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Correlation between insulin-like growth factor 1 gene expression and fennel (*Foeniculum vulgare*) seed powder consumption in muscle of sheep

Mojtaba Shahsavari[a], Mohammadreza Mohammadabadi[a], Amin Khezri[a], Masoud Asadi Fozi[a], Olena Babenko[b], Oleksandr Kalashnyk[c], Valentyna Oleshko[b], and Serhii Tkachenko[b]

[a]Faculty of Agriculture, Department of Animal Science, Shahid Bahonar University of Kerman, Kerman, Iran; [b]Department of Animal Science, Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine; [c]Department of Animal Science, Sumy National Agrarian University, Sumy, Ukraine

ABSTRACT

It has been shown that addition of fennel in the diets of domestic animals has positive and beneficial effects on growth and meat production traits. Thus, the purpose of current study was to investigate the effect of adding fennel in the ration on growth characteristics and on insulin-like growth factor 1 (IGF1) gene expression in muscle tissue of Kermani lamb. Feeding of animals were performed with three levels of fennel including zero, 10 and 20 g/kg dry matter (DM) for 90 days. After slaughter, small pieces of tissues were removed and rapidly transferred to a nitrogen tank. Then, total RNA extracting and the Real-Time PCR reaction was performed. Results showed that as the level of fennel in the diet increases the amount of IGF1 gene expression also increases significantly in humeral muscle and femur (leg) muscle tissues ($p < 0.05$). In animals fed with fennel, femur muscle weight, back muscle weight, lean meat weight, final weight, warm carcass weight and live daily gain were greater than in animals fed with diet without fennel ($p < 0.05$). According to the findings of this investigation, it can be concluded that fennel, by creating positive effects on IGF1 gene expression can be used to improve muscle structure.

KEYWORDS

Growth characteristics; diet; femur; humeral; Real-Time PCR

Introduction

Due to the social and economic conditions of hot, dry and desert areas, the livelihood of a large part of the population of these areas is provided by the native breeds of small ruminants, especially sheep.[1,2] Thus, it is very important to try to improve the production of these animals by improving the quantity and quality and improving genetic development.[3,4] This improvement in performance in sheep herds is usually achieved through improved yields and reproduction.[5] There are about twenty-seven breeds and ecotypes of sheep in Iran, which total more than 50 million heads.[6] One of the most important and famous Iranian sheep is the Kermani sheep.[7] This animal is fully adapted to the hot, dry and unfavorable conditions of the southeastern region of Iran, which is its main habitat and has weak and unstable vegetation and rangeland.[4,8] This sheep is a dual-purpose (meat and wool) and fat-tailed breed that has white wool and medium size and produces most of the life needs of nomads and ranchers.[5,9] Therefore, paying attention to breeding this sheep to develop and improve its phenotypic and genetic traits will have positive effects on the needs of this breed.

One of the alternatives to antibiotics in animal nutrition, as natural antimicrobial growth promoters are the use of phytobiotics and medicinal plants. The replacement of phytogenic compounds (phytobiotics and medicinal plants), including the essential oils, alkaloids, and flavonoids, has many benefits, including prevention of particular diseases,[10] increasing antimicrobial and antioxidants functions,[11,12] progression of liver activities,[13] improvements of enzymes related to digestion,[14] rising zootechnical yield criteria, and hypocholesterolemic effects.[15] Mohammed and Abbas[16] have shown that adding phytobiotics and medicinal plants to livestock and poultry diets improves feed intake, carcass yield and feed conversion ratio. One of the most important medicinal plants that have very strong antimicrobial, hepatoprotective and antioxidant properties and has been distinguished and used by humans since ancient times is

CONTACT Mohammadreza Mohammadabadi mrm@uk.ac.ir; mmohammadabadi@yahoo.ca; mrm2005@gmail.com Department of Animal Science, Shahid Bahonar University of Kerman, 22, Bahman Blvd., Kerman 7616914111, Iran.

fennel (Foeniculum vulgare mill) from Apiaceae family.[8,17] The results of various studies on domestic animals have proven that the addition of fennel in their diets increases digestion and growth output[18], improves meat oxidative quality,[19] grows packed cell volume, hemoglobin and red blood cells numbers,[16] makes better carcass efficiency and weight and length of the small intestine and reduces the total number of bacteria[20] (Saki et al., 2014), gets better efficiency and health situation[10] and improves feed conversion and body weight.[21]

