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Differential protein input in the maternal diet alters the skeletal muscle transcriptome in fetal sheep

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Abstract

Maternal nutrition during pregnancy is one of the major intrauterine environmental factors that influence fetal development by significantly altering the expression of genes that might have a consequence on the physiological, morphological, and metabolic performance of the offspring in the postnatal period. The impact of maternal dietary protein on the expression of genes in sheep fetal skeletal muscle development is not well understood. The current study aims to investigate the impact of high and low maternal dietary protein on the holistic mRNA expression in the sheep fetal skeletal muscle. Dams were exposed to an isoenergetic high-protein diet (HP, 160–270 g/day), low-protein diet (LP, 73–112 g/day), and standard protein (SP, 119–198 g/day) diets during pregnancy. Fetal skeletal muscles were obtained at the 105th day of pregnancy and mRNA expression profiles were evaluated using Affymetrix GeneChip™ Ovine Gene 1.0 ST Array. The transcriptional analysis revealed a total of 323, 354, and 14 genes were differentially regulated (fold change > 2 and false discovery rate ≤ 0.05) in HP vs. SP, LP vs. HP, and SP vs. LP, respectively. Several myogenic genes, including *MYOD1*, *MYH2*, *MYH1*, are significantly upregulated, while genes related to the immune system, such as *CXCL11*, *HLA-E*, *CXCL10*, *CXCL9*, *TLRs*, are significantly downregulated in the fetal muscle of the HP group compared to those of SP and LP group. Bioinformatic analysis revealed that the majority of these genes are involved in pathways related to the immune system and diseases. The results of our study demonstrate that both augmented and restricted dietary proteins in maternal diet during pregnancy alter the expression of genes as well as the offspring's genetic marks.

Introduction

The intrauterine environment plays a crucial role in fetal-placental development that has a consequence in the lifelong health and productivity of offspring. The fetus is particularly sensitive to the mother's diet during pregnancy. Nutrition in pregnancy plays an essential role in placental development, organogenesis, and fetal growth (Lin et al. 2019). Altered maternal nutrition at the time of pregnancy may cause permanent changes in the physiology, structure, and metabolism of the developing conceptus (Wallace et al. 2004). A maternal nutritional restriction has been shown to have negative consequences on fetal development. It has been shown that the diet lack of energy resulted in the development of a higher number of heterogenous fetuses in pigs (Che et al. 2017). In addition, maternal feed restriction during mid- to late gestation critically influences the phenotypic development of the fetus as well as the expression of growth and myogenic genes in the muscle in beef cattle (Paradis et al. 2017). Due to differential maternal nutrition, rapid cell divisions occur in various tissues of the fetal body and may alter the expression of the fetal genome that may have lifelong consequences, a phenomenon known as fetal programming.

Meat is one of the important end products of the livestock industry. Meat-producing animals are typically reared

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for their skeletal muscle. The overall meat production of an animal crucially depends on the number of muscle fibers. It is important noting that since the number of muscle fibers does not increase in the postnatal stage, the fetal stage is extremely important for skeletal muscle development in mammalian livestock (Du et al. 2015). In mammals, embryonic muscle fiber development, also known as myogenesis, is a biphasic process. The primary muscle fibers developed first and serve as a scaffold for the secondary muscle fiber development. Both the primary and secondary muscle fibers are developed in the early- to mid-gestation. Since the majority of muscle fibers are formed at the fetal stage, an adequate amount of nutrients are required for myogenesis (Rehfeldt and Kuhn 2006); hence, the development of both primary and secondary muscle fibers is vulnerable to maternal nutrient alterations (Zhu et al. 2004; Che et al. 2017). On the other hand, compared to the heart and brain, skeletal muscle has a lower priority for nutrient distribution, which makes the myogenesis process even more vulnerable to nutritional deficiency (Zhu et al. 2004). It is important to note that maternal nutritional deviation has no major impacts on the muscle fibers once they become mature (approximately day 105 of gestation in sheep) (Picard et al. 1995).

In utero fetal growth is the result of genetic potential orchestrated by the maternal nutrient supply (Cetin et al. 2007). However, genomic variation focused on DNA markers not always only explains the main portion of the phenotypic variance in complex traits. A puzzling portion of the genetic variation in livestock complex traits is likely nested in the epigenome and remains to be exploited (Ibeagha-Awemu et al. 2008). Studies that are associating nutritional fetal programming to complex traits in livestock were initiated relatively recently. These studies showed both malnutrition and over-nutrition during gestation impact offspring growth performance in different livestock species including in pigs (Altmann et al. 2012), in beef cattle (Wang et al. 2015), and in sheep (Peñagaricano et al. 2014). Among different macro- and micronutrients, protein is one of the key macronutrients involved in both structural (keratin, collagen) and functional (enzymes, protein transport, hormones) biological roles (Mousa et al. 2019). The effect of maternal protein diets on postnatal growth traits in cattle and sheep have been previously reported in the literature (Radunz et al. 2011, 2012). However, research on understanding the genetic mechanisms underlying how maternal protein intake regulates fetal skeletal muscle development in sheep is kind of vague. It is important noting that maternal nutrition during pregnancy has a pivotal role in the regulation of placental-fetal development and muscle growth as well as the quality and quantity of the produced meat. Therefore, this work aimed to report the effect of maternal dietary protein source during early- to mid-gestation on transcriptome expression of fetal skeletal muscle tissues in sheep.

Materials and method

Animals and diets

Three differential protein (standard, high, and low protein) diet was used in the experiment to evaluate the effect of maternal dietary protein on the transcriptome of fetal skeletal muscle. A total of 12 two-year-old Akkaraman ewes were randomly placed in three treatment groups, 4 ewes in each group. The ewes were selected from the university research center according to their age and body weight. Ewe specifications are presented in Table S1. After a 15-day adaptation period, the ewe's daily dry matter (DM) feed consumption were determined and compared with NRC (2007) recommendations for ewes. The ewes had a similar daily DM feed consumption. Three different protein content diets (based on wheat straw, alfalfa, soybean meal, barley, and corn grain) were formulated. The primary sources of proteins in the diet were soybean meal and alfalfa, while the main energy sources were barley and corn grains. Other cellulose/energy sources were wheat straw and alfalfa. The protein content was adjusted by replacing the rate of soybean meal and alfalfa in the diets. The ewes had a very similar daily DM feed consumption. The ewes were fed from 0 to 30 days of gestation period according to NRC (2007) recommendations. During day 31–105 of gestation, ewes fed the following diets—1: standard diet, NRC level of the nutrient composition of feeds (daily, DM feed intake 1252.3 to 1862.4 g, crude protein 119.4 to 198.6 g and 2506.0 to 3909.3 kcal ME/kg), 2: low-protein diet (daily, DM feed intake 1247.9 to 1726.4 g, crude protein 73.1 to 112.2 g and 2520.1 to 3649.0 kcal ME/kg), and 3: high-protein diet (daily, DM feed intake 1252.4 to 1829.8 g, crude protein 160.0 to 271.6 g and 2513.0 to 3880.9 kcal ME/kg). Diets were consisting of alfalfa, wheat straw, barley, corn, and soybean meal. The chemical composition of feed materials was determined according to A.O.A.C. (1990) in the feed analysis laboratory. Diets were prepared as isoenergetic. Detail information regarding diet is presented in supplementary Tables S2 and S3.

Estrus synchronization, semen preparation, and transcervical AI

Progestogenated pessaries (EaziBreed CIDR; 1.38 mg progesterone; Zoetis, Turkey) for 12 days, 2 ml of PGF2α (5 mg/ml Dinoprost tromethamine; Dinolytic; Zoetis, Turkey) treatment on Day 12, and 400 IU of eCG (Folligon, 1000 IU/vial PMSG, MSD Animal Health, Turkey) at the time of pessary removal were used for estrus synchronization during the annual breeding season.

The semen used in the study was taken from four 1 to 3 aged rams by the artificial vaginal method in the presence of a ewe in estrus. Semen obtained by the artificial vaginal method was kept in a collection cup and the motility was determined with a phase-contrast microscope with a hot plate at 37 °C. Ejaculates of four rams with a motility of 70% and above were mixed and used for the insemination of ewes. The spermatologically motility of the appropriate ejaculates was mixed to ensure homogeneity. The density of mixed ejaculates was determined by the hemocytometric method. The semen was stored in a water bath at 35 °C and diluted with Trisma base, D-Fructose, Citric acid, and skimmed milk (Paulenz et al. 2002) to achieve 0.5 mL semen containing 150 × 106 motile spermatozoa. Artificial insemination was intravaginally performed 48 h after sponge removal using diluted fresh semen.

Necropsy and sample collection

The dams were necropsied on day 105 of gestation. A total of 14 fetuses were collected from 12 dams. There were two twine pregnancies: one in standard protein and another low-protein group and twin birth fetuses were removed from the experiment to avoid any growth bias due to multiple births. In addition, one fetus from a high-protein group was also removed from the experimental group to have 3 fetuses in each experimental group. Longissimus dorsi muscles were removed from the left side of the fetus and a muscle tissue sample was collected. All tissues were snap-frozen in liquid nitrogen and kept at − 80 °C until RNA extraction was performed.

