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Muscle fibers and cellular characteristics of male Saanen kids with different slaughter weights

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Abstract

Background Fiber and cellular characteristics of skeletal muscle mass are directly related to growth in farm animals. Therefore, this study aimed to examine the DNA, RNA, and protein contents, as well as the number, size and diameter of muscle fibers in selected skeletal muscles of male Saanen kids with different post-fattening weights. Twenty-four male kids of the Turkish Saanen breed were individually fattened for 60 days after five months of weaning under an intensive management system. The kids were divided into two groups: low-slaughter-weight ($n = 11$; 27.73 ± 0.40 kg) and high-slaughter-weight ($n = 13$; 31.18 ± 0.23 kg) at the end of the fattening. Muscle samples from the central part of the mid-section of LD and ST muscles were excised from the right side of the carcasses. DNA, RNA, and protein content of muscle samples were isolated by commercial purification kits as suggested by the manufacturer. The total number and area of muscle fibers were determined using hematoxylin and eosin histological staining.

Results Kids with high-slaughter-weight had a higher ($p < 0.05$) number of total muscle fibers number in ST muscle than those of low-slaughter-weights. In contrast to muscle fiber cross-sectional area of low-slaughter-weight kids was higher ($p < 0.05$) than high-slaughter-weight kids in both muscles. Moreover, low-slaughter-weight kids had higher ($p < 0.05$) muscle fiber diameter than high-slaughter-weight kids in ST muscle. Kids with high-slaughter-weight had significantly higher ($p < 0.05$) contents of DNA and RNA in ST muscle but not in LD muscle compared to low-slaughter-weight kids. Also, high-slaughter-weight kids had significantly higher ($p < 0.05$) RNA: DNA in ST muscle. However protein: DNA, and protein: RNA ratios in ST muscle of low-slaughter-weight kids were significantly higher ($p < 0.05$) than those of high-slaughter-weights.

Conclusions This study demonstrates that the cellular and morphological mechanisms influencing the slaughter weight of male Saanen kids can be used to select more efficient animals for fattening practices.

Keywords Saanen, Kid, Skeletal muscle, Muscle fiber composition, Cellular characteristics, Fattening

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Background

In livestock farms producing female-sourced products, male offspring pose a significant problem in terms of animal welfare, sustainability, and economic efficiency [1–3]. While female offspring are generally preferred as replacements for their production traits, such as milk, fertility, and raising offspring, a large portion of male offspring are either slaughtered at a very young age and used as pet food or are slaughtered for human consumption after short-term fattening in dairy goat farming [1]. However, the meat yield of male offspring born to dairy goats can be optimized with appropriate fattening programs and management practices. Hereby, the effective use of male offspring in dairy goat farms can increase the overall efficiency of the enterprises and minimize economic losses.

Saanen goat breed is known for its milk yield and is widely raised worldwide. The growth performance of Saanen goats is shaped by the interaction of their genetic potential and environmental factors [4–8]. Although male Saanen kids have a relatively slow growth rate, assessing the fattening performance of this breed is an important issue for Saanen goat dairy farms. Therefore, analysis of meat yield, growth rates, and muscle structure of Saanen kids should be used to assess their fattening potential [8]. Furthermore, analyzing the growth rates and fattening performance of male Saanen kids can assist breeders in developing optimal feeding strategies.

Understanding the causes of differences in live weight at the end of fattening in male kids of dairy goats is critical for improving productivity and profitability in dairy goat farms [8]. Growth traits, including fattening performance, result from the interaction of numerous biological and environmental factors, such as genetics, nutritional programs, health status, and management practices [9]. Also, the number, size, and diameter of fibers in the muscle mass and cellular characteristics such as DNA, RNA, and protein content play a critical role in growth competence [10–12]. Muscle mass is an important biological factor that directly impacts animals' overall health and performance. The cellular characteristics of muscle tissue, such as DNA, RNA, and protein content, as indicators of metabolic activities at the cellular level, reflect the organism's growth and development potential. While DNA is responsible for storing and transmitting genetic information, RNA plays a key role in translating this information into proteins. Proteins, essential components of cellular structures and enzymes, directly influence growth and metabolic processes. Therefore, changes in the ratios of these molecules in skeletal muscle mass can determine the animal's overall growth performance and fattening efficiency.

