We examined atrogin-1/MAFbx mRNA expression in the smooth muscle of gizzards from egg- and meat-type chickens. Gizzard weight relative to body weight was significantly lower in the meat-type chickens than in the egg-type at 14 d of age. In contrast, the level of atrogin-1/MAFbx mRNA in the gizzard was significantly higher in the meat-type chickens than in the egg-type chickens. Thus atrogin-1/MAFbx mRNA expression in the smooth muscle of the gizzard was higher in meat-type chickens than in egg-type chickens, in contrast to its expression in the skeletal muscles.

Key words: atrogin-1/MAFbx; chicken; gizzard; smooth muscle; egg-/meat-type

Meat-type chickens are selected for rapid growth and large muscle mass, whereas egg-type chickens are selected for egg production. The difference in growth between egg-type chickens and meat-type chickens is greater than the above difference, because muscle protein degradation in meat-type chickens is less than in egg-type chickens.1-4 In the skeletal muscle, cell mass is determined by the balance between the rate of protein synthesis and that of degradation. Studies with experimental animal models have consistently demonstrated that protein degradation by the ubiquitin-proteasome system is increased in muscle undergoing atrophy.5-9 Proteolysis under catabolic conditions is also due primarily to activation of the ubiquitin-proteasome proteolytic pathway.8,10,11 In this way, proteins to be degraded are linked to a chain of ubiquitin molecules, which targets them for rapid breakdown by the proteasome.12 Expression of E3 ubiquitin ligase atrogin-1, also known as muscle atrophy F-box (MAFbx), is increased under the catabolic conditions that arise during muscle atrophy.5,7,13,14 and has been found to play a critical role in the regulation of muscle proteolysis.14,15 We have reported that the differences in muscle protein degradation rates between egg- and meat-type chickens are related to the expression of atrogin-1/MAFbx in the smooth muscle.16

Meat-type chickens are bred selectively to yield more meat and to gain weight quickly, which increases their skeletal muscle-to-body weight ratio relative to that of egg-type chickens. Consequently, the weights of other tissues (e.g., the gizzard) relative to body weight are lower than in meat-type chickens than in egg-type chickens. The gizzard is a characteristic avian smooth muscle sac that is used to crush the food and begin protein digestion.17 Although the amount of smooth muscle in the gizzard correlates strictly with factors such as diet composition and structure,18 there is little information as to how the size of the cells in the gizzard smooth muscle is regulated. We have reported that atrogin-1/MAFbx mRNA expression in chickens is higher in the gizzard than in skeletal or cardiac muscle, and that the expression of atrogin-1/MAFbx in the smooth muscle of the gizzard changes when chickens are fasted and then fed again.19 It is likely that the difference in gizzard weight between in egg- and meat-type chickens is related to the expression of atrogin-1/MAFbx in the smooth muscle. To investigate this, we compared the expression of atrogin-1/MAFbx in the smooth muscle of the gizzard in the egg- and meat-type chickens.

Egg- and meat-type chicks (male, 1-d-old) were housed in an electrically-heated brooder, and were provided water and a commercial starter diet (crude protein, 20.7%; metabolizable energy, 2,900 kcal/kg diet; Toyohashi Feed, Aichi, Japan) ad libitum. All the chicks were reared until 14 d of age.

The gizzard was rapidly excised and weighted. A piece of smooth muscle was dissected from the gizzard, frozen in liquid nitrogen, and stored at −80°C.

All of the experimental protocols employed in this study followed the Guidelines for Animal Care and Use of the NARO Institute of Livestock and Grassland Science, and they were approved by the Animal Care and Use Committee of the NARO Institute of Livestock and Grassland Science.

Total RNA was extracted with TRIzol reagent (Invitrogen) following the manufacturer’s instructions. Complementary DNA was synthesized from total RNA using random hexamer primers (TaKaRa, Tokyo, Japan) and ReverTra Ace® (TOYOBO, Tokyo, Japan). The primer sequences were as follows: chicken atrogin-1/MAFbx, forward 5'-CCA ACA ACC CAG AGA CCT GT-3' and reverse 5'-GGA GCT TCA CAC GAA CAT GA-3'; chicken m-calpain large subunit, forward 5'-GAG ATC TCT GCA TCG CTT CC-3' and reverse 5'-CAA CCT TCA CAC CAC CAC TGG AA-3' and reverse 5'-TCA AAG GTA TCC GGC AAA TC-3'; chicken caspase-3, forward 5'-GAG ATC TCT GCA TCG CTT CC-3'; chicken catabepsin B, forward 5'-CAA GCT CAA CAC CAC TGG AA-3' and reverse 5'-TCA AGT AGA TCT GCC GGC AAA TC-3'; chicken caspase-3, forward 5'-TGG CGA TGA AGG ACT CTT CT-3' and reverse 5'-CTG GTC CAC TGT CTT GCA CA-3'; and chicken 18S rRNA,
were expressed as mean

expression is a reliable index of it.15) We have found that proteolysis and the level of atrogin-1/MAFbx gene rate of muscle protein degradation and thus, to muscle undergoing atrophy, and expression of it is related to the level of atrogin-1/MAFbx gene expression in chicken is higher in Egg-Type (open columns) and Meat-Type (closed columns) Chickens.