Extraction of total RNA

miRNeasy Mini kit (Qiagen, Hilden, Germany) was used to isolate the total RNA from tissue samples (~ 30 mg) according to the manufacturers' protocol. An on-column DNA digestion step was performed using the RNase-Free DNase Set (Qiagen, Hilden, Germany) according to the manufacturers' protocol. The purity and concentration of the total RNA were determined with a BioSpec-nano micro-volume UV–Vis spectrophotometer (Shimadzu Co., Kyoto, Japan).

mRNA microarray analysis

Isolated total RNA samples were used to synthesize cDNA and microarray analysis. Labeling and hybridizations were performed based on the protocol provided by Affymetrix. In brief, approximately, 100–300 ng of total RNA was amplified and labeled using the Ambion WT Expression Kit for Affymetrix GeneChip (Invitrogen, Carlsbad, CA, USA) and then hybridized to the GeneChip Ovine Gene 1.0 ST Array (Catalog number: 901972; Affymetrix, Santa Clara, CA, USA). GeneChip Instrument System of Affymetrix was used for washing and scanning (GeneChip Hybridization Oven 640, GeneChip Fluidics Station 450, and GeneChip Scanner 3000 7G). The 169-format GeneChip™ Ovine Gene 1.0 ST Array contains approximately 26 probes that were designed across the full length of 22,047 well-annotated genes for sheep. The design of the array was based on the September 2009 Sheep Reference Genome Assembly OARv2.0 with comprehensive coverage of RefSeq (https://www.affymetrix.com/support). The GeneChip Ovine Gene 1.0 ST Array has 100 percent coverage of NM sequences present on April 3, 2007, RefSeq database (https://www.affymetrix.com/support). Data analysis were performed using Affymetrix Expression Console software (v.1.4; Affymetrix UK Ltd) that provides signal estimation and quality control functionality for the GeneChip Expression Arrays, and the Affymetrix Transcriptome Analysis Console (TAC) Software (Affymetrix UK Ltd), which performs statistical analysis and provides a list of differentially expressed genes. Expression level analysis was performed using the normalization method based on the processing algorithm called limma procedure (Ritchie et al. 2015). Expression was considered significant when the fold change is > 2 and false discovery rate is ≤ 0.05.

Bioinformatics analysis

David Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/) was used to identify the most affected pathways and GO terms affected by the differentially regulated genes. Significantly enriched pathways were selected based on p value ($p \leq 0.05$). The pathways are presented according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/). Differentially expressed genes were then uploaded to STRING version 11 (https://string-db.org/) to identify protein–protein interaction networks and functional enrichment analysis.

Results

Expression of differentially regulated genes

The expression of genes was investigated using a microarray platform. The gene chip contains 508,538 probes covering a total of 22,047 genes all over the ovine genome. To identify the quality of the data and potential experimental bias, fold change and p value of each gene from all the comparisons are plotted in a scatter plot and the scatter plot is presented in supplementary figure, Fig. S1.

Standard protein vs. high protein

Among the genes investigated, a total of 323 genes were expressed differentially in the HP group compared to the SP group. Of these, 37 genes and 286 genes were upregulated and downregulated, respectively (Fig. 1a). Among the upregulated genes, the eukaryotic translation elongation factor 1 alpha 2 (*EEF1A2*) gene showed the highest fold change regulation (− 4.44), while the chemokine (C-X-C motif) ligand (CXCL) family, particularly *CXCL10,* exhibited the

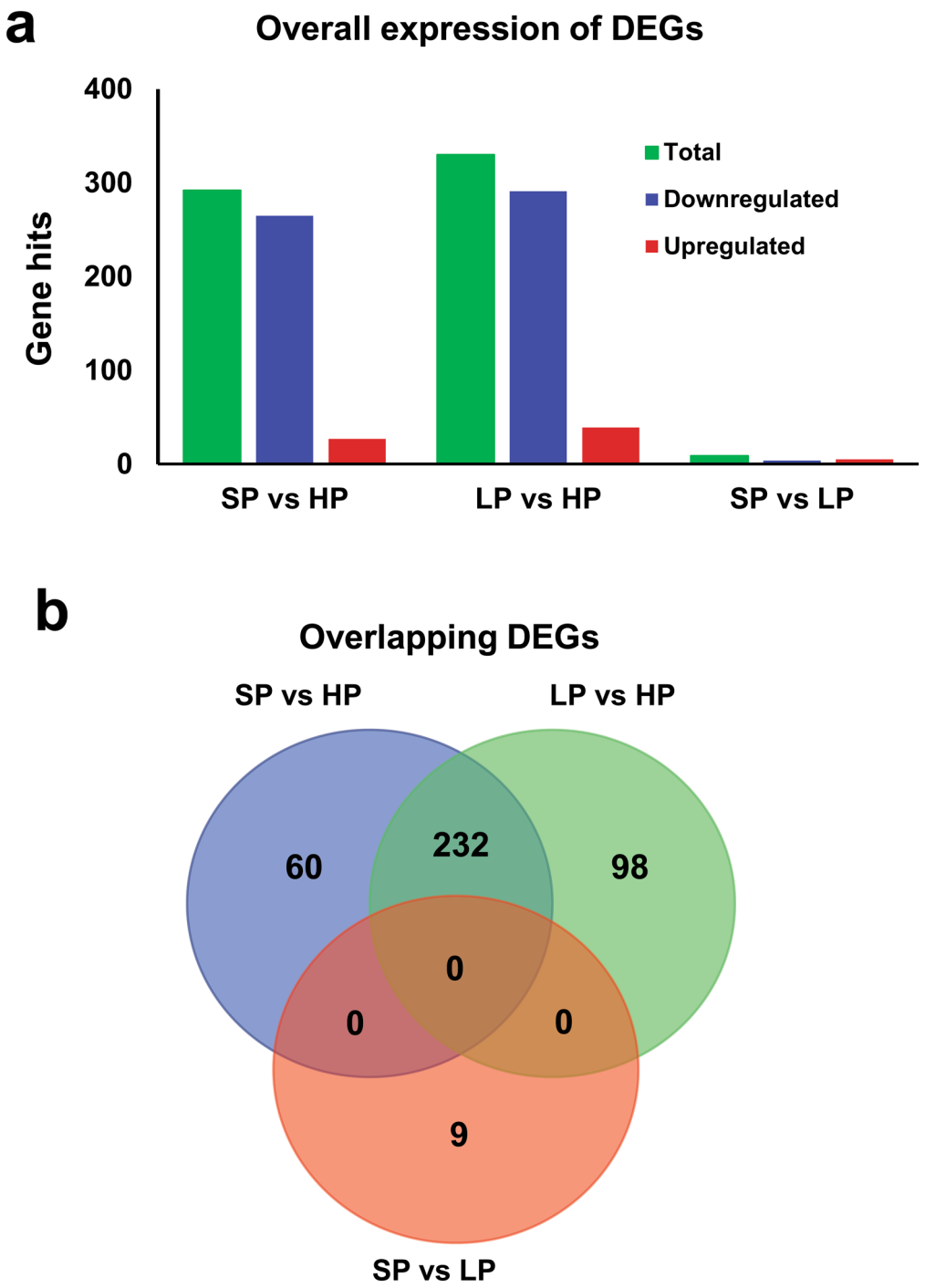


Fig. 1 Differentially regulated genes in different comparisons in fetal skeletal muscles. **a** Graphical representation of significantly differentially regulated genes (fold change > 2, $p < 0.05$) in fetal skeletal muscles derived from high, low, and standard protein groups. **b** Venn diagram representing the overlapping genes among three different comparisons

highest fold change regulation (49.71-fold) among the downregulated genes (Table 1). The complete list of differentially regulated genes in SP vs. HP comparison is presented in supplementary Table S4.

Low protein vs. high protein

The highest number of differentially regulated genes was obtained in LP vs HP comparison. A total of 354 genes were expressed differently in the HP group in comparison to those of the LP group (Fig. 1a). Among the differentially expressed genes, 43 genes were highly expressed, and 311 genes have shown reduced expression levels. Of the upregulated genes, *FOS* showed the highest fold change regulation (−7.77), while *CXCL9* showed the highest fold change regulation (66.73) among the downregulated genes (Table 1). The complete list of the differentially regulated genes is presented in Table S5.

Standard protein vs. low protein

Surprisingly, a few numbers of differentially regulated genes were observed in SP vs LP comparison. We found only 14 genes were differentially expressed in SP vs. LP comparison (Fig. 1a). Among the differentially regulated genes, 6 genes were upregulated, while 8 genes were downregulated. The top upregulated gene was LOC101118184 (docosahexaenoic acid omega-hydroxylase CYP4F3-like) with a fold change of 2.95 and the top downregulated gene was *FABP7* with −2.25-fold change (Table 1). The list of differentially regulated genes is presented in Table S6.