One of the main mediators for prenatal and postnatal growth is insulin-like growth factor 1 (*IGF1*) polypeptide hormone that encodes by *IGF1* gene.[22] In molecular structure, *IGF1* is similar to insulin.[23] Many tissues produce this hormone, while it is usually produced in the liver in reaction to stimulation of growth hormone.[24] In the peripheral tissues, this hormone shows autocrine, paracrine and endocrine effects.[22] The sheep *IGF1* gene is located on chromosome 3 and its size is 59.3 kb. This gene is made up of 5 exons that encode a long protein with 172 amino acids.[22] This gene is a key mitogen.[25] *IGF1*, along with insulin-like growth factor II (*IGF2*), growth hormone (*GH*), growth-hormone-releasing hormone (*GHRH*), and their related receptors (*IGF2R*, *GHR*, *GHRHR* and *IGF1R*) and binding proteins (BPs) takes part in the somatotrophic axis.[23]

Food-restriction[26] and fasting[27] decreased *IGF1* gene expression and refeeding recovered it to the level of fed animals, as was in good agreement with the changes in plasma *IGF1* levels.[28] Although the recovery of plasma *IGF1* concentration by refeeding needed almost 48 h, *IGF1* gene expression was increased within 24 h after refeeding in fasted young animals.[27] The alterations in dietary protein and amino acid levels are also very strong factors to change plasma *IGF1* concentration and *IGF1* gene expression. Plasma concentration of *IGF1* increased with elevating dietary protein levels from deficiency to the required level, and above the level, it decreased significantly.[29] Thus, it can be proposed that fennel can probably increase *IGF1* gene expression by increasing food intake and raising blood protein levels.

Furthermore, *IGF1* with proteins binding IGF in the blood (*IGFBP*) and with insulin-like growth factor I receptors (*IGFIR*) in target tissues interacts. This interaction can moderate the function of *IGF1* hormone.[23,30–32] It is believed that many biological processes like protein synthesis, cell growth and differentiation, skeletal growth, metabolism regulation and embryogenesis can be mediated by *IGF1*.[23,33–35] It is proven that effects of nutritional disorders are mediated by *IGF1* in skeletal muscles.[25] The differentiation and proliferation of myoblasts are stimulated by *IGF1*.[25,36–38] Semsarian et al.[39] and Sacheck et al.[40] demonstrated that *IGF1* barricades protein degeneration and increases protein synthesis at the same time, thus this gene is involved in muscle hypertrophy. It is effectively shown that *IGF1* is regulated by nutrition.[41,42] Moreover, several reports have shown that estrogens can affect IGF action in breast cancer cells by altering the expression of various members of the IGF family. Specifically, estrogens can modulate the expression of IGF ligands, receptors, *IGFBPs*, and downstream signaling mediators such as insulin receptor substrate-1 (IRS-1) and others.[43–48] Therefore, it can be proposed that fennel can probably increase *IGF1* gene expression via its estrogenic content.

Specifically, we hypothesized that fennel can affect growth characteristics and *IGF1* gene expression in the muscle tissue of sheep. As regards effects of adding fennel to ration of farm animals, mainly in sheep and function of *IGF1* gene expression was not investigated, the purpose of the current study was to investigate the effect of adding fennel in the ration on growth characteristics and on *IGF1* gene expression in muscle tissue of Kermani lamb.

Materials and methods

Ethical statement

All ethical laws and native standard in Iran was used for performing this research and the Iranian Council of Animal Care (Guide to the Care and Use of Experimental Animals, 1. IUT, Iran) guide complex was used for animal maintaining.

Animal breeding, rations and data collection

The current study was conducted in the Animal Science Research and Training Station of Shahid Bahonar University of Kerman, Iran. All 30 used animals in this research were male at 6 months old and had approximately similar weight (27.5 ± 0.45 kg). The lambs were randomly divided into three groups with ten animals. Each animal was housed in a 1.2 × 1.5 m independent pen. Each pen had a cemented floor with bedded straw, an open-side barn, in a sheltered and well ventilated. Animals had free access to water and food. All diets were similar in energy and crude protein. Water was replaced twice a day. In this research, 20 days were considered for the adaptation period and 90 days for the data collection period. The following

Table 1. The characteristics of the used primers for the IGF1 target gene and GADPH reference gene.

