Stereological estimation of volume ratios of chest muscle in Atak-s hybrid with Rhode Island Red and Barred Rock pure lines by magnetic resonance imaging

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Abstract: In-vivo determination of the volume ratio of breast muscles among the first local laying hybrid chicken and its parents Rhode Island Red (RIR) and Barred Rock (BAR) by using magnetic resonance (MR) imaging technique with Cavalieri principle and comparing of these data between the three breeds were aimed in this study. Ten pair of 50 weeks old RIR, BAR and ATAK-S hybrid breeds were used as research subjects. After scanning of the whole body with 3.0 Tesla MR scanner, cross-sections were taken from the same levels with the MR images and photographed. The parameters obtained from MR images and physical sections were evaluated with Cavalieri principle by using Stereo Investigator 10.50 software. The ratios of the chest muscle volume to the total body volume on the transversal cross-sectional images for ATAK-S, RIR and BAR are 0.140 ± 0.003, 0.129 ± 0.006 and 0.128 ± 0.002 respectively. As a result, comparative breast volume ratio measurements between three breeds were evaluated (ATAK-S, RIR and BAR) in this study. The volume ratios of the pectoral muscles in ATAK-S hybrid chicken were higher than the parents.

Keywords: ATAK-S, Cavalieri principle, magnetic resonance imaging, pectoral muscle, stereology

Rhode Island Red ve Barred Rock saf hatları ile Atak-s hibritinde göğüs kasları hacim oranlarının manyetik rezonans görüntüleme ile stereolojik açıdan hesaplanması

Öz: Bu çalışmada ülkemizde yetiştirilen ilk yumurtacı yerli hibrit olan ATAK-S ile bu hibritlerin ebeveynleri olan Rhode Island Red (RIR) ve Barred Rock (BAR) hatlarında göğüs kaslarının hacim oranlarının

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manyetik rezonans (MR) görüntüleme ile in-vivo olarak belirlenmesi ve ırklar arası karşılaştırma yapılması amaçlandı. Araştırma materyali olarak 10'ar adet, 50 haftalık RIR, BAR ebeveyn hatları ve ATAK-S hibritleri kullanıldı. 3.0 Tesla MR taramayla elde edilen görüntüler ile fiziksel kesitler Stereo Investigator 10.50 yazılım programında Cavalieri hacim hesaplama metodu kullanılarak değerlendirildi. Transversal kesit görüntüleri üzerinden yapılan ölçümlerde göğüs kas hacminin toplam vücut hacmine oranı ortalama olarak ATAK-S için; 0,140±0,003, RIR için; 0,129±0,006, BAR için; 0,128±0,002 olarak belirlendi. Sonuç olarak ATAK-S, RIR ve BAR ırkları arasında yapılan bu karşılaştırmalı hacim oranı ölçüm çalışmasında, üç ırkın göğüs hacim oranları değerlendirildi. ATAK-S hibritinde göğüs kası hacim oranlarının ebeveyn hatlara göre daha yüksek olduğu görüldü.

Anahtar sözcükler: ATAK-S, Cavalieri prensibi, göğüs kasları, manyetik rezonans görüntüleme, stereoloji

Introduction

In late 1960's, studies on the development of domestic parenthood and hybrids with planned breeding have been initiated in laying hen in Turkey. Studies focused on the development of domestic layer parents accelerated in 1995 under the guidance of Ankara Poultry Research Institute (ATAE). However, a specific study comparing the performance characteristics of outsourced hybrid genotypes with those of ATAE had not been designed. Therefore, breeds on pure lines had been brought in from Canada in 1995 and three domestic commercial layer hybrids were registered as ATAK-S, ATAK and ATABEY (10, 18). Testik and Yiğitoğlu (21) stated that it would be useful to carry out a comprehensive study by the Ministry of Agriculture, ATAE, universities and the private sector in order to determine the performance of the ATAK-S layer genotype under various conditions.