Overlapping of differentially regulated genes

Next, we evaluated the overlapping possibilities of differentially regulated genes obtained from different group comparisons and the results are presented in Fig. 2b. When we compared the differentially regulated genes derived from SP vs. HP and LP vs. HP, we found that a total of 232 genes are detected in common which means 232 genes are differentially regulated in the HP group. We did not find any common genes by comparing SP vs. HP and SP vs. LP or SP vs. LP and LP vs. HP as the number of differentially regulated genes was very low (Fig. 1b). In addition, we did not find any differentially regulated gene that is common in all three comparisons. To understand the biological function of 232 overlapping genes, we performed pathway analysis and gene ontology. Pathway analysis revealed that a total of 39 pathways are significantly ($p < 0.05$) affected by these genes where Staphylococcus aureus infection, Cell adhesion molecules (CAMs), Phagosome, Antigen processing and presentation, and Viral myocarditis are the top pathways (please see Supplementary Table S7 for the full list). On the other hand, the functional annotation and gene ontology analysis detected a total of 71 biological processes, 12 cellular components, and 6 molecular functions are significantly affected by these genes. As expected, the majority of these biological processes, cellular components, and molecular functions are also detected in SP vs. HP and LP vs. HP comparison (Supplementary Table S8).

Table 1 Top differentially regulated genes from each comparison

	Upregulated		Downregulated	
	Gene name	Fold change	Gene name	Fold change
SP vs HP	*EEF1A2*	4.44	*CXCL10*	−49.71
	LOC442994	3.68	*CXCL9*	−41.21
	PPP1R1A	3.12	*CXCL11*	−32.16
	CKMT2	2.77	*UBD*	−25.53
	LOC101109939	2.49	*LOC101109492; LOC101108696*	−23.89
LP vs HP	*FOS*	4.77	*CXCL9*	−66.73
	FGL1	3.51	*UBD*	−48.04
	EEF1A2	3.35	*CXCL10*	−45.74
	LYNX1	3.03	*HLA-E*	−40.24
	METTL21C	3	*CXCL11*	−30.8
SP vs LP	*CYP4F3; LOC509506*	2.95	*FABP7*	−2.25
	TUBA8	2.37	*GSTA1-1; GSTA2*	−2.2
	SPTLC3	2.13	*LOC101108945; LOC105602254; LOC105613207*	−2.07
	MYLK3	2.12	*C7H14orf37; C10H14orf37*	−2.06
	RAB2	2.1		

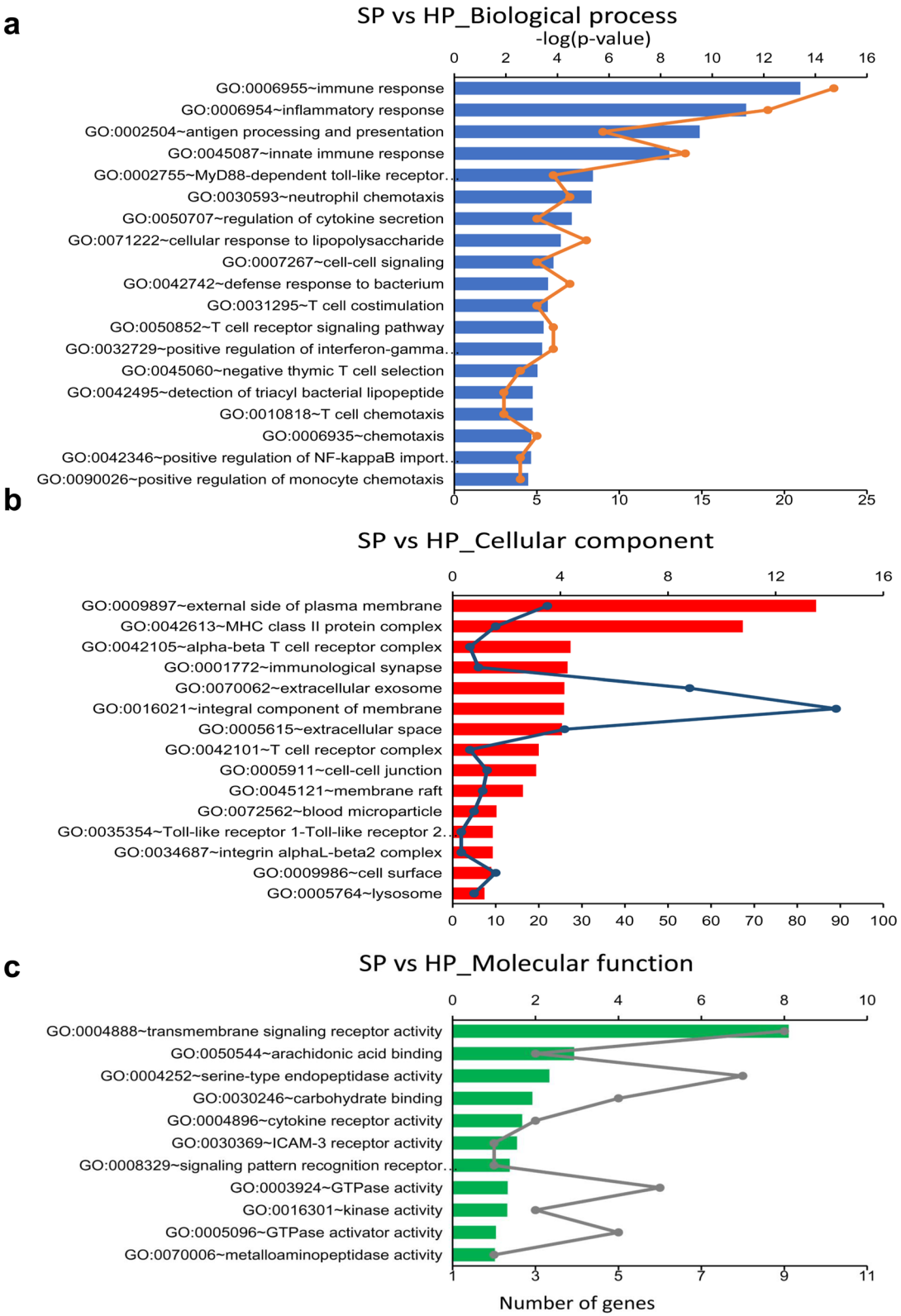


Fig. 2 Functional annotation cluster and gene ontology analysis of the genes derived from SP vs. HP comparison. **a** Biological process, **b** Cellular component, and **c** Molecular function. The bar diagram is representing the -log(*p* value) of each GO category, while the line diagram is representing the number of genes involved in each category

Pathway analysis of differentially regulated genes

To understand the biological relevance, we submitted the list of these differentially regulated genes to David bioinformatics, and the involvement of genes in different pathways was presented according to the KEGG pathway system.

Standard protein vs. high protein

A total of 44 canonical pathways are affected by the differentially regulated genes in SP vs. HP comparison. Among these pathways, 41 pathways were significantly regulated (Table S9). The top 15 pathways are presented in Table 2. Among the top 15 pathways, the majority of the pathways are related to disease and immune systems including Staphylococcus aureus infection, phagosome, tuberculosis, antigen processing and presentation, and inflammatory bowel disease (Table 2). It is important to note that the *Ovar-DRB1* gene is present in 12 pathways out of 15 top canonical pathways. The *Ovar-DRB1* gene is known to be played a crucial role in adaptive immune response and antigen processing and presentation of peptides.

Low protein vs. high protein

The differentially regulated genes obtained from LP vs. HP comparison are involved in regulating a total of 49 canonical pathways where 46 pathways were significantly enriched (Table S10). Interestingly, out of the top 15 pathways in LP vs. HP comparison, a total of 12 pathways are common with SP vs. HP comparison (Table 3). *OVAR-DRB1* gene seems to be the most important gene in the pathways of LP vs. HP comparison as it is appeared in 13 pathways out of 15 top pathways.