Target gene	Primer sequence (5′-3′)	NCBI accession number	Tm* (°C)	product size (bp)
IGF1	forward 5'- ATTACAGCTGCCTGCCCCTT -3' reverse 5'- CACATCTGCTTACACCTTACCCG -3'	NM_001009774.3	57	265
GAPDH	forward 5'- ACCACTTTGGCATCGTGGAG -3' reverse 5'- GGGCCATCCACAGTCTTCTG -3'	NM_001190390.1	57	76

* Tm = melting temperature

measures were performed for all animals before starting the experimental period: shearing, anti-parasitic drugs consumption, and vaccination for anthrotoxemia.

Feeding of animals was performed with three levels of fennel including zero, 10 and 20 g/kg dry matter (DM) for 90 days. Chemical construction and components of used fennel (DM basis) were 91% DM, 87.03% OM, 15% CP, 9.76% and 12.12 MJ/kg ME. Standard methods of AOAC (2000) was used for analyzing nitrogen (method 976.05; Kjeldahl Vap50 Gerhardt, Germany), DM, ash (method 942.05; Shimifan F-47, Tehran, Iran) and ether extract (method 920.39; Soxhlet Model 2000 Automatic Gerhardt, Germany). Van Soest et al.[49] was used to determine the NDFom and ADFom. After complete mixing of the diet, feeding was done twice a day at 8:00 and 16:00 and lambs were fed to allow approximately 5% orts (A scrap or remainder of food from a meal). Daily feed consumption and orts were recorded daily.

Some characteristics associated with the growth of muscle, such as the femur (leg) muscle weight, back muscle (loin) weight, lean meat weight, the weight of neck, weight of shoulder, weight of brisket, final weight, warm carcass weight, dry matter intake and live daily gain were measured.

The lambs were weighed at 14-day intervals before the morning feeding for calculation of the feed conversion (FC) and live weight gain. The live daily gain of lambs was calculated as the difference between the initial and final weights over the interval of the performance phase (80 days).

At the end of the trial (days 80), all animals were slaughtered in the slaughterhouse. After the lambs were bled, they were pelted and the head severed at the atlas joint, and the weights of the head, feet, pelt, and internal organs were recorded. The carcasses were hung by the Achilles tendon after slaughter. Warm and cold (i.e., after 24 h chilling at 4 °C) carcass weights without head were recorded. The carcass was dissected medially into two parts, and the right side was dissected into six cuts (neck, shoulder, brisket, loin, legs and fat tail) according to Kashan et al.,[50] and weighed separately. Then individual cuts were dissected into lean meat, bone, trimmings, and weighed separately.

Gene expression analysis

Sampling from tissues including humeral muscle and femur (leg) muscle was performed and the total number of samples was equal to 180 samples (3 repeats for each of the tissues×2 tissues ×3 groups ×10 animals). For storing, the samples were immediately placed in liquid nitrogen and then at a temperature of −80 °C. Then, total RNA extraction was done using a One Step RNA Reagent Kit (Biobasic Co. Ltd., Iran). Agarose gel electrophoresis was used to evaluate the quality of the extracted RNA. In this method, the presence of two bands 18S and 28S and the absence of genomic DNA confirmed the desired quality of extracted RNA. To synthesize cDNA from extracted total RNA, standard kit (#K1631, Fermentase Co., Iran) and an oligo d(T) primer were used. The characteristics of the used primers for the *IGF1* target gene and *GADPH* reference gene were given in Table 1.

The Real-Time PCR reaction was performed at a final volume of 15 μl using Rotor-Gene Q MDx device (QIAGEN Hilden, Germany). Each Real-Time PCR reaction consisted of 7.5 µL from 2X SYBR Green PCR Master Mix (Fermentase Co., Tehran, Iran), 1.5 µL of template cDNA, 1 µL from 10 µM forward and reverse primers, 4.7 µL of ddH2O and 0.3 µL of ROX. The PCR cycling condition included initial denaturation for 5 min at 95 °C, performing 38 cycles including denaturation for 20 s at 95 °C, annealing for 30 s at 57 °C, and synthesis for 30 s at 72 °C. For confirming the specificity of amplification, at the end of the amplification cycle melting curve analysis was performed. The gradient protocol was used to determine the annealing temperature of target and reference genes. For evaluating Real Time PCR data, Pfaffl method[51] was employed:

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta CT_{\text{target(control}-\text{sample)}}}}{(E_{\text{ref}})^{\Delta CT_{\text{ref(control}-\text{sampl)}}}}$$

where, E_{target} and E_{ref} are the PCR outputs of the investigated gene and the reference or internal control

gene, respectively. ΔCT is the result of subtraction of *Ct* (threshold) of *IGF1* gene from *Ct* of *GADPH* gene.