At the cellular level, muscle fiber numbers, cross-sectional area, and diameter determine muscle tissue's structural properties, such as size and functional capacity. Muscle mass size and volume vary as a function of muscle fiber count and diameter [12]. Therefore, the fiber properties of muscles are one of the fundamental components that determine an organism's growth and development processes [10, 13]. Morphological characteristics of muscle mass, such as muscle fiber count and diameter, directly affect meat yield [11, 12]. A higher number and diameter of muscle fibers translates to higher muscle mass and, consequently, a higher final weight.

In this context, examining the morphology and cellular characteristics of Saanen kids' muscle fibers can provide important information to optimize fattening performance. We hypothesize that higher slaughter weight is associated with increased muscle fiber number, greater cellular activity, and, consequently, higher translational capacity. Also, this study addresses a gap in the literature by investigating the relationships among slaughter weight, muscle fiber morphology, and RNA, DNA, and protein levels in Saanen goat kids. Therefore, this study aimed to examine the DNA, RNA, and protein contents, as well as the number, size, and diameter of muscle fibers in the longissimus dorsi (LD) and semitendinosus (ST) muscles of male Saanen kids raised under similar conditions and with different post-fattening live weights, and to determine their relationship with post-fattening live weight differences.

Materials and methods

Animals and ethical statement

The animal study protocol was approved by the Local Animal Care and Ethics Committee of Ondokuz Mayıs University, Samsun, Türkiye, which also approved the experimental procedures, ensuring compliance with EC Directive 86/609/EEC for animal experiments (protocol code: 2022/54 and date: 12 December 2022). Twenty-four singleton male Saanen kids with similar birth weights (2.50 ± 0.05 kg) were used as experimental animals. The kids were bought from the livestock farm of the Ondokuz Mayıs University, Samsun, Türkiye (41°21'40.5"N 36°11'01.1"E and 22 m above sea level).

Growth and finishing of kids

All kids were weaned at 4 months of age and fattened until 6 months of age. The kids were fed ad libitum concentrates containing 12% crude protein (Samsun Yem A. S., Samsun, Türkiye) and alfalfa hay during the fattening period. The growth (from birth to weaning) and fattening periods of kids were in detail described in our previous article [8]. All kids were fasted for one day at the end of the fattening period and divided into two groups according to body weight: low-slaughter-weight (n = 11; 27.73 ± 0.40 kg) and high-slaughter-weight (n = 13; 31.18 ± 0.23 kg), with a statistically significant difference between the groups ($P < 0.05$).

Muscle sample collection

All kids were then transported to a local slaughterhouse, and stunned prior to slaughter. Standard commercial slaughter procedures were carried out in accordance with ethical requirements. Following slaughter, three pieces of muscle samples, approximately 50 g with rectangular prism shape, were taken from the mid-sections of LD and ST muscles. Fat and connective tissues were removed from muscle samples, wrapped with sterilized aluminum foil, snap-frozen in liquid nitrogen, and stored at − 80 ∘C until DNA, RNA and protein isolation.

Determination of muscle cellular characteristics

DNA and RNA of muscle samples were isolated by a commercial DNA (PureLink™, DNA Mini Kit Invitrogen™, K182001) and RNA (PureLink™, RNA Mini Kit, Invitrogen™, 12183018 A) purification kit as suggested by the manufacturer. The purity and quantity of isolated DNA and RNA were measured by using NanoDrop™ 2000/2000c spectrophotometers (Thermo Fisher Scientific), and 1% w/v agarose gel was used to check the DNA and RNA quality. Total protein of muscle samples was isolated by radioimmunoprecipitation assay (RIPA) with some modifications [14]. Total protein in the extracts was determined in triplicate in suitable dilutions of both fractions by the method of Bradford [15] using bovine serum albumin as a standard. The quality of proteins was checked in 12% w/v SDS-PAGE gel electrophoresis.