Table 2 Differentially regulated genes and pathways affected in SP vs HP comparison

Pathway name	Number of genes	*p* value	Top 10 genes
oas05150: Staphylococcus aureus infection	21	1.69E-18	C3AR1, ITGAL, DQA, C5AR1, ITGB2, **OVAR-DRB1**, C1S, OVAR-DRB3, C1QC, LOC101123419
oas04145: Phagosome	23	3.04E-12	MRC1, DYNC1I1, DQA, MSR1, LOC101108171, TLR2, ITGB2, TLR4, **OVAR-DRB1**, CTSS
oas04514: Cell adhesion molecules (CAMs)	22	3.89E-12	ITGAL, PTPRC, DQA, CD8B, LOC101108171, ITGB2, **OVAR-DRB1**, ITGA4, OVAR-DRB3, CDH5
oas05140: Leishmaniasis	17	4.14E-12	PTPN6, DQA, PTGS2, TLR2, ITGB2, TLR4, ITGA4, **OVAR-DRB1**, STAT1, OVAR-DRB3
oas05152: Tuberculosis	23	2.37E-11	MRC1, DQA, IL18, TLR1, TLR2, ITGB2, TLR4, **OVAR-DRB1**, CTSS, STAT1, TLR6, OVAR-DRB3
oas04612: Antigen processing and presentation	16	9.01E-11	DQA, LOC101108171, CD8B, CTSS, **OVAR-DRB1**, OVAR-DRB3, CD74, B2M, LOC101107908, OVAR-DQA1
oas05323: Rheumatoid arthritis	17	1.06E-10	ITGAL, DQA, IL18, TLR2, ITGB2, TLR4, **OVAR-DRB1**, CCL5, OVAR-DRB3, OVAR-DQA1
oas05321: Inflammatory bowel disease (IBD)	15	1.56E-10	DQA, IL18, TLR2, TLR4, **OVAR-DRB1**, STAT1, OVAR-DRB3, IL12RB2, OVAR-DQA1, LOC101109746
oas05416: Viral myocarditis	15	2.25E-10	OVAR-DQA1, ITGAL, CD86, DQA, RAC2, LOC101109746, LOC101108171, LOC101109747, ITGB2, **OVAR-DRB1**
oas05322: Systemic lupus erythematosus	18	5.65E-09	DQA, **OVAR-DRB1**, C1S, OVAR-DRB3, C1QC, LOC101123419, C1QA, C1QB, OVAR-DQA1, CD86
oas04672: Intestinal immune network for IgA production	12	9.37E-09	OVAR-DQA1, CD86, DQA, LOC101109746, LOC101109747, ICOS, LOC101108696, ITGA4, **OVAR-DRB1**, OVAR-DRB3
oas04620: Toll-like receptor signaling pathway	15	1.28E-08	PIK3CG, LOC101114285, TLR1, TLR2, CXCL9, TLR4, CXCL11, TLR6, CCL5, STAT1, TLR7, TLR8, CXCL10
oas05332: Graft-versus-host disease	12	1.37E-08	OVAR-DQA1, CD86, DQA, LOC101109746, LOC101108171, LOC101109747, LOC101108696, **OVAR-DRB1**, LOC101109492, OVAR-DRB3
oas04610: Complement and coagulation cascades	13	3.31E-08	C3AR1, C7, C5AR1, CR2, F13A1, SERPING1, C1S, C1QC, LOC101123419, C1QA
oas05330: Allograft rejection	12	3.36E-08	OVAR-DQA1, CD86, DQA, LOC101109746, LOC101108171, LOC101109747, LOC101108696, **OVAR-DRB1**, LOC101109492, OVAR-DRB3

Top enriched KEGG pathways obtained from DAVID (*p* values Fisher's exact test $p < 0.05$)

Table 3 Differentially regulated genes and pathways affected in LP vs HP comparison

Pathway name	Number of genes	*p* value	Top 10 genes
oas05150: Staphylococcus aureus infection	21	1.30E-17	C3AR1, ITGAL, DQA, C5AR1, ITGB2, **OVAR-DRB1**, C1S, OVAR-DRB3, C1QC, LOC101123419, C1QA, OVAR-DQA1
oas04514: Cell adhesion molecules (CAMs)	24	4.14E-13	ITGAL, PTPRC, DQA, MPZ, CD8B, LOC101108171, ITGB2, **OVAR-DRB1**, ITGA4, OVAR-DRB3
oas05140: Leishmaniasis	18	1.57E-12	PTPN6, DQA, ITGB2, TLR4, **OVAR-DRB1**, ITGA4, STAT1, OVAR-DRB3, FOS, OVAR-DQA1
oas04612: Antigen processing and presentation	18	2.88E-12	DQA, CD8B, LOC101108171, **OVAR-DRB1**, CTSS, OVAR-DRB3, LOC101107641, CD74, B2M, LOC101107908, OVAR-DQA1
oas04145: Phagosome	24	3.14E-12	MRC1, DQA, MSR1, OLR1, LOC101108171, ITGB2, TLR4, **OVAR-DRB1**, CTSS, TLR6
oas05332: Graft-versus-host disease	15	1.60E-11	DQA, LOC101108171, **OVAR-DRB1**, OVAR-DRB3, LOC101107641, LOC101107908, OVAR-DQA1, CD86, LOC101109746, LOC101109747, IFNG
oas05323: Rheumatoid arthritis	18	4.96E-11	ITGAL, DQA, ITGB2, TLR4, **OVAR-DRB1**, CCL5, OVAR-DRB3, LOC101114535, FOS, OVAR-DQA1
oas05164: Influenza A	23	5.76E-11	PIK3CG, DQA, IFIH1, OAS1, TLR4, **OVAR-DRB1**, STAT1, CCL5, NLRP3, TLR7, OVAR-DRB3
oas05416: Viral myocarditis	16	7.46E-11	ITGAL, DQA, LOC101108171, ITGB2, **OVAR-DRB1**, OVAR-DRB3, LOC101107641, LOC101107908, OVAR-DQA1, CD86
oas04940: Type I diabetes mellitus	15	1.25E-10	DQA, LOC101108171, **OVAR-DRB1**, OVAR-DRB3, LOC101107641, LOC101107908, OVAR-DQA1, CD86, LOC101109746, LOC101109747
oas05152: Tuberculosis	23	1.79E-10	MRC1, DQA, TLR1, ITGB2, TLR4, **OVAR-DRB1**, CTSS, STAT1, TLR6, OVAR-DRB3, CD74, OVAR-DQA1
oas05168: Herpes simplex infection	24	3.66E-10	DQA, IFIH1, LOC101108171, OAS1, **OVAR-DRB1**, STAT1, CCL5, OVAR-DRB3, LOC101107641, CD74
oas05321: Inflammatory bowel disease (IBD)	15	6.02E-10	IL12RB2, OVAR-DQA1, DQA, LOC101109746, LOC101109747, LOC101108696, IFNG, IL-1B, IL2RG, TLR4
oas04620: Toll-like receptor signaling pathway	17	6.50E-10	PIK3CG, LOC101114285, TLR1, CXCL9, TLR4, TLR6, CCL5, STAT1, CXCL11, TLR7, TLR8, CXCL10
oas05330: Allograft rejection	14	7.08E-10	OVAR-DQA1, CD86, DQA, LOC101109746, LOC101108171, LOC101109747, LOC101108696, IFNG, **OVAR-DRB1**, LOC101109492

Top enriched KEGG pathways obtained from DAVID (*p* values Fisher's exact test $p < 0.05$)

Standard protein vs. low protein

Due to the marginal differences in mRNA abundance between SP and LP group, no canonical pathways are found to exhibit diet-dependent alterations in the fetal skeletal muscle.

Functional annotation, cluster and gene ontology analysis

To explore the biological significance of the differentially regulated genes in different comparisons, we next investigated the detailed annotation of biological process, cellular components, and molecular function of these genes using gene ontology (GO) analysis.

Standard protein vs. high protein

A total of 105 biological processes are regulated by the differentially regulated genes derived from SP vs. HP comparison. Out of them, 72 processes are found to be significantly regulated (Table S11). Many of the top biological processes are linked to immunity including immune response, inflammatory response, antigen processing and presentation, innate immune response, MyD88-dependent toll-like receptor signaling, regulation of cytokine secretion, cellular response to lipopolysaccharide, etc. (Fig. 2a). A total of 115 genes are involved in all the biological processes. The most frequently appeared genes are *TLR2* (appeared in 22 BP), *TLR4* (appeared in 21 BP), *CCL5* (appeared in 17 BP), *TLR7* & *FCER1G* (appeared in 13 BP), *CXCL10* (appeared in 11 BP), *CXCL9*, *CXCL11*, *TLR1*, *TLR6* (appeared in 9 BP).

GO analysis of cellular components revealed that the differentially regulated genes derived from SP vs. HP comparison are involved in regulating 15 cellular components, among them 14 are significantly enriched (Table S11). Many of the top cellular components are related to the cell membrane and receptor–ligand complex. For instance, the external side of plasma membrane, MHC class II protein complex, alpha–beta T cell receptor complex, immunological synapse, extracellular exosomes, an integral component of membrane, T cell receptor complex, etc. are the top cellular components (Fig. 2b). Although the integral component of the membrane is the 6th top cellular components according to the *p* value, this category contains the highest number of genes (89) from our gene list (Table S4). Another important cellular component GO term is extracellular exosome which contains 55 genes from our gene list.

A total of 11 molecular functions GO terms are regulated by the differentially regulated genes derived from AP vs HP comparison where 9 GO terms are significantly regulated including transmembrane signaling receptor activity, arachidonic acid-binding, serine-type endopeptidase activity, carbohydrate-binding, cytokine receptor activity, etc. (Fig. 2c). The full list of the GO term of molecular function in this comparison is listed in (Table S11). Important genes involved in this category are *MRC1*, *TLR1*, *TLR2*, *TLR4*, *GZMK*, *ITGAL*, etc.