Statistical analysis

To analyze the data, the mixed procedure of SAS[52,53] was used in the form of a completely randomized design. The PairWise Fixed Reallocation Randomization Test© (REST, 2009) was used to check the data distribution in terms of normality. Means comparison was carried out using the LSD test ($p < 0.05$). REST software (REST, 2009) and Pfaffl formula[51] were used for evaluating the results of Real-Time PCR.

To evaluate the major effect of fennel seed powder level and effect of the tissue by the fennel × tissue interaction the below statistical model was used:

$$X_{\mathrm{ijm}} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{m(ij)}$$

Where, μ = mean, α_i = main effect of tissue at level i, β_j = main effect of fennel at level j, $\alpha\beta_{ij}$ = interaction effect of tissue at level i and fennel at level j, $\varepsilon_{m(ij)}$ = the effect of all other extraneous variables on subject m in treatment group ij, and X_{ijm} = dependent variable score for subject m in treatment group ij.

To assess gene *IGF1* interactions with other studied genes in sheep (Ovis aries) and also other organisms, the STRING program (http://string-db.org) was used. STRING is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations; they stem from computational prediction, from knowledge transfer between organisms, and from interactions aggregated from other (primary) databases.

Results

The relationship of some characteristics associated with the growth of muscle and fennel feeding were shown in Table 2. In animals fed with fennel, femur (leg) muscle weight, back muscle (loin) weight, lean meat weight, final weight, warm carcass weight, dry matter intake and live daily gain were greater than in animals fed with diet without fennel ($p < 0.05$), while this relationship was the opposite for neck weight. Although this difference for the weight of shoulder, the weight of brisket, weight of the neck and initial weight was not significant.

The mean cycle threshold (Ct) value for *IGF1* differs from 23 to 25 in different studied tissues. The results of studying the interactions between tissue and fennel feeding level showed that these interactions were always significant. Results showed that as the level of fennel in the diet increases from 0 g/kg DM to 10 g/kg DM and from 10 g/kg DM to 20 g/kg DM, the amount of *IGF1* gene expression also increases significantly ($p < 0.05$) in studied tissues; humeral muscle and femur (leg) muscle (Fig. 1). Comparison of *IGF1* gene expression between the two studied tissues; femur (leg) muscle and humeral muscle showed that expression of *IGF1* gene in humeral muscle was significantly higher ($p < 0.05$) than in femur (leg) muscle both for 10 g/kg DM fennel and for 20 g/kg DM fennel (Fig. 1).

Table 2. The result of fennel feeding on some characteristics associated with growth of muscle in studied animals.

	Fennel powder (g/kg DM)				
Calculated parameters	0	10	20	*p*-Value	SEM
Neck weight (kg)	2.01	1.86	1.90	0.506	0.10
Brisket weight (kg)	3.32	3.45	3.61	0.690	0.13
Shoulder weight (kg)	2.96	3.10	3.02	0.506	0.10
Dry matter intake (kg/day)	1.367^{b}	1.406^{a}	1.433^{a}	0.01	0.015
Initial weight (kg)	27.0	27.9	27.7	0.638	0.49
Final weight (kg)	44.7^{b}	45.8^{a}	46.5^{a}	0.024	0.23
Live daily gain (g)	221^{b}	224^{b}	235^{a}	0.035	3.06
Warm carcass weight (kg)	20.74^{b}	21.20^{ab}	21.99^{a}	0.039	0.36
Weight of back muscle (loin) (kg)	3.67^{b}	3.84^{ab}	4.07^{a}	0.043	0.11
Weight of femur muscle (kg)	5.53^{b}	5.86^{ab}	5.96^{a}	0.039	0.12
Weight of lean meat (kg)	14.28^{b}	14.70^{ab}	15.43^{a}	0.029	0.34

[a,b]Values within a row with different superscripts differ significantly at $p < 0.05$.