Cavalieri principle is a technique which is developed to estimate the volume of any structure with an unbiased way (7). It was firstly introduced by the Italian mathematician Bonaventura Cavalieri in the 17th century. The basis of this method had been laid by the famous astronomer Johannes Kepler. The ratio of volume component to total volume in organ is also frequently used parameter (3, 14). In the Cavalieri principle, entire organ is cut into equal and parallel sections for volume estimation (7). Surface areas of the each section facing the same direction can be calculated by this method (2).

The starting point of the first image in computed tomography (CT) and magnetic resonance imaging (MRI) is chosen completely randomly. In addition, the intervals can be known in advance while images are taken. These are useful features for the convenient application of the Cavalieri principle. It

was seen that 8-15 images were enough for coefficient of error for the volume calculations on CT and MR images (1, 4). The most commonly used projection area calculation method is the point grid field in stereology. This chart is a systematic matrix consisting of points separated by equal intervals from each other. Surface areas of both macroscopic and microscopic structures can be calculated with it (3).

Magnetic resonance imaging is a non-invasive in vivo diagnostic technique. It is the most widely used imaging method for determination of normal and pathological structures of soft tissues with high tissue contrast feature (9, 17). Studies on the examination of muscles, especially the chest muscles, have not only scientific function, but also commercial importance in terms of various parameters for poultry farming. It has also been determined that MRI calculation of muscle mass in poultry is a very effective way to evaluate carcass yield (8).

The aim of this study is to calculate the breast muscle volume fraction values using the Cavalieri principle on the cross-sectional images after performing whole body MRI scans of the native hybrid layer hen ATAK-S and the parental lines RIR and BAR.

Materials and Methods

For the study, ten for each ATAK-S, RIR and BAR chicken groups obtained from Ankara Poultry Research Institute were examined. 50 weeks old chickens were weighed with bench scale (Kern FKB, Germany). Before MRI protocol, in order to remove artifacts due to motion during imaging, sodium pentotal injection (i.v.) was administered for euthanasia to the animals from the wing vein (*v. ulnaris*). Images were obtained by 3 Tesla MR (Siemens Timtrio Magnetom, Germany) using standard body coil. Animals were placed in the prone position and scanned on T1-weighted, T2-weighted with three planes. Technical sequence parameters were standardized as following; TE: 13 ms; TR: 600 ms; slice thickness: 0.67 mm; field of view: 256×256 for T1-weighted and TE: 355 ms; TR: 3000 ms; slice thickness: 0.69 mm; field of view: 266×266 for T2-weighted protocol.

After MRI process, a detailed anatomic dissection has been performed to two subjects from each group. Volume and weight of chest muscles were measured after they were removed from the body and then photographs were taken. The whole body of the rest eight of each group were frozen to -18°C for 2 days for physical sectioning. Afterwards they were embedded into water for 2 days in order to provide a proper slicing. Bodies were sliced at 1 cm thick with band saw (Scheppach Basato 4, Germany) from same levels with the matching MR image sections. The obtained sections were recorded by taking images from

the same image quality and height over the fixed photo setup supported by the Canon Power shot S70 camera.

Stereo Investigator software (10.50 32.bit, Macro Bright Field, Inc.) at the Ankara University Faculty of Veterinary Medicine was used for the digital stereological calculations. In accordance with the Cavalieri principle, volume calculations were made on MR and physical section images either. For this purpose, different lenses were calibrated separately for each image. The muscle mass values of the chest, arm, thigh and neck region and all remaining body were calculated. Total volume was determined by the sum of the calculated data. The volume calculation using the Cavalieri principle was carried out by the following formula.

$V = A_p \times m \times t \times \sum P$

In the equation; "V" is the measured volume, "m" is the section evaluation range, "A_p" is the area of the point number, "t" is the section thickness, and "$\sum P$" is total number of points (12, 13). Area of each points (A_p) were calculated as 25 mm^2. The point counting grid was randomly placed on the sections during the calculation. The volume fraction values that calculated on the obtained volume data, were determined according to the formula given below (14).

$$V_{(x, y)} = \frac{\text{Volume X sectin in volume Y}}{\text{Volume Y}}$$

In order to see the reliability of the method, the coefficient of errors (CE) were calculated. During the CE calculation, the formula set by Gundersen and Jensen (13) was automatically calculated.