Low protein vs. high protein

GO analysis revealed that the genes derived from LP vs. HP comparison are involved in regulating a total of 110 biological processes, 15 cellular components, and 14 molecular functions (Table S12). Among 110 biological processes, a total of 77 biological processes are significantly regulated by the differentially expressed genes. Similar to the significantly regulated biological processes in SP vs HP comparisons, we found immune-related biological processes are significantly impacted by the differentially regulated genes derived from LP vs. HP comparison. Top biological processes in this comparison include immune response, inflammatory response, antigen processing and presentation, innate immune response, defense response to a virus, defense response to a bacterium, neutrophil chemotaxis, etc. (Fig. 3a). The topmost important genes in these biological processes are *Ovar-DRB1*, *Ovar-DRB3*, *FYB*, *TLR1*, *TLR2*, *TLR4*, *TLR6*, *TLR8*, *CXCL9*, *CXCL10*, *CXCL11*, *CXCL13*, *PLAC8*, *ITGAL*, etc. (Table S12). Out of 15 cellular components, a total of 13 components were significantly regulated by the differentially regulated genes derived from LP vs. HP comparison (Table S12). Top cellular components in this comparison are the external side of the plasma membrane, MHC class II protein complex, immunological synapse, alpha–beta T cell receptor complex, extracellular space, cell–cell junction, extracellular exosomes, etc. (Fig. 3b). Among the 14 molecular functions, a total of 12 molecular functions were found to be significantly regulated by the differentially regulated genes in this comparison (Table S12). Top significantly regulated molecular functions are transmembrane signaling receptor activity, GTPase activity, double-stranded RNA binding, carbohydrate-binding, oxygen transport activity, GTP binding, etc. (Fig. 3c).

Standard protein vs. low protein

No biological processes, cellular components, and molecular functions were found to be significantly regulated by the genes derived from SP vs. LP comparisons.

Protein–protein interaction (PPI) networks of DEGs

To shed a light on the interaction of differentially regulated genes in different comparisons, corresponding proteins of differentially regulated genes were analyzed using STRING to draw a protein–protein interaction (PPI) pattern.

Standard protein vs. high protein

The PPI network analysis showed that the majority of the corresponding proteins are highly interacted (Fig. 4 and Fig. S2). The PPI analysis of differentially regulated genes using STRING revealed that out of 323 corresponding proteins, a total of 269 proteins are interacting with each other while 54 protein showed no interaction. While the expected number of edges is 108 for similar protein sets, a total of 1106 edges were found in our corresponding protein set indicating the PPI has significantly higher interactions than expected. The cluster analysis showed that the proteins are clustered in three distinct groups (Fig. 4).

In the largest interaction (marked by the blue circle), *CYTH4*, *LCP2*, *CD53*, *ITGB*, *PTPN22*, *PTPRC*, *TYROBP*, and *TLR7* are the central genes, while in the second largest cluster (indicated by the purple circle), *TLR4*, *CXCL10*, *CCR5*, *CXCL11*, and *CCL8* are distributed in the central part. In the smallest cluster (indicated by the red circle), *Ovar-DRB1*, *Ovar-DRB3*, and *EMOES* are the central genes (Fig. 4). The whole PPI is presented in Fig. S2.

Low protein vs. high protein

The PPI networks of the genes derived from LP vs. HP comparison are presented in Fig. 5 and Fig. S3. The PPI analysis revealed that among the 354 differentially regulated genes, a total of 304 corresponding proteins were found to be interacting with each other producing 1399 edges. The number of expected edges is 159 indicating that corresponding proteins of our gene set are significantly

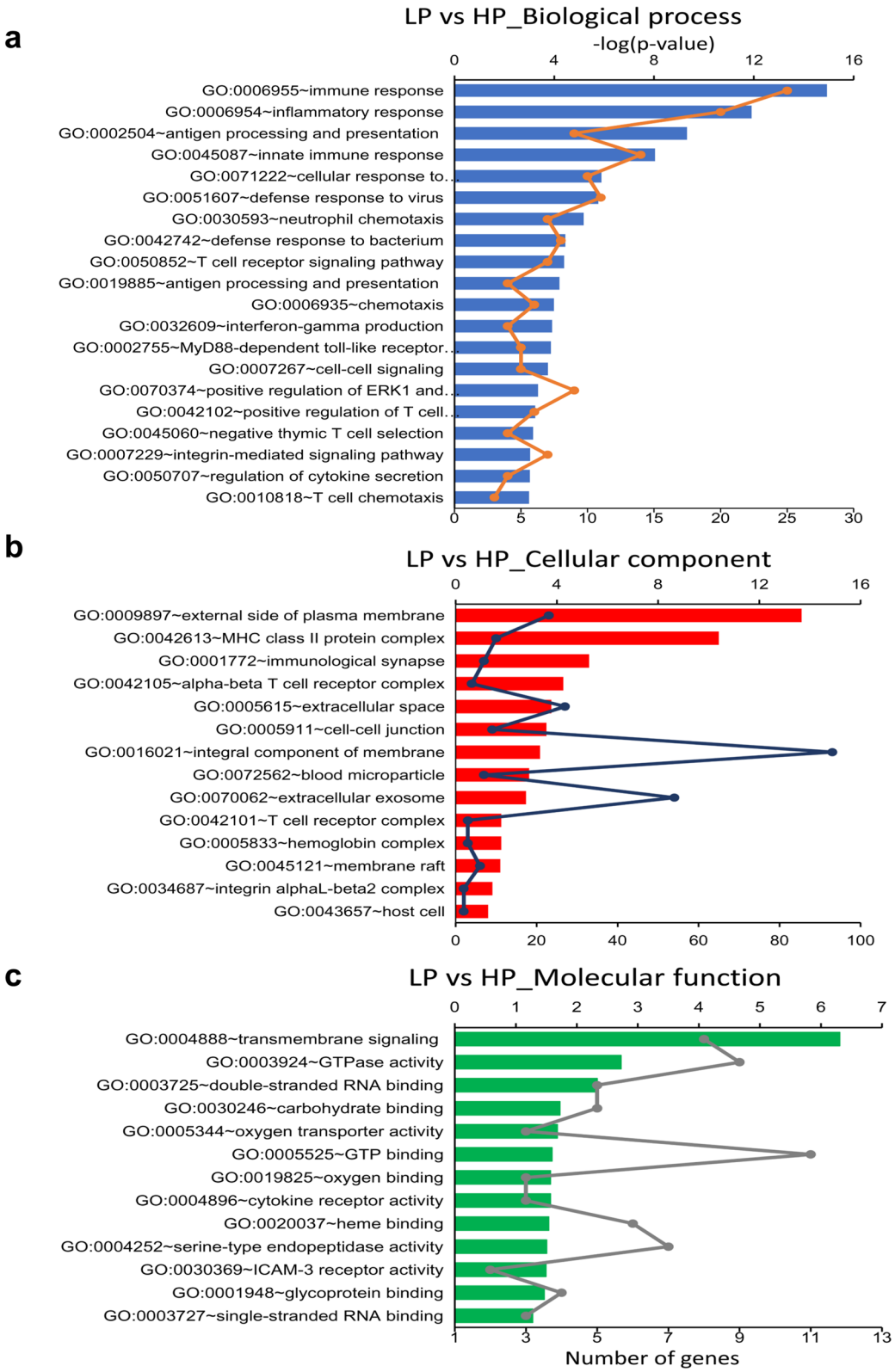


Fig. 3 Functional annotation cluster and gene ontology analysis of the genes derived from LP vs. HP comparison. **a** Biological process, **b** Cellular component, and **c** Molecular function. The bar diagram is representing the -log(*p* value) of each GO category, while the line diagram is representing the number of genes involved in each category