Histological analysis of the muscle fibers

The muscle fibers in the LD and ST muscles were assessed by the hematoxylin and eosin histological staining technique (Fig. 1). Muscle tissues were dehydrated in gradient ethanol (75%; 1 h, 80%; 1 h, 90%; 1 h, 100%; 1 h, and 100%; 1 h), transparentized in pure xylene (15 min), and finally embedded in molten paraffin wax at 70 °C for 1 h, three times. Paraffin blocks were sectioned with a microtome at a thickness of 10 µm, and at least three sections per sample were floated in a 45 °C water bath. Afterwards, the sections were mounted onto glass slides and dried overnight on a slide warmer set at 50 °C. The sections were then stained with hematoxylin for 5 min and stained with eosin for 1 min [16]. Each section was then dehydrated, transparentized, and sealed with Entellan (Merck, 1.07961.0100). A microscope (Zeiss, PrimoStar HD light microscope equipped with AxioCam ERc 5s 5mp digital microscope camera) was used to view the sections, and ZEN 2012 SP2 (Carl Zeiss, Jena, Germany) image analysis programme was used to scan the images. Three tissue sections were obtained from each muscle sample as parallel measurements. For each section, four random visual fields were selected and measured, resulting in a total of 12 visual fields for each muscle sample. The diameter, cross-sectional area, and number of muscle fibers per mm² were quantified using ImageJ software (version 1.53c; U.S. National Institutes of Health, Bethesda, MD, USA). In calculating diameter and cross-sectional area, at least 200 muscle fibers were used for each section, and the number of muscle fibers per mm² was evaluated as the arithmetic mean obtained from each section. Muscle fiber measurements for each animal were not considered repeats, and average values were analyzed on an animal-by-animal basis.

Statistical analysis

The optimum sample size was determined using mean and standard deviation values of related literature by a simple randomized sampling method, and results showed that six repeats in each group were enough for the trait, which had the maximum variance. The observed power of the test was obtained as 92.07%, which shows that the used sample size was adequate to get reliable results. To compare the L and H groups within the muscles independent sample t-test was used. The statistical analyses were performed using SPSS 17.0 package program (SPSS Inc., 2008, 17.0.1, Wacker Drive, Chicago, Illinois 60606, USA). Relationships between traits were determined with the Pearson correlation analysis. Results were computed as mean ± SE and statistical significance was determined at the level of $p < 0.05$. The assumptions of normality and

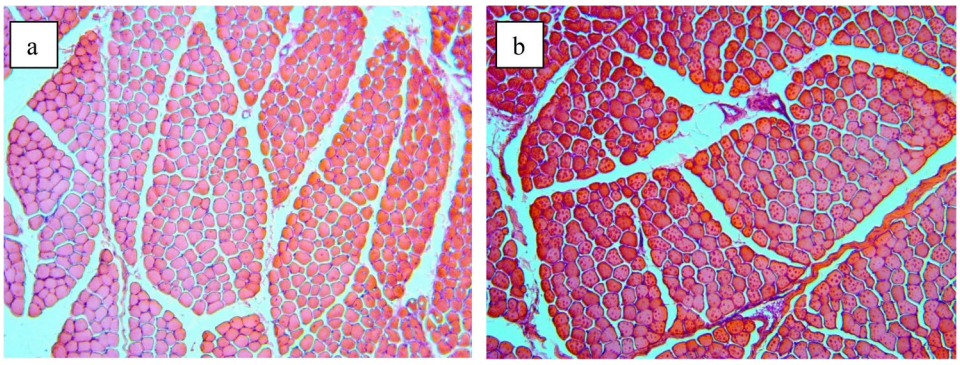

Fig. 1 Pictures of hematoxylin and eosin histological staining (**a**) Longissimus dorsi and (**b**) Semitendinosus muscles

Table 1 Muscle fiber characteristics in longissimus-dorsi (LD) and semitendinosus (ST) muscles of low-slaughter-weight (L) and high-slaughter-weight (H) Saanen kids

Variable	Muscles	Groups	
		L ($n=11$)	H ($n=13$)
TMFN (per mm^2)	LD	695.40 ± 43.80	749.00 ± 31.50
	ST	645.88 ± 5.19[b]	702.10 ± 20.50[a]
MFCSA (μm^2)	LD	910.10 ± 42.10[a]	806.70 ± 2.20[b]
	ST	907.90 ± 23.70[a]	817.50 ± 28.10[b]
MFD (μm)	LD	22.07 ± 1.27	20.28 ± 2.46
	ST	21.57 ± 0.65[a]	19.80 ± 0.55[b]

[a, b]Mean values with different superscripts in the same row indicate a significant difference ($p \leq 0.05$)