Descriptive statistics were calculated and presented as "Mean ± Standard Deviation (SD)". Before performing the statistical analysis, data was examined with Shapiro-Wilk test for normality as parametric test assumptions. Data was analyzed using the GLM for Repeated Measures procedure of SPSS 14.01 (SPSS Inc., Chicago, IL, USA). The model included the breeds (ATAK-S, BAR, RIR) as between subject factor and the treatment (MRI and Physical cross section) as within subject factor, and the 2-way interaction term. Post hoc testing was carried out for the significant interaction term using simple effect analysis. A probability value of less than 0.05 was considered significant, unless otherwise noted.

Results

In the study, chest muscles of ATAK-S, RIR and BAR groups were examined and morphometric analyses of the volume fraction values of these muscles were estimated.

It was seen that breast muscles of all three breeds were well developed and formed the largest muscle mass in the body when compared with the other parts of muscles in terms of weight and volume. It was determined that chest muscles were consisted of pectoral

muscle (*m. pectoralis*) and superficial pectoral muscle (*m. pectoralis superficialis*). Both muscles were located in the coracoclavicular membrane and originated with two branches from the sternal costae. While one of the branches lied on the ventrolateral side of the sternum and the other one was on the lateral side of the humerus (Figure 1). The average weights of breast muscle for ATAK-S, RIR and BAR were calculated as 261.6 g, 211.6 g, and 237.2 g respectively. The average volumes of breast muscle for ATAK-S, RIR and BAR were estimated as 251 ml and 232 ml and 248 ml respectively after dissection.

Muscle tissues were hypointense and fats were slightly hyperintense on T1-weighted images. Bone tissues were displayed hypointense in pneumatic bones such as humerus, and isointense in bones with bone marrow such as femur and sternum. It was observed that soft tissues gave better anatomical details on T1-weighted images.

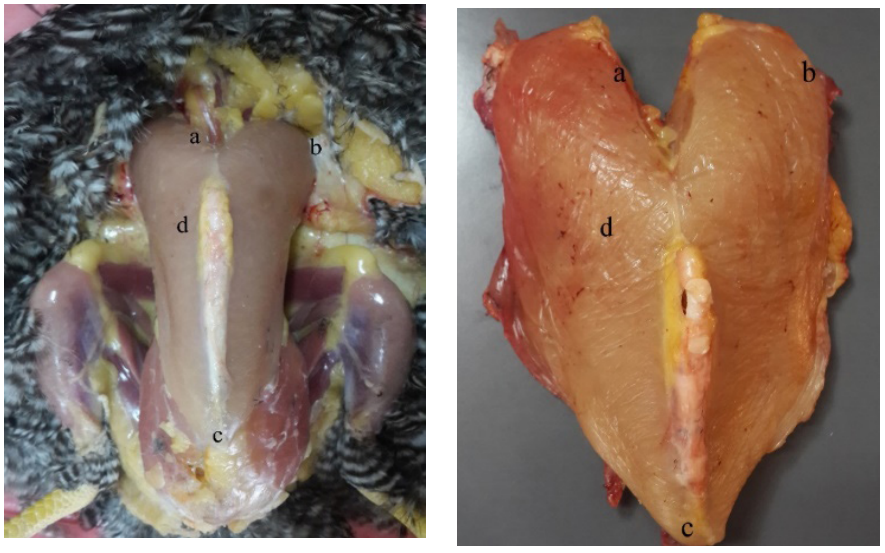

Figure 1: Example of a chest muscle obtained after dissection. a, coracoclavicular border; b, initial limit of humerus: c, end point of sternum: d, musculus pectoralis superficialis.