IGF1 gene and other predicted genes interactions and the definition of predicted functional partners using the STRING program are given in Fig. 2. *IGF1* had an interactions with Tyrosine-protein kinase receptor (*IGFR1*) score 0.999, Insulin-like growth factor-binding protein 4 (*IGFBP4*) score 0.991, insulin-like growth factor-binding protein 3 (*IGFBP3*) score 0.988, insulin-like growth factor-binding protein 5 (*IGFBP5*) score 0.986, Tyrosine-protein kinase receptor (*INSR*) score 0.984, Insulin-like growth factor-binding protein 1 (*IGFBP1*) score 0.983, Vascular endothelial growth factor A (*VEGFA*) score 0.969, Hepatocyte growth factor (*HGF*) score 0.952, Insulin-like growth factor-binding protein 2 (*IGFBP2*) score 0.948 and Tyrosine-protein kinase receptor or Insulin receptor-related receptor (*INSRR*) score 0.939. As shown in Fig. 2, among the genes that are associated with the *IGF1* gene, *IGFR1*, *IGFBP4*, *IGFBP3* and *IGFBP5* genes have the most and closest relationship with *IGF1* gene. For example, the association and interaction of the *IGF1* gene and *IGFR1* gene has been established and reported based on curated databases and experimentally determined (known Interactions), based on gene neighborhood, gene fusions and gene co-occurrence (predicted

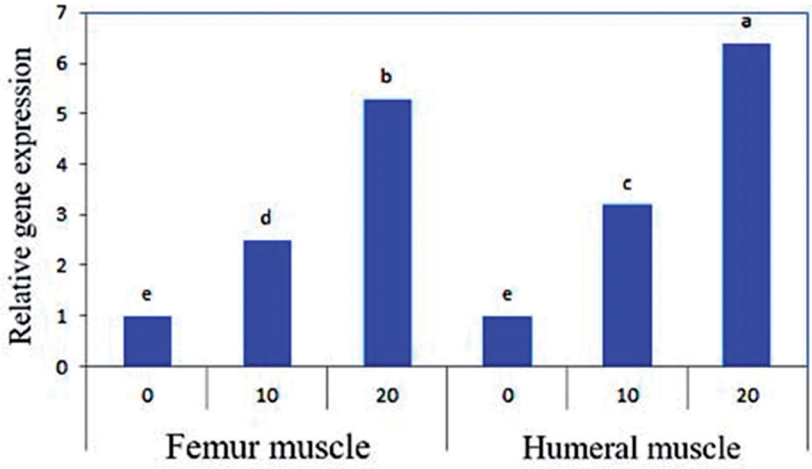

Figure 1. Compare the effect of adding fennel at 3 levels (0, 10 and 20 g/kg DM) on *IGF1* gene expression in the humeral muscle and femur muscle in studied animals. LSD test ($p < 0.05$) was used for comparison of means (mean of three replications). The mean of at least one common alphabet is not statistically significant ($p < 0.05$).