TMFN total muscle fiber number, *MFCSA* muscle fiber cross-sectional area, *MFD* muscle fiber diameter

Table 2 Total DNA, RNA and protein amounts in longissimus-dorsi (LD) and semitendinosus (ST) muscles of low-slaughter-weight (L) and high-slaughter-weight (H) Saanen kids

Variable	Muscles	Groups	
		L ($n=11$)	H ($n=13$)
DNA (μg/g)	LD	562.5 ± 22.2	615.2 ± 38.1
	ST	485.6 ± 18.6[b]	564.4 ± 14.6[a]
RNA (μg/g)	LD	1448 ± 129	1677 ± 128
	ST	1318.3 ± 54.5[b]	1836 ± 128[a]
Protein (mg/g)	LD	256.24 ± 9.48	280.6 ± 14.8
	ST	272.0 ± 12.1	265.02 ± 9.87

[a, b]Mean values with different superscripts in the same row indicate a significant difference ($p \leq 0.05$)

Table 3 Ratios of DNA, RNA and total protein in longissimus-dorsi (LD) and semitendinosus (ST) muscles of low-slaughter-weight (L) and high-slaughter-weight (H) Saanen kids

Variable	Muscles	Groups	
		L ($n=11$)	H ($n=13$)
RNA: DNA	LD	2.586 ± 0.207	2.818 ± 0.223
	ST	2.743 ± 0.140[b]	3.292 ± 0.255[a]
Protein: DNA	LD	467.9 ± 35.2	472.2 ± 32.8
	ST	570.3 ± 36.8[a]	475.8 ± 25.2[b]
Protein: RNA	LD	192.7 ± 19.1	175.2 ± 12.1
	ST	213.2 ± 17.1[a]	153.0 ± 11.5[b]

[a, b]Mean values with different superscripts in the same row indicate a significant difference ($p \leq 0.05$)

homoscedasticity were tested using Shapiro-Wilk and Levene tests and after the ensuring about the assumptions were fulfilled the mentioned test were used.

Results

Muscle fiber characteristics in longissimus-dorsi (LD) and semitendinosus (ST) muscles of low-slaughter-weight and high-slaughter-weight Saanen kids are presented in Table 1. There were no significant differences between low- and high-slaughter-weight kids in terms of the total muscle fiber number (TMFN) of LD muscle, but TMFN of low-slaughter-weight kids were significantly lower ($p < 0.05$) than high-slaughter-weight kids in ST muscle. Moreover, muscle fiber cross-sectional area (MFCSA) of low-slaughter-weight kids were significantly higher ($p < 0.05$) than those of high-slaughter-weights in both muscles. Although muscle fiber diameter (MFD) in the LD muscle were similar between low- and high-slaughter-weight kids, MFD of low-slaughter-weight kids was higher ($p < 0.05$) than high-slaughter-weight kids in the ST muscle.

Total DNA, RNA and protein amounts, and ratios to each other in LD and ST muscles of low-slaughter-weight and high-slaughter-weight Saanen kids are presented in Tables 2 and 3, respectively. There were no significant differences between low- and high-slaughter-weight Saanen kids in terms of total DNA amount of LD muscle, but high-slaughter-weight kids had significantly higher ($p < 0.05$) total DNA amount in ST muscle compared to low-slaughter-weight kids (Table 2). Similarly, while the total amount of RNA in LD muscle was the same between low- and high-slaughter-weight kids, total RNA amount in ST muscle of high-slaughter-weight kids were significantly higher ($p < 0.05$) than those of low-slaughter-weights (Table 2). There were no significant differences between low- and high-slaughter-weight Saanen kids in

terms of total protein amount in both muscles. Although RNA: DNA, protein: DNA, and protein: RNA ratios in LD muscle were the same between low- and high-slaughter-weight kids, DNA, RNA, and total protein ratios of low- and high-slaughter-weight kids were significantly different from each other (Table 3). High-slaughter-weight kids had significantly higher ($p < 0.05$) RNA: DNA in ST muscle compared to low-slaughter-weight kids. However protein: DNA, and protein: RNA ratios in ST muscle of low-slaughter-weight kids were significantly higher ($p < 0.05$) than those of high-slaughter-weights (Table 3).