Şekil 1: *Diseksiyon sonucu elde edilen göğüs kası örneği. a, coracoclavicular sınır; b, humerus'un başlangıç sınırı: c, sternum'un bitiş noktası: d, musculus pectoralis superficialis.*

Similarly, it was determined that transversal sections provide better anatomical detail for the boundaries of muscle masses compared to sagittal and dorsal sections. Volume and volume fraction ratio values were calculated over the sections according to the Cavalieri principle (Figure 2). Muscle, bone and fat tissues were easily distinguished from each other without any coloration in the photographs and estimations were made according to the Cavalieri principle (Figure 3).

The average values of chest muscle volume fraction obtained from the transverse sections of MR images of ATAK-S, RIR and

BAR were determined as 0.140 ± 0.003, 0.129 ± 0.006 and 0.128 ± 0.002, respectively. Volume fraction values between ATAK-S and RIR were statistically significant (p< 0.001). Similarly, the volume fraction values between ATAK-S and BAR were statistically

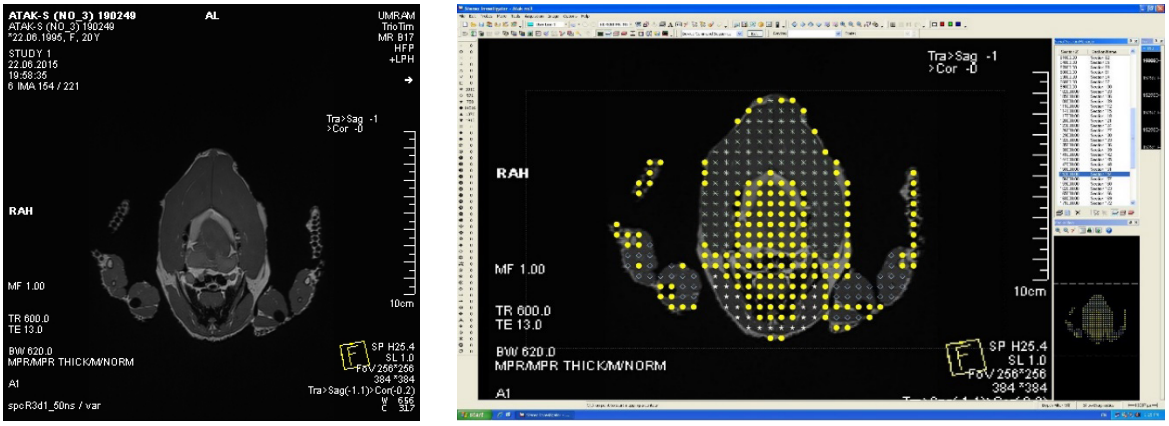

Figure 2: Volume calculation with Cavalieri principle applied on MRI.