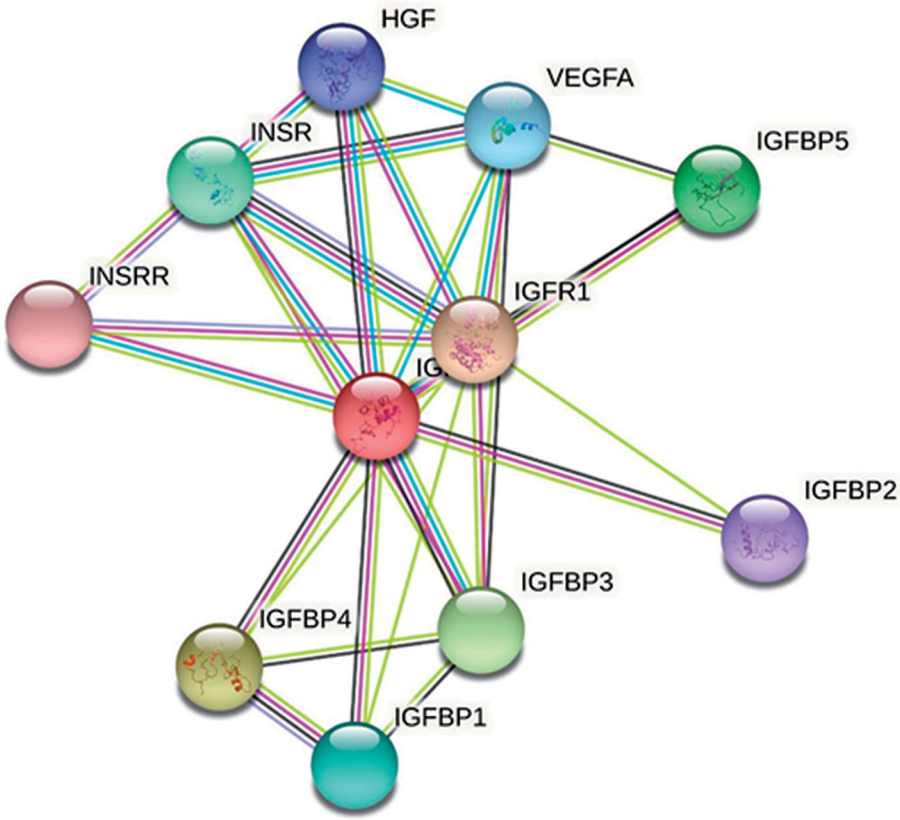

Figure 2. *IGF1* gene and other predicted genes interactions and the definition of predicted functional partners using the STRING program in sheep. Tyrosine-protein kinase receptor (IGFR1) score 0.999, Insulin-like growth factor-binding protein 4 (IGFBP4) score 0.991, insulin-like growth factor binding protein 3 (IGFBP3) score 0.988, insulin-like growth factor binding protein 5 (IGFBP5) score 0.986, Tyrosine-protein kinase receptor (INSR) score 0.984, Insulin-like growth factor-binding protein 1 (IGFBP1) score 0.983, Vascular endothelial growth factor A (VEGFA) score 0.969, Hepatocyte growth factor (HGF) score 0.952, Insulin-like growth factor-binding protein 2 (IGFBP2) score 0.948 and Tyrosine-protein kinase receptor or Insulin receptor-related receptor (INSRR) score 0.939.

Interactions) and based on text mining, co-expression and protein homology. While genes *IGFBP2* and *INSRR* have the least and most distant association with the *IGF1* gene. For example, the association and interaction of the *IGF1* gene and *INSRR* gene has been established and reported only based on

experimentally determined, text mining and protein homology.

Discussion

In this research, we studied the effect of different levels (0, 10 and 20 g/kg DM) of fennel in the ratio on growth characteristics and on expression of *IGF1* gene in the humeral muscle and femur (leg) muscle of Kermani sheep. The mean of cycle threshold (Ct) value for *IGF1* differs from 23 to 25 in different studied tissues, which indicates the high level of the transcript abundance for *IGF1* in these tissues.[54] Although other factors such as the amount of cDNA, instrument settings and Real-Time PCR performance also affect the mean of cycle threshold (Ct) value. *IGF1* gene was expressed in both studied tissues (humeral muscle and femur muscle) of Kermani lambs. In confirmation of our results, various studies showed the *IGF1* gene expresses in the muscle of sheep.[25,55–58] Su et al.[56] studied *IGF1* gene expression in longissimus dorsi of Hu sheep and showed that *IGF1* gene expression gradually increased from birth and reached the peak at 6 months of age and significantly ($p < 0.01$) is greater than from birth to 4 months. Wang et al.[59] demonstrated that there is a correlation between *IGF1* gene expression and the development stage of cells and their origin. They also showed that this gene is upregulated in muscle cells of sheep and rats. On the other hand, Ernst et al.[60] reported that the expression of *IGF2* gene in satellite cells of turkey from the stage of proliferation to differentiation is decreasing. Furthermore, Crown et al.[61] and Kocamics et al.[62] reported that the expression of *IGF1* gene is not recognizable in satellite cells of humans and avians, respectively. Wang et al.[59] studied the expression patterns of *IGF* in vitro for muscle cells and showed that myogenesis is autocrine-regulated by *IGFs*. Su et al.[56] demonstrated that *IGF1* gene expression at various steps of growth after birth in the longissimus dorsi of ram is higher than ewe and proposed that there is a correlation between these differences at expression levels and steps of growth. Vice versa, in another study, Xu[63] reported that there is no significant difference in *IGF1* gene expression level of longissimus dorsi between females and males. Researchers suggested that different *IGF1* gene expression levels between the muscle of male and female animals are probably due to the different species and varieties of studied animals.[56,64,65]

