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EFFECTS OF PHOTOPERIOD VALIDATION ON SKELETAL MUSCLE REMODELING AND HORMONAL REGULATION IN MALE JAPANESE QUAIL

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I. INTRODUCTION

The Japanese quail (*Coturnix japonica*) has long been popular as egg-laying. However, the industrial value of the male quail is not fully utilized in Japan due to insufficient research on using their muscles for food. Quails are migratory birds; therefore, their behavior and physiological functions change in response to differences in daylight hours [1]. Our previous study showed that under long daylight conditions (breeding season), weight of their testes increases and that of the pectoralis major muscle decreases [2]. The purpose of this study was to elucidate the mechanism by which muscle weight decreases despite an increase in blood testosterone concentration in male Japanese quails.

II. MATERIALS AND METHODS

Four-week-old male quails (n=10) that had not reached sexual maturity were reared for 4 weeks under a 6/18 h light/dark cycle. After they were 8 weeks old, they were divided into two groups such that the average weight and standard deviation were equal. The same conditions were used for the short-day group (SD). The light-stimulated period was changed to a 20/4 h light/dark cycle for 2 weeks in the long-day group (LD) until the quails were 10 weeks old.

At the end of the study, testes, liver, and skeletal muscles of both groups were collected. RNA extraction reagents (RNeasy Fibrous Tissue Mini Kit: Quiagen) were used to extract total RNA from skeletal muscle samples according to the standard protocol. Library preparation and RNA-sequencing (RNA-seq) were outsourced to KURABO Co., Ltd. The library was prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina), and sequencing was performed using the Illumina NovaSeq platform to generate 150-bp paired-end reads. Gene expression differences between the LD and SD were analyzed using DESeq2, with a significance threshold set at $padj < 0.05$ and log2 fold change ≥ |1.0|. Gene Ontology (GO) and pathway enrichment analyses were performed using the DAVID software to identify biologically relevant processes and pathways that were significantly enriched. A testosterone and corticosterone ELISA Kit (Cayman Chemical) were used to measure hormone levels in the serum, which were quantified spectrophotometrically according to the manufacturer's instructions. Student's t-test was used to detect differences between groups due to differences in light stimulus duration.

Data are expressed as the mean ± standard deviation. A significant difference was defined for P values smaller than 0.05: *, $p<0.05$; ** and $p<0.01$.

III. RESULTS AND DISCUSSION

The weight of testis, liver, and pectoralis major muscle weights between LD (breeding season) and SD (non-breeding season) groups were compared (Table 1). The LD exhibited significantly increased testis weight ($p<0.001$) and elevated liver weight ($p=0.052$), but reduced muscle mass ($p=0.054$). Serum testosterone and corticosterone levels were also higher in LD.

To explore the mechanisms behind muscle mass reduction in LD, RNA-seq analysis was performed (Figure 1). Results showed significant clustering of genes related to the extracellular matrix, chromosomes, chromosomal regions, and chromosome centromeric region, indicating active skeletal muscle remodeling. Furthermore, the ubiquitin-proteasome related genes were notably upregulated in LD. Quantitative PCR analysis revealed that Atrogin-1 and MuRF-1 expressions increased over 15-fold in LD, whereas androgen receptor (AR) expression remained unchanged between groups.

These findings suggest testosterone-induced sexual maturation occurs in LD but is accompanied by testosterone resistance in skeletal muscle. Concurrently, corticosterone-induced catabolism, particularly affecting type II muscle fibers, likely underpins the observed muscle atrophy [3]. This mechanism is consistent with decreased carnosine levels and reduced gene expression, as these components are abundant in type II fibers. Thus, raising male

Japanese quail under SD conditions can prevent muscle atrophy by suppressing sexual maturation and maintaining muscle mass, potentially enabling the production of meat enriched with functional components such as carnosine.

Table 1 – Body weight, liver, each tissue weight, hormone levels and carnosine in LD and SD.

	SD			LD			p value
Body weight (g)	113	±	6.56	108	±	8.43	0.212
Liver (g)	2.46	±	0.335	1.95	±	0.0872	0.0512
Testis (right) (g)	0.118	±	0.12	0.783	±	0.127	< 0.001
Pectoralis M. (right) (g)	9.94	±	0.873	8.4	±	1.37	0.0535
Testosterone (ng/ml)	124	±	71.9	4860	±	3500	0.0733
Corticosterone (pg/ml)	1.41	±	0.189	1.62	±	0.149	0.0814
Carnosine (mM)	6.60	±	1.42	4.30	±	0.998	0.0475

Abbreviation: SD, Short day. LD, Long day. All data are represented as the mean ± SD.

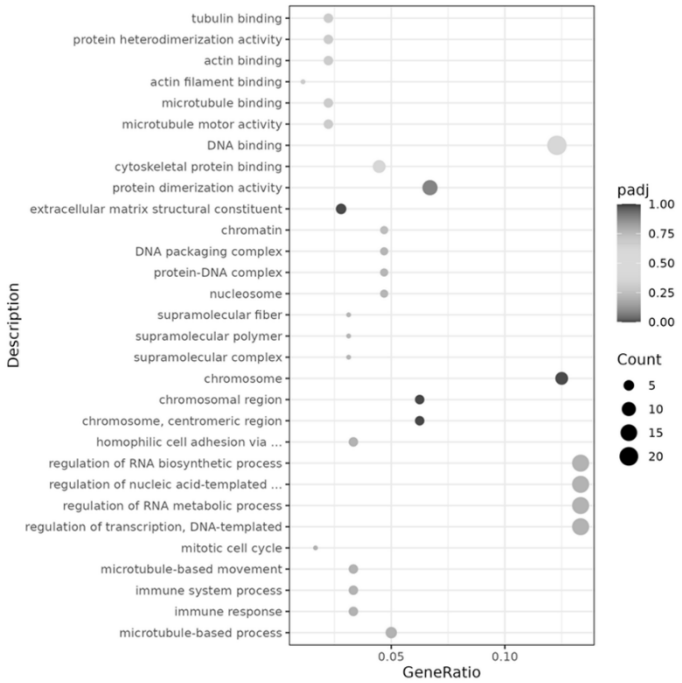


Figure 1. GO Enrichment Analysis Scatter Plot on quail pectoralis M. (SD vs LD)

IV. CONCLUSION

This study examined the effects of photoperiod variation on the pectoral muscles of male Japanese quail (Coturnix japonica), an underutilized resource for meat production. Under long-day (LD) conditions, quail exhibited sexual maturation and reproductive behavior, whereas short-day (SD) conditions suppressed maturation. Despite elevated testosterone levels under LD, pectoral muscle mass decreased. RNA sequencing revealed upregulation of ubiquitin-proteasome system-related genes, suggesting muscle remodeling. Increased corticosterone levels were also observed, indicating muscle catabolism. These results suggest that LD induces muscle breakdown to reallocate energy toward reproduction.

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