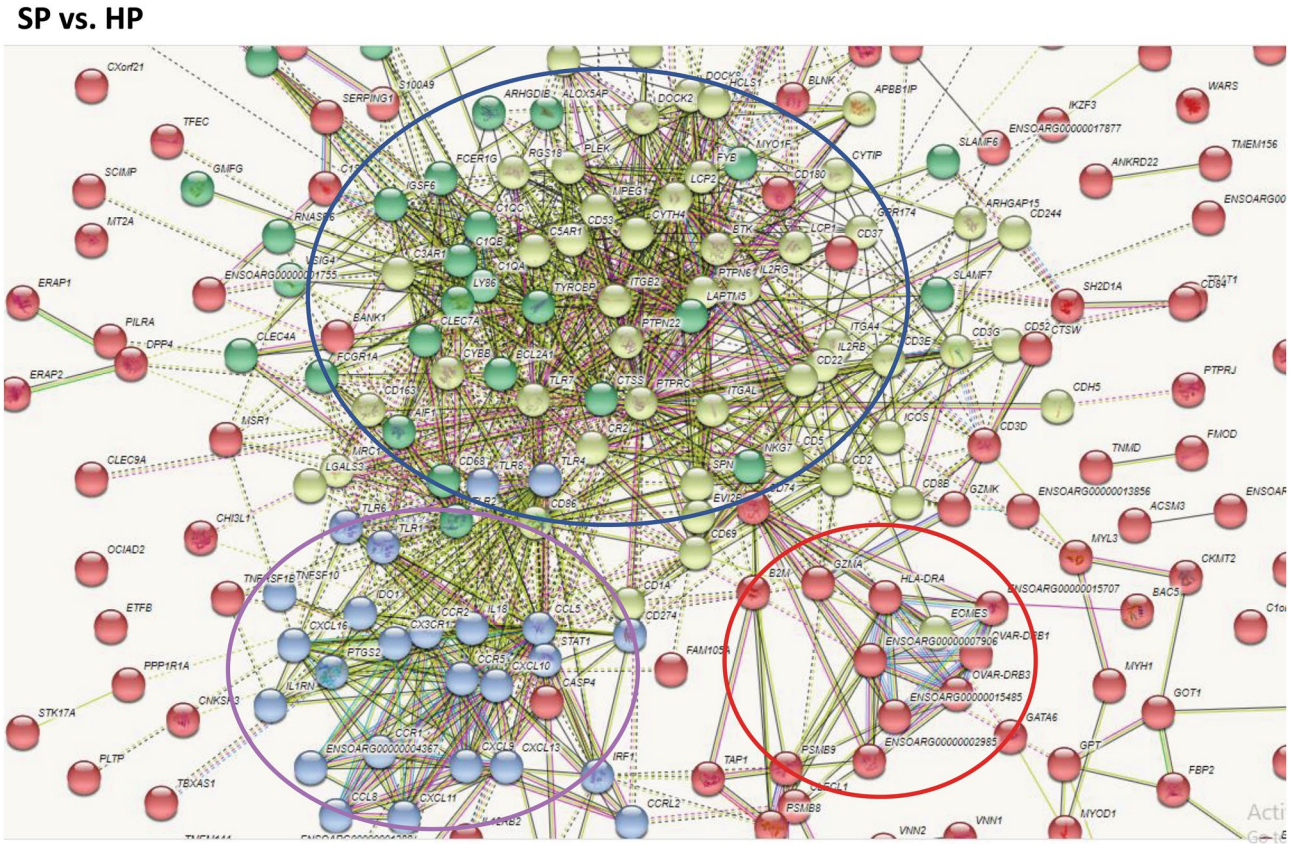


Fig. 4 Protein–protein interaction networks of differentially regulated genes obtained from SP vs. HP comparison. Three distinct gene clusters are marked by round circles. Different colored lines represent different types of evidence used to predict the association of differentially regulated genes. Sky blue line: curated databases, purple: experimentally validated, green: gene neighborhood, red: gene fusions, blue: gene co-occurrence, yellow: text mining, black: co-expression, light blue: protein homology. The minimum required interaction score was set to 0.400 that represents the medium confidence level. In the network, the total number of nodes is 238 and the number of edges is 1024 with a PPI enrichment *p* value: $< 1 \times 10^{-16}$

highly interacted (PPI enrichment p value is 1×10^{-16}). Cluster analysis of the corresponding proteins in the PPI interaction formed four clusters (Fig. 5). The central genes in the largest cluster (indicated by the blue circle) are *PTPRC*, *ITGB2*, *C5AR1*, *TLR7*, *PLEK*, *PTPN6*, *FYB*, and *ITGAL*. In the second largest cluster, *OVAR-DRB1*, *OVAR-DRB3*, *HLA-DRA*, *HLA-DMA*, and *PSMB8* are the central genes. While *IFNG*, *CXCL9*, *CXCL10*, *IL-1B*, *TLR1*, *CXCL11* genes are the central genes in the third-largest cluster, *MX2*, *PARP14*, *IFIT1*, *OAS1* genes are the central genes in the smallest cluster (indicated by the purple circle) (Fig. 5). The full PPI interaction network is presented in Fig. S3.

Standard protein vs. low protein

No PPI network was detected among the corresponding proteins of the genes derived from SP vs. LP comparison.

Discussion

Pregnancy is an extremely complex and tightly coordinated physiological process that is highly influenced by environmental factors. Maternal diet during pregnancy is one of the most important factors that could reprogram the fetal growth and development. Dietary protein is an important source of essential amino acids that is necessary for proper growth and development (Sohel et al. 2018). During pregnancy, starting from conception to the term, dietary protein is crucial for healthy fetal development (Manta-Vogli et al. 2020). There is a growing number of evidence indicating that the nutritional deviations at different stages of pregnancy can alter fetal muscle development which in turn has important implications in the meat animal production industry (Peñagaricano et al. 2014; Sohel 2020). In the current study, we sought to determine whether maternal dietary protein level during early- to

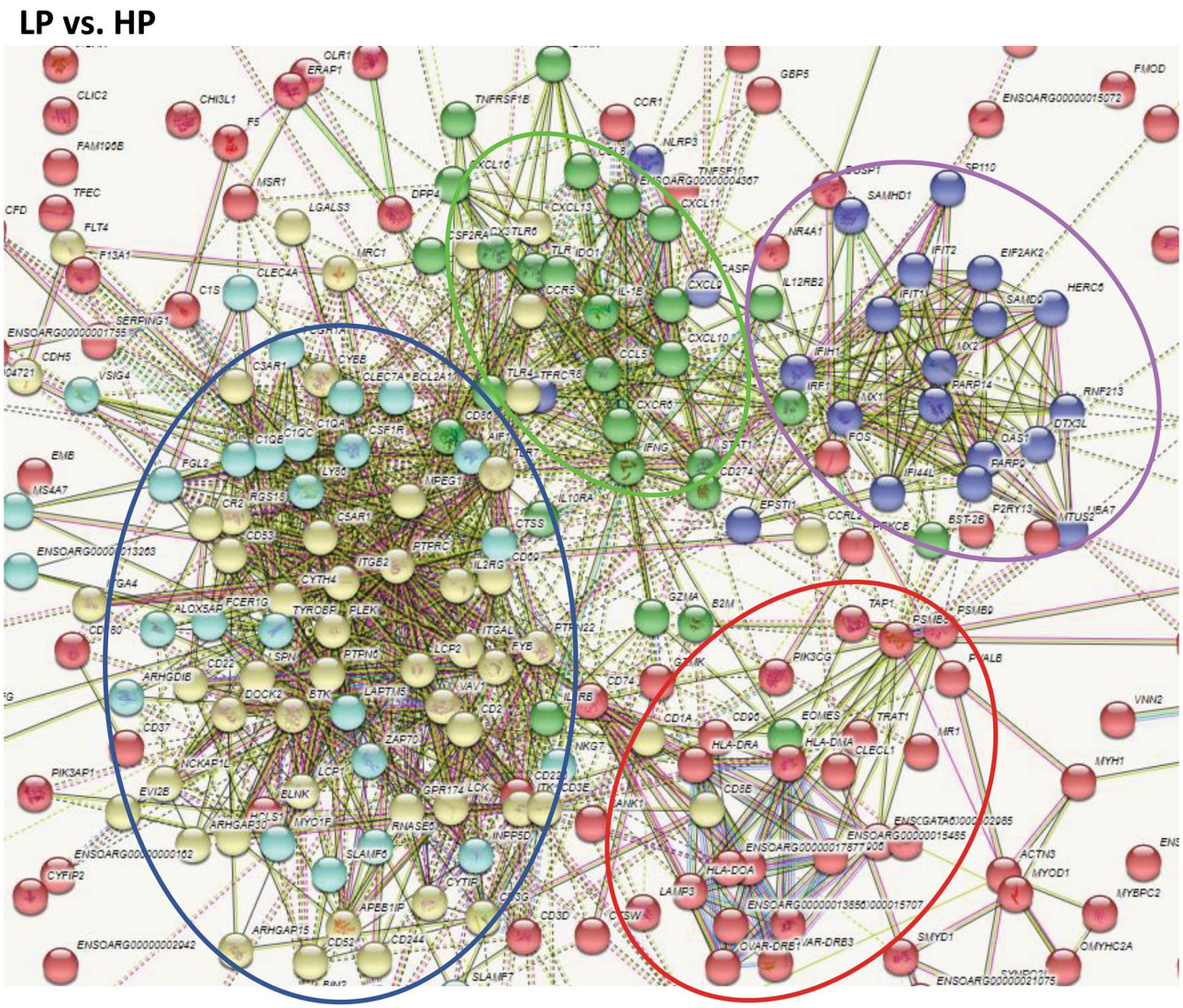


Fig. 5 Protein–protein interaction networks of differentially regulated genes obtained from LP vs. HP comparison. Four distinct gene clusters are marked by round circles. Different colored lines represent eight types of evidence used to predict the association of differentially regulated genes. Sky blue line: curated databases, purple: experimentally validated, green: gene neighborhood, red: gene fusions, blue: gene co-occurrence, yellow: text mining, black: co-expression, light blue: protein homology. The minimum required interaction score was set to 0.400 that represents the medium level of confidence. In this network, the total number of nodes is 272 and the number of edges is 1269 with a PPI enrichment p value: $< 1 \times 10^{-16}$

mid-gestation has impacts on the transcriptomic profile of fetal muscle tissues in sheep. A maternal diet formulated to provide an equal amount of dry matter and metabolizable energy intake differed only in the crude protein intake. In particular, three different protein levels such as standard protein (SP: 100% of the requirement), high protein (HP: 134–138% of the requirement), and low protein (LP: 60–61% of the requirement) were used in the maternal diet to evaluate our hypothesis. Interestingly we found that the maternal dietary protein intake during early- to mid-gestations remarkably alters the expression of genes in the fetal longissimus dorsi muscle. It is important to note that the maternal HP diet had a significant impact on the gene expression in fetal muscle, while the maternal LP diet had less impact on the expression of genes in fetal muscle. The results of our study uphold our hypothesis that maternal dietary protein intake during early- to mid-gestation can reprogram the transcriptional abundance in the fetal muscle tissue.