Increasing the level of fennel in the diet showed a positive correlation with increasing the expression level of *IGF1* gene in our current study (Fig. 1). Thissen et al.[41] reported that for the normal synthesis and secretion of *IGF1*, adequate nutrition is essential. Oldham et al.[66] demonstrated that nutrition regulates *IGF1* peptide concentrations in plasma of sheep, while the first 24–72 h of fasting have no effect on the concentration of muscle *IGF1* mRNA. In another study, Jeanplong et al.[25] showed that *IGF1* gene expression level in the semitendinosus muscle of underfed sheep in comparison with normally fed sheep is lower and concluded that nutritional status affects muscle *IGF1*.[67] Many researchers[25,68–70] showed that there is a positive association between *IGF1* mRNA concentration and peptide frequency in skeletal muscle within disorders causing atrophy or hypertrophy of muscle. It is proven that there is a correlation between muscle loss and reduction in *IGF1* gene expression level.[25] These results are compatible with the autocrine/paracrine *IGF1* task in motivating anabolic operations and development of muscle. Muscle development, perception of amino acid, increasing protein synthesis and preventing protein degeneration are *IGF1* anabolic effects.[25,39,71] In vivo reproduction and differentiation of satellite cells is motivated by *IGF1*.[25,36,37,70,72] Masoudzadeh et al.[8] reported that adding fennel to rations of sheep is useful for progressing muscle structure (increasing muscle fiber size and muscle volume). Considering these reported results and affect of fennel on promoting the expression of *IGF1* gene and the *IGF1* gene task in increasing size of muscle, particularly in the early months of growth, this result can be reached that adding fennel in the ration of lamb diets is useful and could be act as a natural appropriate and advantageous grower for sheep breeding.

Considering that the animals fed with 1 g/kg DM and 2 g/kg DM fennel ate dry matter rather than animals used ration without fennel (Table 2), this result can be reached that adding fennel to the diet of animals can increase dry matter intake. This may be because fenchone and anethole (two of the main compounds found in fennel) make the diet more palatable and increase the animal's consumption. Anethole is a main component of fennel. It is an organic compound that is widely used as a flavoring substance and is a derivative of phenylpropene, a type of aromatic compound that occurs widely in nature, in essential oils. Fenchone is an organic compound classified as a monoterpenoid and a ketone. It is a colorless oily liquid. Fenchone is a constituent of the essential oil of fennel. Fenchone is used as a flavor in foods and in perfumery. Anethole and estragole have been reported to have stimulating effects on appetite.[73] In Holstein

dairy calves, the addition of 0.4% and 0.8% fennel (based on the dry matter) to the starter diets has been shown to increase dry matter intake.[74] Unlike other studies, Zolfaghari Moheb et al.[75] reported that adding by-products of fennel to the ratio of lambs in the growing stage does not affect dry matter intake consumption. In another study, Asemi Esfahani et al.[76] also showed that adding anise seed powder (0.25 and 0.5%) in rations of the suckling calf does not affect dry matter intake consumption.

As shown in Table 2, with the increase in fennel consumption at the level of g/kg DM, compared to the control, the lean meat weight, femur muscle and back muscle (loin) has increased, which may be due to the enhanced *IGF1* gene expression in the respective muscle tissue. However, more feed intake and estrogenic effect of fennel can be other factors of this increase that should be considered and in future studies, the share of each of these factors should be separated. Fennel probably affects muscle tissue through the estrogenic effects of its essential oils, like that anethole compounds.[77] Because estrogens can affect the protein kinase B manufacture, leading to more glucose entering the muscle tissue.[78] Also, since steroid hormones receptors exist on every bone cell, they can enhance lean muscle mass and bone mass.[79] In another study, Saeedi et al.[74] showed that the bodyweight of Holstein dairy calves is affected by the positive effects of estrogen in fennel.