Şekil 2: *MRG üzerinde uygulanan Cavalieri prensibi ile hacim hesaplaması.*

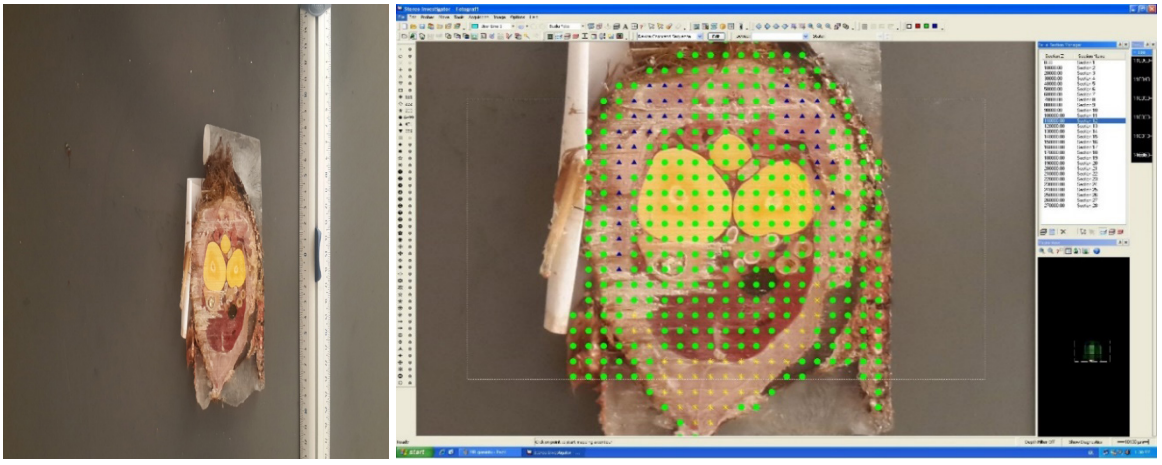

Figure 3: Calculation of the volume with the Cavalieri principle applied on frozen section.

Şekil 3: *Gerçek kesit görüntüsü üzerinde uygulanan Cavalieri prensibi ile hacim hesaplaması.*

significant (p< 0.001), but the values between BAR and RIR were not statistically significant (p> 0.05) (Table 1).

The average values of chest muscle volume fraction obtained from the transverse sections of physical images for ATAK-S, RIR and BAR were 0.108 ± 0.004, 0.081 ± 0.001 and 0.084 ± 0.002, respectively (Table 1). The volume fraction values between the ATAK-S and RIR, ATAK-S and BAR, BAR and RIR was statistically significant (p< 0.001) (Table 2).

Table 1: Descriptive statistics of ratios of chest muscle volume to total body volume in MRI and physical cross sections.

Tablo 1: *MRG ve fiziksel kesitlerdeki göğüs kas hacminin toplam vücut hacmine oranlarının tanımlayıcı istatistikleri.*

	Treatment		
Breed		MRI	Physical
	N	Mean ± SD	Mean ±SD
ATAK	10	0.14 ± 0.003	0.108 ± 0.004
BAR	10	0.128 ± 0.002	0.084 ± 0.002
RIR	10	0.129 ± 0.006	0.081 ± 0.001

When the MR and cadaver cross-section images were compared (Figure 4), the volume fraction values of the chest muscles for three breeds were statistically significant ($p < 0.001$ Table 2).

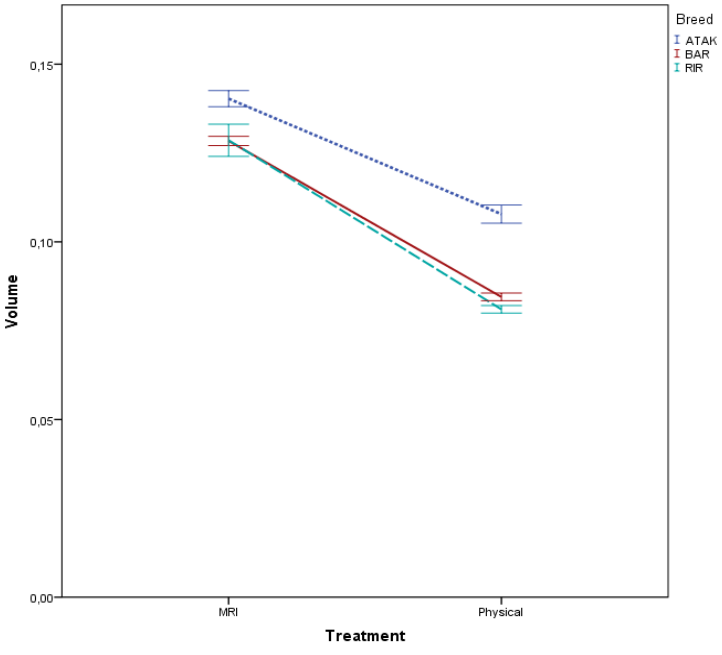

Figure 4: MRI and physical cross section volume estimations of breeds.

Şekil 4: *Irklara göre MRG ve fiziksel kesit hacim verileri.*

Table 2: Effects of treatment and breeds.