Excess or deficiency in maternal nutrition during pregnancy can severely affect the development potential of the offspring. Optimal maternal nutrition is extremely important for placental growth and nutrient transport as well as vascular development (Belkacemi et al. 2010). Any deviation

in the maternal diet could lead to suboptimal fetal growth and development that have greater consequences in the adult life of the offspring, particularly, in the case of economically important domestic animals. The majority of the studies focusing on the impact of maternal nutrition are concentrated on fetal organ development, growth retardation, meat quality, skeletal muscle development in offspring. For instance, it has been reported that the dietary protein is an important source of essential amino acids for pregnant sows and the growing fetus, necessary not only for growth and production but also for the physiological functions (Zhang et al. 2019). Excess protein levels in the maternal diet modulate the hepatic growth axis by downregulating several genes including *IGFBP-3* in weaning piglets (Cong et al. 2019). The restriction of metabolizable protein in the maternal diet in mid- to late gestation of primiparous heifers resulted in progeny with decreased soft tissue fat percentage, increased soft tissue moisture, and carcass water (Webb et al. 2019). Interestingly, the authors mentioned that the progeny can handle the light to moderate gestational metabolizable protein restriction as it has minimal impact on the meat characteristics and compositions (Webb et al. 2019). However, the underlying molecular mechanisms are poorly understood. With the development of throughput technologies, it is now possible to investigate the genome-wide impact of maternal nutritional deviation on the fetal skeletal muscle development. In the current study, we employed a microarray platform to evaluate the differential regulation of gene expression in the fetal skeletal muscle due to differential maternal dietary protein.

The structural unit of the skeletal muscle is known as myofibers or muscle fibers (Frontera and Ochala 2015). Myogenesis is the process to form new muscle fibers from dedicated pluripotent stem cells. It is important to note that all muscle fibers are formed during the prenatal stage of development, specifically in early- to mid-gestation, in livestock (Yan et al. 2013). The early- to mid-gestation stage of muscle development is crucial in mammalian livestock as there is no chance to increase the muscle number after birth. Therefore, in the current study, we choose to investigate the effects of differential maternal protein intake on the expression of genes in fetal skeletal muscle in this important period of development. We found several genes were differentially regulated in the fetal skeletal muscle due to over or restricted maternal protein intake. When ewes were fed a high-protein (HP, ~ 140% higher, requirement 100%) diet, a total of 323 genes were differentially regulated including 37 upregulated and 286 downregulated in the fetal skeletal muscle compared to that of standard protein (SP) group. The higher number of downregulated genes in HP group possibly suggests that maternal high-protein diet during the early- to mid-gestation may preferentially decrease the expression of genes in the fetal skeletal muscle that could have beneficial effects in adult life. However, the precise reason for this is yet to investigate as we did not find any related references in the existing literature.

Several important genes related to muscle development such as *MYH1*, *MYL6B*, *MYL3*, *MYOD1*, and *MYO6* were found to be significantly upregulated in the fetal muscle of the HP group suggesting that a high maternal protein diet may induce the expression of myogenic differentiation and development-related genes in the growing fetus. *MYOD1* is known as myogenic regulatory factors and perform a crucial role in myogenesis (Maltin et al. 2001). *MYOD1* has not only been shown to be important for the formation and survival of myoblasts but also played a central role in the terminal differentiation of myotubes (Maltin et al. 2001). Protein restriction in the maternal feed resulted in the downregulation of the *MYOD1* gene in the fetal muscle in pig (Perruchot et al. 2015). It has been shown that moderate-higher maternal protein intake is involved in upregulating the *MYOD* gene in the porcine fetal skeletal muscle (Kalbe et al. 2017). Therefore, we postulate that higher expression of *MYOD1* in the fetal muscle in our study could be because of higher maternal protein intake during early- to mid-gestation. It is important to note that maternal low energy diet during 55–90 days of gestation resulted in myosin heavy chain (MYH) genes in the fetal skeletal muscle (Zou et al. 2017). In addition, genes involved in protein metabolism including *PPP1R1A* are also found to be upregulated in the fetal muscles of the HP group. Increased expression of genes related to myogenesis and protein metabolism in the fetal muscle of the HP group indicating an upregulation of myogenesis.

Among the downregulated genes, several cytokines and chemokines (mostly related to the inflammatory response) have shown higher fold change regulation. For instance, chemokine (C-X-C motif) ligand-9 and 10 (*CXCL9* and *CXCL10*) showed the highest fold change. *CXCL-9, -10*, and *-11* can be found in different kinds of cells (Vazirinejad et al. 2014) including mesenchymal cells (Treacy et al. 2012). Members of the CXCL family are proinflammatory chemokines in nature that have antimicrobial properties and they may act as the first line of defense against pathogens (Yung and Murphy 2012). Interestingly, the relationship between dietary intake of macronutrients and the expression of several proinflammatory genes has been shown in many studies. For instance, high fat and glucose/carbohydrate diets are linked with higher expression of proinflammatory cytokines in various tissues (Dai et al. 2007; Takanabe-Mori et al. 2010; Kim et al. 2011). On the other hand, a high-protein diet and moderate exercise could lead to a decreased expression of proinflammatory cytokines (Griffin et al. 2012; Kitabchi et al. 2013; Carlin et al. 2016). In particular, voluntary and short-term exercise significantly reduce the expression of *CXCL9* (Griffin et al. 2012) and *CXCL10* (Carlin et al. 2016) in the obis animal model indicating that the

induction of proinflammatory cytokines can be blocked by dietary feed intake and exercise. Several TLRs including *TLR1*, *TLR2*, *TLR4*, *TLR6*, *TLR7*, and *TLR8* are also found to be significantly downregulated in the fetal skeletal muscle of the HP group. The lower expression of all the proinflammatory and immune-related genes in the HP group of our experiment indicates a lower inflammation rate in the fetal skeletal muscles.

Surprisingly, we found a few genes (14) that were differentially regulated in the low-protein (LP, ~ 60% protein, requirement 100%) group compared to that of the SP group. In contrast to our results, several studies have shown that maternal nutrition/protein restriction could alter the expression of a higher number of genes in the skeletal muscles of porcine (Kalbe et al. 2017), goat (Costa et al. 2019), and cattle (Maresca et al. 2018; Sanglard et al. 2018) fetuses. Although the precise reason is not known, we speculate that because of the hardy nature of native Akkaraman sheep, moderately low protein in the maternal diet did not show a severe impact on the fetal skeletal muscle gene expression. On the other hand, as expected, when we compared LP vs. HP groups, we got a similar gene expression profile as SP vs. HP group comparison.