In our recent study, as shown in Table 2, animals fed with fennel consumed more dry matter than animals in the control group, and in terms of *IGF1* gene expression levels, animals fed with fennel (1 g/kg DM and 2 g/kg DM) in comparison with the control group had higher gene expression in the humeral muscle and femur muscle tissues (Fig. 1). Therefore, this result can be reached that adding fennel to the diet of lambs can increase muscle production and meat production in general. Although some of this increase occurs through increased feed intake or estrogenic effect of fennel, a percentage of this enhancement can also occur via increased expression of *IGF1* gene in the muscle tissues. In our research, animals fed with a diet containing 2 g/kg DM fennel had higher back muscle (loin) weight, lean meat weight, femur muscle weight and weight of warm carcass than controls, which may be due to the higher final weight of animals receiving fennel. A report has shown that adding herbal antioxidants to the goat diet reduces eye muscle area,[80] which is not consistent with our results. On the other hand, many researchers[25,68–70] showed that there is a positive association between *IGF1* mRNA concentration and peptide frequency in the skeletal muscle causing hypertrophy of muscle. Musarò et al.[81] showed that overexpression of *IGF1* in muscle causes muscle hypertrophy in mice, and vice versa, Mavalli et al.[82] reported that inactivation of *IGF1* receptors in muscle impairs muscle growth by reducing the number and size of muscle fibers. Yu et al.[83] reported that raised expression of Parkinson Protein 7 (*PARK7*; also known as *DJ-1*) in the muscles of callipyge lambs could be conducted to progress muscle growth in reply to the normal *IGF1* signaling present in young growing lambs. Considering that fennel has significantly increased the expression of *IGF1* gene and also the role of this gene in increasing the size and number of muscle fibers, especially at young ages, it can be concluded that the use of fennel at the 2 g/kg DM level in sheep diet can be recommended as a natural and advantageous growth stimulant in the industry of sheep breeding.

According to the tasks and characteristics of different genes (Fig. 2), *IGF1* has an interaction with Tyrosine-protein kinase receptor (*INSR*), Tyrosine-protein kinase receptor (*IGFR1*), and Insulin-like growth factor-binding proteins 4 (*IGFBPs*). In this regard, Wathes et al.[84] studied negative energy balance (NEB) effects on expression of *IGF1* gene expression and its correlated gene and showed that in postpartum dairy cows, the reason for entering the period of negative energy balance (NEB) is a decrease in the amount of circulating *IGF1*, because, in this period, cows have to repair their uterus on a large scale. In the endometrium of severe negative energy balance (SNEB) expression of Insulin-like growth factor-binding protein 1 (*IGFBP1*) and Insulin-like growth factor-binding protein 4 (*IGFBP4*) genes increased and Insulin-like growth factor-binding protein 6 (*IGFBP6*) gene expression decreased. They reported a significant association between *IGF1* gene expression and estradiol receptor 1 (*ESR1*), growth hormone receptor (*GHR*), *IGFBP6* and Insulin-like growth factor-binding protein 2 (*IGFBP2*) genes expression. *IGF1*, along with insulin-like growth factor II (*IGF2*), growth hormone (*GH*), growth hormone-releasing hormone (*GHRH*), and their related receptors (*IGF2R*, *GHR*, *GHRHR* and *IGF1R*) and binding proteins (BPs) take part in the somatotrophic axis.[23] It can be concluded that when muscles are injured, the *IGF1* gene directly or indirectly plays an important role in better muscle regeneration, repair and growth.

Comparison of the findings of the present investigation with the findings of other studies may suggest that the *IGF1* gene is associated with other genes via

several mechanisms and has pleiotropic effects and can have major and minor effects on different muscles of the body. Moreover, the findings of the current study showed that fennel enhances the *IGF1* gene expression in muscle tissue.

Conclusion

According to the findings of the current investigation, this result can be reached that fennel, by creating positive effects on *IGF1* gene expression can be used to improve muscle structure (by increasing fiber size and increasing muscle mass). Because fennel has increased *IGF1* gene expression in muscle tissue, it can be used to increase muscle mass and animal growth in the sheep industry. Although considering the findings of the current study, this result can be reached that adding fennel is practical for different purposes in the sheep industry, but it is suggested that in future research, various physiological, epigenetic and genetic conditions should be considered to overtake conclusive decision. In addition, the findings of this study investigated the effects of different levels of fennel on gene expression in sheep opens the novel orientation to more wide investigation in this sector.

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ORCID

Mojtaba Shahsavari http://orcid.org/0000-0001-5646-8428
Mohammadreza Mohammadabadi http://orcid.org/0000-0002-1268-3043
Amin Khezri http://orcid.org/0000-0002-5371-9831
Masoud Asadi Fozi http://orcid.org/0000-0002-4150-1835
Olena Babenko http://orcid.org/0000-0002-8404-3272
Oleksandr Kalashnyk http://orcid.org/0000-0003-2354-3473
Valentyna Oleshko http://orcid.org/0000-0002-7323-7813
Serhii Tkachenko http://orcid.org/0000-0002-1437-8392

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