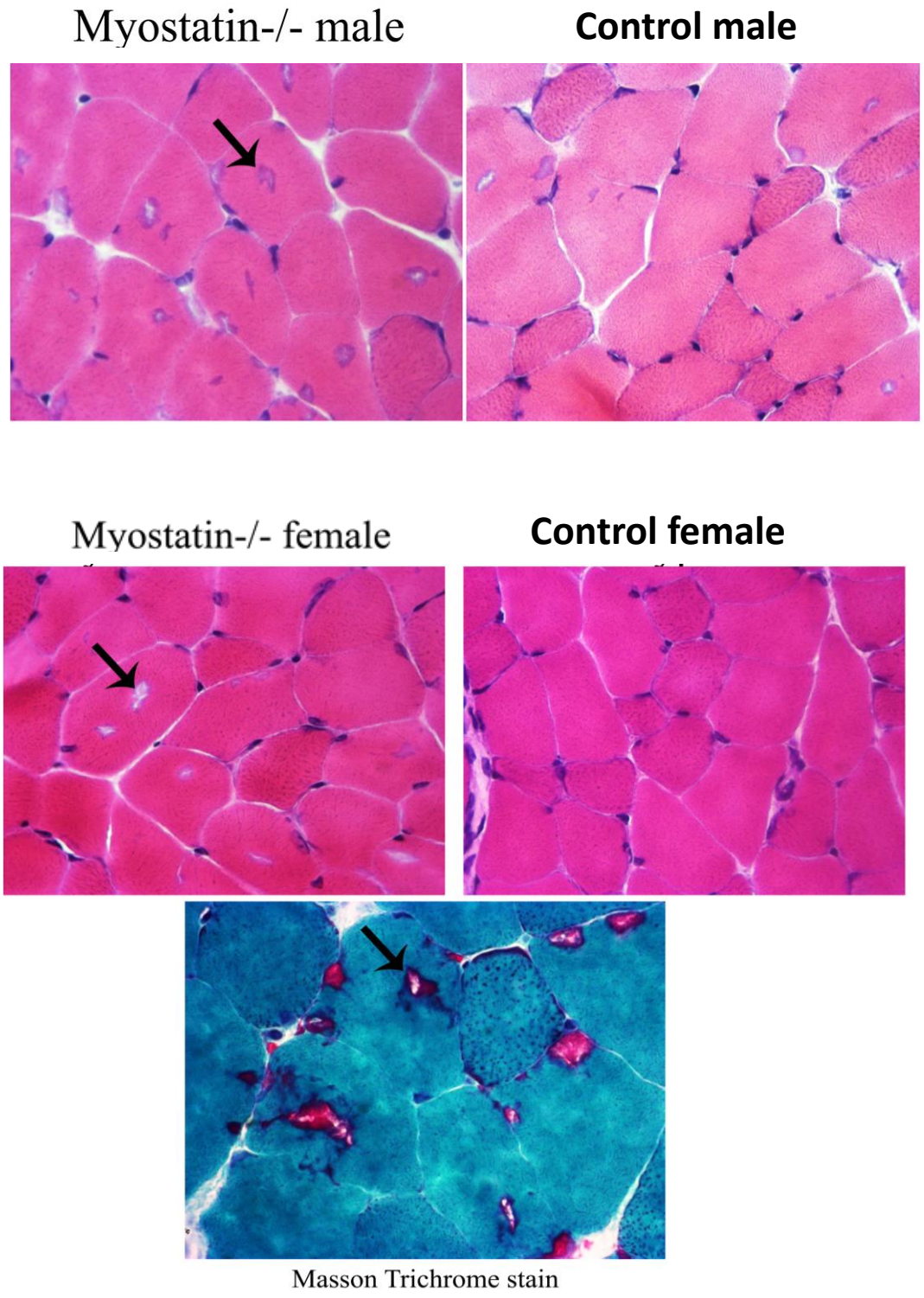


Fig. 2. Histological examination of muscles of male and female Myostatin$^{-/-}$ and control (wild type) chickens show significant hypertrophy of the muscle fibers and cytoplasmic inclusion structure (black arrows) in both sex of Myostatin$^{-/-}$. First four images stained by H&E, while the fifth stained by Masson Trichrome stain. X= 400.

IV. Discussion

Myostatin has a key role in how muscles grow as it has been shown to have an inhibitory effect on muscle hypertrophy and hyperplasia. The Myo-Pro is able to conjugate and suppress myostatin in mice (Yang et al. (2001) , Lee and McPherron, 2001). This result gives high muscle mass (22-44%) due to increased diameter (hypertrophy) and number of muscle fibre (hyperplasia). The increased muscle mass is due primarily to hypertrophy, since knockout mice exhibit approximately 11% more muscle fibers with 43% larger fiber cross sectional area (Amthor et al. 2009). In the present study, injection of Myo-Pro resulted in a significant increased *gastrocnemius* muscle weight as compared to the wild type. To identify whether this increase is due to hypertrophy or hyperplasia, histological examination was performed to measure the total fiber number and cross sectional area of the muscle fibers. Surprisingly, we found significant hypertrophy in the muscle fibers in both sex of transgenic chickens lack Myostatin (Myostatin$^{-/-}$) as compared to control chickens. This means that the increased musculature in myostatin inhibition was mainly due to muscular hypertrophy. Strikingly, we also found inclusion structure seem to be aggregation of sarcoplasmic reticulum in the cytoplasm of muscle fibers in Myostatin$^{-/-}$ chicken.

The exact nature of this aggregation is still unknown but it is worth to be examined carefully using transmission electron microscope to identify their structure.

Some pervious results suggested that there might be an increase in fiber numbers shortly after birth (Rehfeldt et al. 2000) and this is possibly occur a result of maturation and elongation of the existing myotubes during the initial postnatal muscle growth period (Ontell and Kozeka 1984).

The exact mechanism by which myostatin acts to retard muscle growth is not well known. It has been investigated that myostatin suppress 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). In addition, myostatin promoter has a binding region for glucocorticoids, called the glucocorticoid response element region (Joulia-Ekaza and Cabello 2006, Gilson et al., 2007,). This feature explains the negative effects of injecting glucocorticoids since glucocorticoids promote proteolytic pathway initiation in muscles.

V.Conclusion

Injection of Myo-Pro gene causing marked changes in skeletal muscle in Fayoumi chickens

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