The results of RNA quantification are expressed as ratios to relative to the 18S rRNA levels in the egg-type chickens, whose expression level was taken to be 1. Values are means ± SD (n = 5). *p < 0.01.

forward 5′-AAA CGG TTA CCA CAT CCA AG-3′ and reverse 5′-CCT CCA ATG GAT CCT GTG TA-3′. mRNA levels were measured by real-time PCR with a LightCycler® instrument (Roche Diagnostics, Mannheim, Germany) and the QuantiTect SYBR Green PCR system (Qiagen, Tokyo, Japan). The level of 18S rRNA was measured as internal control.

Data were analyzed by Student’s t-test. A p of value <0.05 was considered statistically significant. Results were expressed as mean ± SD.

We compared the expression of atrogin-1/MAFbx in the gizzard smooth muscle of the egg- and meat-type chickens. The body weight of meat-type chickens was significantly greater than that of egg-type chickens at 14d of age (egg-type, 125 ± 8 g versus meat-type, 439 ± 56 g, p < 0.01). The gizzard weight of the meat-type chickens was significantly greater than that of egg-type chickens at 14d of age (egg-type, 5.7 ± 0.3 g versus meat-type, 12.2 ± 0.9 g, p < 0.01). Meat-type chickens usually have a higher proportion of muscle, especially breast muscle, relative to body weight than egg-type chickens. By contrast, at 14d of age, the gizzard to body weight was lower in the meat-type chickens than that in the egg-type chickens (Fig. 1A).

Atrogin-1/MAFbx, a muscle-specific ubiquitin ligase, is highly expressed in skeletal and cardiac muscle undergoing atrophy, and expression of it is related to the rate of muscle protein degradation and thus, to muscle size. Atrogin-1/MAFbx plays a critical role in muscle proteolysis and the level of atrogin-1/MAFbx gene expression is a reliable index of it.15) We have found that expression of atrogin-1/MAFbx in chicken is higher in the smooth muscle of the gizzard than in skeletal or cardiac muscle,19) but no information is available on genetic differences in the gizzard between egg- and meat-type chickens. The level of atrogin-1/MAFbx mRNA in the gizzard was higher in the meat-type chickens than in the egg-type chickens (Fig. 1B). This is the first study to find that expression of an ubiquitin ligase (atrogin-1/MAFbx) correlates with the difference in gizzard weight relative to body weight observed in egg- and meat-type chickens. We have reported that the differences in muscle protein degradation rates between egg- and meat-type chickens are related to the expression of atrogin-1/MAFbx in the skeletal muscle.16)

Multiple proteolytic systems play major roles in various cases of protein loss and muscle wasting. Intercellular proteolytic processes that occur in skeletal muscle involve various proteases, including lysosomal acidic cathepsins20) and Ca2+-dependent calpains.21,22) Proteins are also degraded by the ATP-dependent ubiquitin-proteasome system.23) This system constitutes an essential pathway that mediates accelerated proteolysis in various animal models of muscle wasting,5,23) but the precise roles played by these degradation systems in the breakdown of smooth muscle proteins are yet to be determined. We measured the mRNA levels of three proteolytic-related genes in gizzard smooth muscle of egg- and meat-type chickens. mRNA expression of the m-calpain large subunit, cathepsin B, and caspase-3 mRNA in the gizzard smooth muscle was higher in the meat-type chickens than in the egg-type chickens (Fig. 2, p < 0.01). We have reported that the lower rate of muscle protein degradation in meat-type chickens than in egg-type chickens due at least in part to the lower expression level of atrogin-1/MAFbx (but not m-calpain large subunit, cathepsin B, or caspase-3) mRNA in the skeletal muscle of meat-type chickens.10) Calpain, cathepsin B, and caspase-3 are expressed in the smooth muscle of the chicken gizzard,19) but no comparison of enzyme activity or expression with regard to strains have been reported. The present study detected significant differences in the levels of calpain, cathepsin B, and caspase-3 mRNA in the gizzard smooth muscle of egg- and meat-type chickens.

In conclusion, our findings indicate that atrogin-1/MAFbx expression in the smooth muscle of the gizzard

![Fig. 1](image1.png)  
**Fig. 1.** Comparison of Gizzard Weight Relative to Body Weight (A) and Atrogin-1/MAFbx mRNA Levels in Gizzard Smooth Muscle (B) of Egg- and Meat-Type Chickens.

The results of RNA quantification are expressed as ratios to relative to the 18S rRNA levels in the egg-type chickens, whose expression level was taken to be 1. Values are means ± SD (n = 5). **p < 0.01.

![Fig. 2](image2.png)  
**Fig. 2.** Comparison of mRNA for Proteolytic-Related Genes in Gizzard Smooth Muscle of Egg-Type (open columns) and Meat-Type (closed columns) Chickens.

The results of RNA quantification are expressed as ratios to relative to the 18S rRNA levels in the egg-type chickens, whose expression level was taken to be 1. Values are means ± SD (n = 5). **p < 0.01.
is higher in meat-type chickens than in egg-type chickens, whereas the opposite is true for skeletal muscle. This indicates that the levels of proteolytic-related genes expression differ between these two types of chickens, and this might contribute to the differences in gizzard weight relative to body weight between egg- and meat-type chickens.

References