The analysis of Pearson correlation coefficients on the pooled data showed that there were positive correlations ($p < 0.05$) among chest depth (CD), chest circumference (CC) and DNA in ST muscle (Table 4). Similarly, positive correlations ($p < 0.05$) between CD and RNA was observed in ST muscle. A negative correlation ($p < 0.05$) was calculated between CD and Protein: RNA in ST muscle (Table 4).

Chest measurement of low-slaughter-weight and high-slaughter-weight Saanen kids are presented in Fig. 2. High-slaughter-weight kids had significantly higher ($p < 0.05$) CD (27.50 ± 0.90 vs. 25.05 ± 0.34), chest width (CW) (13.50 ± 0.19 vs. 14.39 ± 0.21), and CC (71.41 ± 0.29 vs. 77.08 ± 0.66) than those of low-slaughter-weights.

Discussion

Muscle fiber composition and determinants of muscle growth

Muscle fiber composition and cellular characteristics are influenced by genetic predisposition and feeding program, growth rate, activity level, and health status [11, 17–19]. Energy and protein balance in the early postnatal period can affect the proliferation and differentiation of satellite cells, thus altering fiber size in the long term [20, 21]. The different slaughter weights in the study may reflect differences in individual metabolic responses or nutrient utilization efficiency despite similar rearing conditions. In this context, interventional feeding studies are necessary to determine which periods have a greater impact on hypertrophy (fiber thickening). This study examined muscle fiber and cellular characteristics of LD and ST muscle in male Saanen kids with different slaughter weights. The results suggest that muscle growth does not occur through a single mechanism; instead, differences in fiber number, fiber hypertrophy, and cellular translational capacity act together or separately. First, the higher total muscle fiber count in the ST muscle of kids with high-slaughter-weight suggests that increased fiber count determines muscle mass development. On the other hand, in kids with lower slaughter weights, MFCSA in LD and ST muscles and MFD in the ST muscle were found to be higher. When these two results are evaluated together, it can be interpreted that muscle growth in kids reaching high slaughter weights occurs partially by fiber size or preservation of fiber number, while at low slaughter weights, muscle volume increases by thickening of existing fibers (hypertrophy). Similarly, Greenwood et al. [10] reported that muscle fiber number and size play a critical role in muscle mass and growth performance. On the other hand, the higher muscle fiber diameter and cross-sectional area in kids with low-slaughter-weight suggest that fiber thickening (hypertrophy) may be a more dominant mechanism in muscle growth. Indeed, Sen et al. [12], while evaluating muscle development in native goat breeds, reported that similar differences vary according to breeds and environmental factors. These two different growth strategies may affect meat yield and quality; Animals with higher fiber counts may exhibit more balanced muscle mass over the long term, while animals with larger fibers may exhibit increased protein content and tissue hardness muscle mass per fiber.

Nucleic acid and protein content as indicators of cellular activity

LD and ST muscles differ regarding embryological development, functional load, and metabolic profiles [18, 22–24]. When evaluated by nucleic acid content, DNA and RNA are significantly higher in the ST muscle of high-slaughter-weight kids in the current study. DNA and RNA content can be explained by increased cell number and metabolic activity in skeletal muscle. Marguerat and Bähler [13] stated that DNA and RNA levels are related

Table 4 Pearson correlation coefficients between cellular characteristics and chest measurements for the pooled data

Variable	DNA	RNA	Protein	RNA: DNA	Protein: DNA	Protein: RNA
LD						
CD	-0.039	0.351	0.412	0.379	0.368	-0.195
CW	-0.203	0.171	0.481	0.255	0.439	-0.007
CC	0.039	0.468	0.297	0.401	0.126	-0.351
ST						
CD	0.527*	0.551*	-0.195	0.260	-0.357	-0.605*
CW	0.355	0.122	0.112	-0.019	-0.200	-0.226
CC	0.543*	0.381	0.047	0.204	-0.276	-0.430

LD longissimus-dorsi muscle, *ST* semitendinosus muscle, *CD* chest depth, *CW* chest width, *CC* chest circumference. * $p \leq 0.05$

