International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2017; 2(3): 01-03

www.veterinarypaper.com
Received: 01-03-2017
Accepted: 02-04-2017

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A study on the effect of green microalgae (*Chlorella vulgaris*) on the histology of skeletal muscle fibres in Japanese quails (*Coturnix coturnix japonica*)

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Abstract
Muscle development is influenced by a number of environmental factors and in a range of species. Understanding growth and development of muscles is one of the most important goals in animal production and human medicine. The quail has been used as an animal model for the muscle growth and development because of its relatively rapid generation time, easy access to study embryonic muscle development and conservation of muscle developmental processes in mammals. Muscle growth and ultimate muscle mass are largely determined by both; initial number of muscle fibres and growth, size and length of individual muscle fibres during post-natal period. Thus, both number and size of muscle fibres are co related with growth rate and muscle mass. Muscle fibre formation (myogenesis) occurs only during early embryonic development when it is under the control of the MyoD gene family consisting of myogenin, MyoD1, myf-5 and myf – 6.

Keywords: Muscle fibre, myogenesis, Japanese quail, MyoD, muscle fibre hypertrophy, *Chlorella vulgaris*

1. Introduction
The poultry species have been on earth for nearly 150 million years. Dating back to the original wild jungle fowl, now we include ducks, geese, turkeys, guinea fowls and quails under the general term poultry. The field of poultry has accelerated the standards of human nutrition and food security by being a principal source of high quality protein in the form of eggs and meat. It acts as a key alternative, thus avoiding the need of traditional food sources. It is a good source of foreign exchange which occurs as a result of exporting poultry products to other countries. The major difficulties in poultry production include, production related constraints, lack of knowledge and skills, inadequate capital at all levels and marketing. Japanese quail was introduced in India by Central Avian Research Institute during 1974 and the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) introduced the Japanese quail in Chennai in the year 1983. At present, the Japanese quail constitutes the third largest avian species in number next only to chicken and duck in commercial poultry production in India. Microalgae represent an abundant biological resource acting as alternative feed supplements. The dietary supplementation of *C. vulgaris* in Japanese quails have profound significance in weight gain. The myogenic regulatory factors play a primary role in muscle cell differentiation and determination. The somites give rise to the skeletal muscles and also express the myogenic regulatory genes. Muscle develops from mesoderm, the middle germ layer of the embryo, followed by differentiation in several steps. The present study was aimed to determine as to how the weight gain occurred in quails, whether due to increase in individual muscle fibre thickness or due to increase in the number of muscle fibres.

2. Materials and Methods
The biological experiment was carried out in Japanese quails (*C. japonica*) from day old to sixth week of age to study the effect of dietary supplementation of the green microalgae (*C.vulgaris*) on the productive performance.

2.1 Place of Work
The biological experiment is carried out at the Poultry Research Station, Madhavaram, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) for a period of 12 weeks.

2.2 Experimental Diet
The green microalgae, *C. vulgaris* in dried form used in this study was procured from the National Institute of Ocean Technology (NIOT), Pallikaranai, Chennai. The experimental diet was formulated as per the standards prescribed by the Central Feed Technology Unit, Kattupakkam to the Japanese quails throughout the experiment. The experimental diet was supplemented with 5g, 10g, 20g and 40g of *C. vulgaris* per kg of feed. Control diet had no *C. vulgaris*. The proximate analysis of the microalgae was estimated as per the method of AOAC (1995).

2.3 Experimental Design
The Japanese quail chicks are to be weighed individually and randomnly distributed in five treatments. Each treatment had three replicates of 20 chicks each.

Treatment	Number of Replicates	Number of Japanese Quails		*C. Vulgaris* (G Per Kg Of Feed/ %)
		Per Replicate	Per Treatment	
T_1 (Control)	3	20	60	Nil
T_2	3	20	60	5 / 0.5%
T_3	3	20	60	10 / 1%
T_4	3	20	60	20 / 2%
T_5	3	20	60	40 / 4%

2.4 Management of the Experimental Japanese Quails
The experimental Japanese quails were housed in their respective cages and reared from 0 to sixth week of age. A constant photoperiod along with uniform brooding was maintained using 200 watt incandescent bulbs and lighting provided throughout the day during the starter phase (day 0 to second week). Free access was given to water and feed. Standard medications and management practices were followed for all the experimental groups throughout the study period.

2.5 Histopathological Studies
At the end of day one, three and six weeks of age, two birds (one male and one female) from each replicate (total six quails per treatment) were selected randomly and subjected to humane method of slaughter. Six hours prior to slaughter, feed was removed and the quails had free access to drinking water. Immediately prior to slaughter, the individual weight of the quail was recorded as pre slaughter live weight. Blood samples without anticoagulant were collected for hormonal profile studies. Breast and thigh muscle tissue samples were collected for histopathological studies.