Tablo 2: *Yaklaşımların ve ırkların etkileri.*

Source of Variation			df	Mean square	F	P
Treatment			1	0.026	2116.61	<0.001
Breed			2	0.001	198.24	<0.001
Treatment * Breed			2	0.003	25.58	<0.001
	Pairwise Comparisions					
	Treatment	Breed				
	MRI	ATAK vs. BAR				<0.001
	MRI	ATAK vs. RIR				<0.001
	MRI	BAR vs. RIR				0.916
	Physical	ATAK vs. BAR				<0.001
	Physical	ATAK vs. RIR				<0.001
	Physical	BAR vs. RIR				0.003
	Breed	Treatment				
	ATAK	MRI vs. Physical				<0.001
	BAR	MRI vs. Physical				<0.001
	RIR	MRI vs. Physical				<0.001
Error Term (Treatment)			27	1,21E-05		
Error Term (Breed)			27	5.73E-06		

Discussion and Conclusion

The Archimedes method, the oldest and most common method for volume measurements, is based on the principle of immersing objects into water. However, this method could be misleading for the structures with complex internal anatomy. For this reason, different methods have been developed. One

of them is the Cavalieri principle which is an unbiased stereological method. It is possible to estimate volume or volume fraction values with area calculations on twodimensional cross section images with specified thickness by this method (16).

Body fat ratios, chest muscle volumes and body compositions were evaluated by computerized tomography in broiler chicken (5) and turkey (6). In previous studies, Eric et al. (11) used the Cavalieri principle on tomography images for measuring chest volume in human. Scollan et al. (19) suggested that reconstructed images from MRI can be used as an effective method for determining body composition in studies of chest muscle mass estimation in chickens. Similarly, Mitchell et al. (15) evaluated MRI images and 3D reconstructed data's for analysing of body composition in pigs. Similar to Scollan et al. (19) and Mitchell et al. (15), high resolution cross sectional images obtained in our study was revealed that MRI is an effective imaging technique for the discrimination of both muscle and fat tissues. It was seen that much more detailed sections can be obtained in MRI than CT. Parallel to mentioned researches above, it was seen that the Cavalieri principle gives proper, accurate and unbiased results for volume measurement on section images.

Scollan et al. (19) and Mitchell et al. (15) were emphasized the importance of in vivo MR data acquisition in validating and evaluating the results. When frozen cadaver sections were evaluated, some disadvantages such as the difficulty of obtaining proper cadaver sections, necessity of animal sacrification and alterations in the tissue shrinkage rates according to the cadaver's storage method can affect volumetric data.

Silvia et al. (20) calculated chest volume ratios on real time ultrasound images on chickens and made estimates for carcass morphometry. They have emphasized that ultrasound is quicker and practical than MR and CT with high contrast in addition to being in-vivo. However, they have suggested that more effective image analysis systems than ultrasound could be developed. Walton et al. (22) compared ultrasonography with MRI on quadriceps muscle images and stated that MRI could be a good alternative method for visualization and determination of muscles. Researchers also integrated the Cavalieri method to MRI and indicated that this modification could be unbiased. It is thought that MRI can be rather used as a convenient alternative to both tomography and ultrasonography either and it is one of the most effective methods for imaging muscle and

similar soft tissues when compared to other imaging modalities. Our study also indicated that Cavalieri principle is the most convenient method to estimate volume calculations from MR images.

In conclusion, the comparative volume calculation of breast muscles between ATAK-S, RIR and BAR indicated that ATAK-S have a higher volume fraction value than the parent breeds in this study. From this point, it can be said that the ATAK-S hybrid reveals heterozygosis in terms of chest muscle ratios. It is predicted that this specific property can be quite useful if integrated to slow-growing broiler chicken studies. Our study also indicated that MRI is an effective and preferred imaging method in studies to be performed on carcass morphometry with advantages such as being non-invasive and providing in-vivo and high resolution images. It is thought that MRI combined with stereological methods can be used as a reliable method in further studies of body composition determination especially planned for broiler species.

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