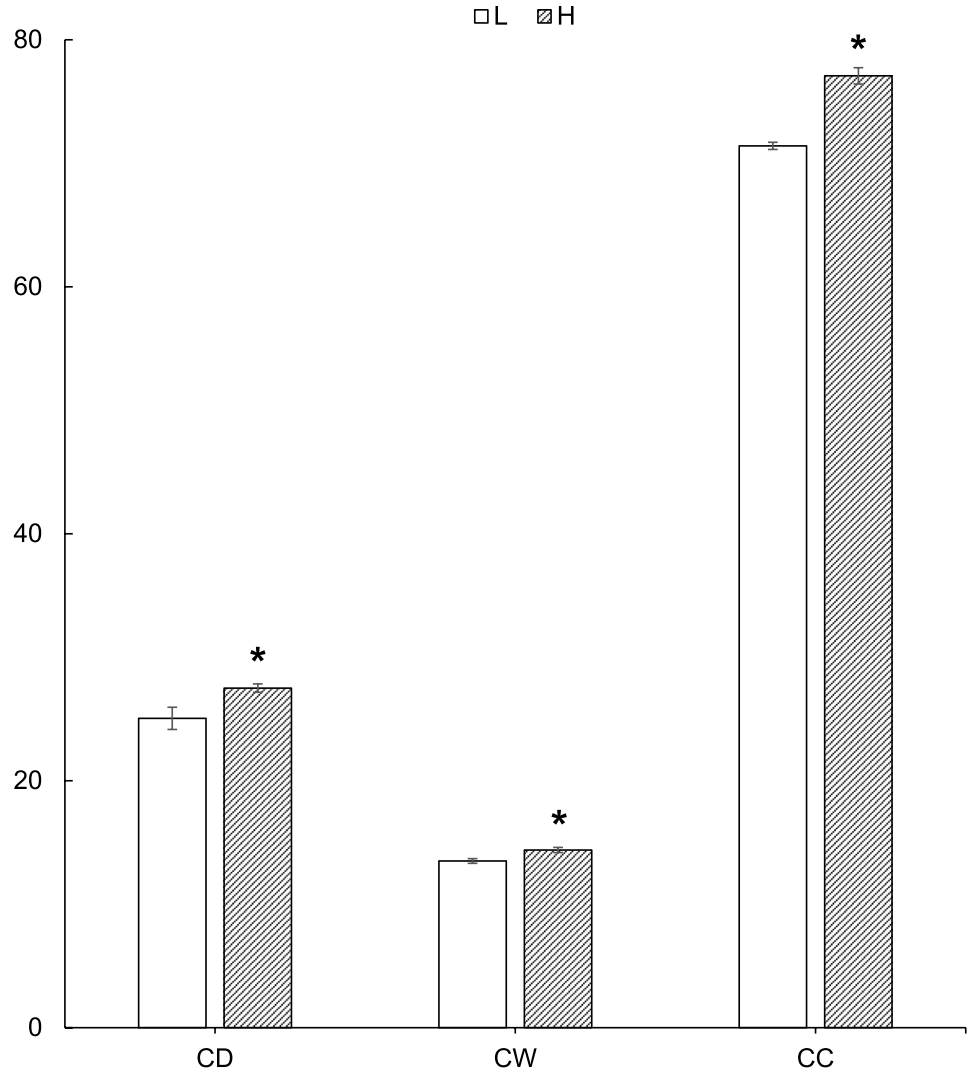

Fig. 2 Chest measurement of low-slaughter-weight (L) and high-slaughter-weight (H) Saanen kids. CD= chest depth, CW= chest width, CC= chest circumference. * $p < 0.05$

to cell size and metabolic activity, suggesting that the balance between genetic information and protein synthesis underlies growth. In this study, the higher DNA and RNA contents in the ST muscle of high-slaughter-weight kids compared to low-slaughter-weight kids, when considered together with the increased RNA: DNA ratio, suggest that the muscle tissue in high-slaughter-weight kids has a higher transcriptional and translational capacity and that these animals have a more active protein synthesis mechanism at the cellular level. High RNA: DNA ratios may be associated with increased ribosome density and enhanced protein synthesis at the cellular level, creating a favorable environment for rapid growth and tissue regeneration. In contrast, the higher protein: DNA and protein: RNA ratios in the low-slaughter-weight kids may suggest a higher protein content per cell or a greater concentration of existing cellular components toward protein accumulation. Therefore, differences in protein, DNA, and RNA ratios may be related to the greater muscle fiber cross-sectional area and fiber diameter in low-slaughter-weight goat kids. The less pronounced DNA/RNA/protein differences in the LD muscle suggest that the ST muscle is more susceptible to growth and cellular adaptation. It is widely reported in the literature that more functionally active or loaded muscles respond more rapidly to cellular adaptation [25, 26]. Furthermore, muscle-region-specific myosin isoform distribution, vascularization, and satellite cell populations may differ, potentially leading to one muscle developing distinct growth mechanisms within the same organism. These results of our study suggest that differences in muscle cellular characteristics between low- and high-slaughter-weight Saanen kids likely reflect interactions between nutritional management and genetically regulated muscle growth pathways,