The tissues were fixed in 10 per cent formalin and subjected to 48 hours of fixation. The tissues were trimmed to four microns in thickness and treated with Neutral Buffered Formalin (40 per cent formalin – 100 mL and distilled water – 900 mL) for one hour. Dehydration of tissues were done with ascending grades of alcohol in the following order :

50% Isopropanol – overnight
70% Isopropanol – 2 hours
90% Isopropanol – 2 hours
Absolute Alcohol – 2 hours

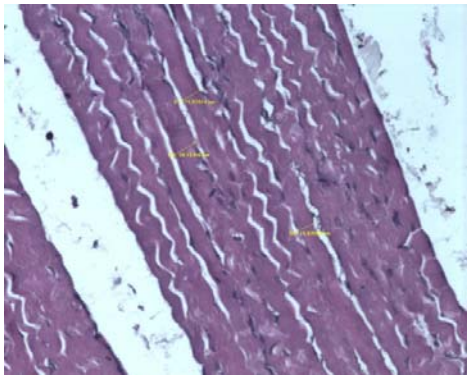
Clearing of tissue sections were done with xylene and absolute alcohol in 1:1 ratio followed by embedding which consisted of three changes in molten paraffin wax inside a hot air oven at 70 °C and then poured into a 'L' shaped block the next morning. These blocks were then trimmed and placed in ice for 10 minutes in order to achieve hardening. Tissue sections of 5µm were cut with rotary microtome and the tissue ribbons were placed in a thermostatically controlled water bath at 50 °C after sectioning, in order to remove the folds.

Forceps, brushes and tearing needles were used for removing folds and air bubbles that may form, while floating out the sections from the water bath. The tissue sections were then placed on clean, grease free slides with egg albumin as an adhesive.

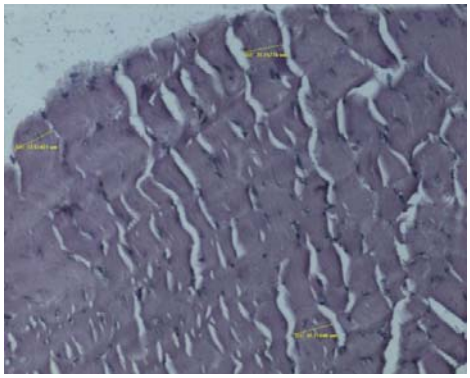
The sections are then stained with haematoxylin which stains the nucleus followed by counter staining with eosin which stains the cytoplasm. The stained slides were then air dried and mounted with distyrene plasticizer xylene (DPX) and observed under the microscope for the increase in the thickness of individual muscle fibre.

3. Results and Discussion
It was found that there was a considerable increase in individual muscle fibre thickness among the birds that were fed with 0.5% of *C. vulgaris* when compared to control and other concentration of algae supplemented diets. The individual muscle fibre thickness was found to be 33.1 µm in the 0.5% algae fed group when compared to that of the control group muscle fibres which was 16.9 µm in thickness during their third week of age. Studies were done in their sixth week of age, when they attain the desirable market size in order to observe the fibre thickness. The fibre thickness in 0.5% algae fed group was 36.3 µm in thickness as that of 20.3 µm in the control. Thus, we conclude that the increase in weight gain due to the dietary supplementation of *C.vulgaris* occurs as a result of increase in the thickness of the muscle fibre and not due to the number of myofibrils.

3.1 Tissue sections taken during three weeks of age

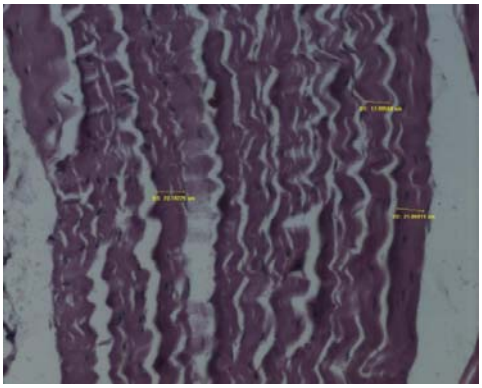


Control

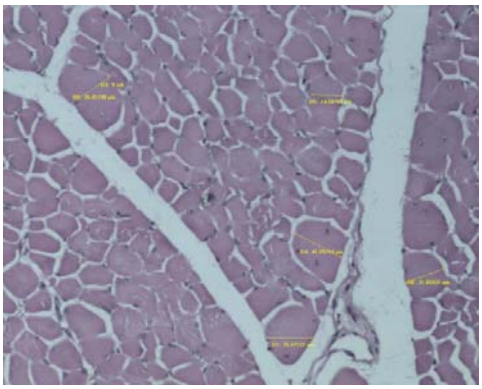


0.5% algae fed group

3.2 Tissue sections taken during six weeks of age



Control



0.5% algae fed group

4. Acknowledgement

I hereby wish to express my sincere thanks to my research supervisor Dr. K. Revathi, Associate Professor and Head, Ethiraj College for Women for her constant support and guidance. My immense gratitude to Dr. M. Babu, Former Director of TANUVAS and his team for all their encouragement.

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