such as variation in myogenic regulatory factor expression that influences muscle fiber development and overall growth performance.

Associations between muscle cellular traits and body measurements

The positive correlations between muscle cellular characteristics and body measurements, such as chest circumference, width, and depth, are also noteworthy. In particular, the positive correlations between chest depth and DNA and RNA content support the direct link between weight gain and muscle development. Koşum et al. [4] and Akdağ et al. [5] reported that growth performance in Saanen kids can vary significantly with environmental conditions and nutrition. In this context, the data obtained suggest that differences in slaughter weight are due to nutrition and variations in muscle cellular properties.

When targeting slaughter weight under farm conditions, not only body weight but also muscle fiber morphology and cellular capacity should be considered. If total meat yield is the production goal, management strategies may be preferred to promote fiber proliferation in early life. On the other hand, if meat quality (tenderness, fiber size, fat distribution) is essential, managing hypertrophic responses becomes crucial. Moreover, since growth differences appear to be driven by nutrition and cellular growth capacity, these factors may also influence the timing of puberty in males and, consequently, the age at which candidates can enter artificial insemination (AI) stations for routine semen collection. Therefore, management strategies that optimize early growth should be evaluated not only for meat yield but also for their potential to advance AI station entry age without compromising reproductive soundness. Accordingly, meat-oriented enterprises can integrate myogenic profiles into the selection of breeding males and semen for artificial insemination, provided that fertility and meat quality traits are concurrently managed within a balanced selection index.

Conclusions

In conclusion, this study demonstrates that muscle development in male Saanen kids can occur through different biological mechanisms. While increased muscle fiber count and a higher RNA: DNA ratio are the primary growth mechanisms in high-slaughter-weight kids, muscle fiber thickening and higher protein ratios in low-slaughter-weight kids suggest that growth occurs through an alternative strategy. These results suggest that not only weight but also the muscle's cellular profile can serve as selection criteria for growth performance targets.

Authors' contributions

Conceptualization, U.Ş., İ.C., H.Ö. and O.O.; methodology, U.Ş., İ.C., H.Ö., Ö.F.Y., H.M.Y., A.K., İ.C.K. and O.O.; software, Ş.U., İ.C. and H.Ö.; validation, U.Ş., İ.C., H.Ö., Ö.F.Y., H.M.Y., A.K., İ.C.K. and O.O.; formal analysis, Ş.U., İ.C., O.O. and H.Ö.; investigation, U.Ş., İ.C., H.Ö., Ö.F.Y., H.M.Y., A.K., İ.C.K. and O.O.; resources, U.Ş., İ.C., H.Ö. and O.O.; data curation, U.Ş., İ.C., H.Ö., Ö.F.Y., H.M.Y., A.K., İ.C.K. and O.O.; writing—original draft preparation, U.Ş., İ.C., H.Ö. and O.O.; writing—review and editing, U.Ş., İ.C., H.Ö., Ö.F.Y., H.M.Y., A.K., İ.C.K. and O.O.; visualization, U.Ş., İ.C., H.Ö. and O.O.; supervision, U.Ş.; project administration, U.Ş., İ.C., H.Ö. and O.O.; funding acquisition, U.Ş., İ.C., H.Ö. and O.O. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The animal study protocol was approved by the Local Animal Care and Ethics Committee of Ondokuz Mayıs University, Samsun, Türkiye, which also approved the experimental procedures, ensuring compliance with EC Directive 86/609/EEC for animal experiments (protocol code: 2022/54 and date: 12 December 2022). The study did not involve any invasive procedures, and all animal handling complied with the ethical standards and guidelines for the care and use of animals in research.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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