To discover the biological relevance of the differentially expressed genes, we performed pathway analysis and gene ontology analysis using DAVID bioinformatics. The results showed that several pathways related to disease and immune response are affected by a high-protein diet. For instance, Staphylococcus aureus infection, phagosome, leishmaniasis, tuberculosis, antigen processing and presentation, toll-like receptor pathway, etc. are the top pathways affected by a high-protein diet. The genes involved in these pathways are downregulated in our experiment (Tables S4 and S5) probably indicating that maternal high-protein diet decreases the expression of genes related to disease in the fetal skeletal muscle. *Ovar-DRB1*, *TLR2*, and *TLR4* are the top genes found in several top pathways (Tables 2 and 3). Although we found several muscle-specific genes were upregulated in the HP group, no pathways were identified as significantly impacted by these genes. Similar pathways were found to be affected by the genes derived from LP vs. HP comparison. Several biological processes, cellular components, and molecular functions in fetal skeletal muscle were affected by the maternal high-protein diet. The majority of these biological processes and cellular components are directly related to immune response and inflammatory responses. It is interesting noting that the corresponding proteins of the differentially regulated mRNAs in our data set were highly interconnected. For instance, the SP vs. HP comparison generated 323 differentially regulated proteins where only 54 proteins are not interconnected and the remaining proteins are highly interconnected and generated a total of 1106 edges while the expected number of edges is 108. Similar protein–protein interactions were obtained from the differentially regulated genes derived from LP vs. HP comparison, although no interactions were obtained in SP vs. LP, Interestingly, highly interconnected proteins are clustered into distinct modular clusters (Figs. 4 and 5). The corresponding proteins of *CYTH4*, *LCP2*, *CD53*, *ITGB*, *PTPN22*, *PTPRC*, *TYROBP*, *TLR7*, *PTPRC*, *ITGB2*, *C5AR1*, *TLR7*, *PLEK*, *PTPN6*, *FYB*, *ITGAL*. *OVAR-DRB1*, *OVAR-DRB3*, *HLA-DRA*, *HLA-DMA*, *PSMB8*, *IFNG*, *CXCL9*, *CXCL10*, *IL-1B*, *TLR1*, *CXCL11* genes were found to be the central genes in different modular clusters in the PPI interactions. It is important noting that these genes are also significantly important in top pathways and other biological processes impacted by the differential protein input. It is evident from our results that the maternal high-protein diet decreased the abundance of several genes in the fetal skeletal muscle that is involved in immunity-related several pathways and biological processes. In addition, it also increases the expression of genes related to myogenesis and protein synthesis in the fetal skeletal muscles. One of the limiting factors in the current study was lack of phenotypic measurements of the investigated fetus. The expression changes as well as the affected pathways could better explained if we have the phenotypic measurements of the investigated fetus.

Conclusion

In the current study, we have identified and characterized the effects of differential protein input to the maternal diet during early- to mid-gestation on the transcriptomic profile of fetal skeletal muscle in sheep. Differential protein in the maternal diet caused notable changes in the expression of several genes. In particular, the maternal high-protein diet primarily altered the expression of genes related to myogenesis and immunity in the skeletal muscle of a growing fetus. Bioinformatics analysis revealed that these genes are involved in regulating several pathways and biological processes specific to disease and immunity. Interestingly, maternal low-protein diet has very minute effects on the expression of genes in the fetal skeletal muscles. The results of our study provide evidence that the level of protein in the maternal diet during early- to mid-gestation has differential effects on the global expression of genes in the fetal skeletal muscle of sheep that may have important implications in the food animal production. However, the epigenetic effects of the differential protein in the maternal diet on the modification of fetal genome warrant further investigations. Currently, we are evaluating the effects of differential protein in the maternal diet on the body weight gain, body composition, meat quality, and global expression of genes in the skeletal muscle in the postnatal period.

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Author contributions MUC, MMHS, and BA conceived and designed the study. KG and MA contributed insemination and necropsy. KG, MA, KA, and MK performed the sample collection. YK and MK carried out animal nutrition experiments. MUC, MMHS, and BA contributed genetic experiments. MUC and MMHS performed the research and analyzed the data. MMHS and MUC drafted the paper. All authors have read and approved the manuscript.

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Data availability All data generated and analyzed in this study are presented in the figures/tables and supplementary information documents.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest exists.

References

Altmann S, Murani E, Schwerin M et al (2012) Maternal dietary protein restriction and excess affects offspring gene expression and methylation of non-SMC subunits of condensin I in liver and skeletal muscle. Epigenetics 7:239–252. https://doi.org/10.4161/epi.7.3.19183

A.O.A.C. (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemist, Washington, DC

Belkacemi L, Nelson DM, Desai M, Ross MG (2010) Maternal undernutrition influences placental-fetal development1. Biol Reprod 83:325–331. https://doi.org/10.1095/biolreprod.110.084517

Carlin JL, Grissom N, Ying Z et al (2016) Voluntary exercise blocks Western diet-induced gene expression of the chemokines CXCL10 and CCL2 in the prefrontal cortex. Brain Behav Immun 58:82–90. https://doi.org/10.1016/j.bbi.2016.07.161

Cetin I, Alvino G, Radaelli T, Pardi G (2007) Fetal nutrition: a review. Acta Paediatr 94:7–13. https://doi.org/10.1111/j.1651-2227.2005.tb02147.x

Che L, Yang ZG, Xu MM et al (2017) Maternal nutrition modulates fetal development by inducing placental efficiency changes in gilts. BMC Genomics 18:213. https://doi.org/10.1186/s12864-017-3601-1

Cong R, Qu X, Zhang H et al (2019) Maternal high-protein diet modulates hepatic growth axis in weaning piglets by reprogramming the IGFBP-3 gene. Eur J Nutr. https://doi.org/10.1007/s00394-019-02097-z

Costa TC, Moura FH, Souza RO et al (2019) Effect of maternal feed restriction in dairy goats at different stages of gestation on skeletal muscle development and energy metabolism of kids at the time of births. Anim Reprod Sci 206:46–59. https://doi.org/10.1016/j.anireprosci.2019.05.006

Dai X, Ou X, Hao X et al (2007) Effect of T0901317 on hepatic pro-inflammatory gene expression in ApoE-/- mice fed a high-fat/high-cholesterol diet. Inflammation 30:105–117. https://doi.org/10.1007/s10753-007-9026-2

Du M, Wang B, Fu X et al (2015) Fetal programming in meat production. Meat Sci 109:40–47. https://doi.org/10.1016/J.MEATSCI.2015.04.010

Frontera WR, Ochala J (2015) Skeletal muscle: a brief review of structure and function. Behav Genet 45:183–195

Griffin TM, Huebner JL, Kraus VB et al (2012) Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: effects of short-term exercise. Arthritis Rheum 64:443–453. https://doi.org/10.1002/art.33332

Ibeagha-Awemu EM, Kgwatalala P, Zhao X (2008) A critical analysis of production-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. Mamm Genome 19:591–617. https://doi.org/10.1007/s00335-008-9141-x

Kalbe C, Lösel D, Block J et al (2017) Moderate high or low maternal protein diets change gene expression but not the phenotype of skeletal muscle from porcine fetuses. Domest Anim Endocrinol 58:63–75. https://doi.org/10.1016/j.domaniend.2016.08.003

Kim MS, Choi MS, Han SN (2011) High fat diet-induced obesity leads to proinflammatory response associated with higher expression of NOD2 protein. Nutr Res Pract 5:219–223. https://doi.org/10.4162/nrp.2011.5.3.219

Kitabchi AE, McDaniel KA, Wan JY et al (2013) Effects of high-protein versus high-carbohydrate diets on markers of β- Cell function, oxidative stress, lipid peroxidation, proinflammatory cytokines, and adipokines in obese, premenopausal women without diabetes. Diabetes Care 36:1919–1925. https://doi.org/10.2337/dc12-1912

Lin Y-J, Huang L-T, Tsai C-C et al (2019) Maternal high-fat diet sex-specifically alters placental morphology and transcriptome in rats: assessment by next-generation sequencing. Placenta 78:44–53. https://doi.org/10.1016/J.PLACENTA.2019.03.004

Maltin CA, Delday MI, Sinclair KD et al (2001) Impact of manipulations of myogenesis in utero on the performance of adult skeletal muscle. Reproduction 122:359–374

Manta-Vogli PD, Schulpis KH, Dotsikas Y, Loukas YL (2020) The significant role of amino acids during pregnancy: nutritional support. J Matern Neonatal Med 33:334–340

Maresca S, Lopez Valiente S, Rodriguez AM et al (2018) Effect of protein restriction of bovine dams during late gestation on offspring postnatal growth, glucose-insulin metabolism and IGF-1 concentration. Livest Sci 212:120–126. https://doi.org/10.1016/j.livsci.2018.04.009

Mousa A, Naqash A, Lim S (2019) Macronutrient and micronutrient intake during pregnancy: an overview of recent evidence. Nutrients. https://doi.org/10.3390/nu11020443

National Research Council (NRC) (2007) Nutrient requirements of small ruminants. National Academy of Sciences, Washington, DC

Paradis F, Wood KM, Swanson KC et al (2017) Maternal nutrient restriction in mid-to-late gestation influences fetal mRNA expression in muscle tissues in beef cattle. BMC Genomics 18:1–14. https://doi.org/10.1186/s12864-017-4051-5

Paulenz H, Ådnøy T, Fossen OH et al (2002) Effect of deposition site and sperm number on the fertility of sheep inseminated with liquid semen. Vet Rec 150:299–302. https://doi.org/10.1136/vr.150.10.299

Peñagaricano F, Wang X, Rosa GJ et al (2014) Maternal nutrition induces gene expression changes in fetal muscle and adipose tissues in sheep. BMC Genomics 15:1034. https://doi.org/10.1186/1471-2164-15-1034

Perruchot MH, Lefaucheur L, Louveau I et al (2015) Delayed muscle development in small pig fetuses around birth cannot be rectified by maternal early feed restriction and subsequent overfeeding during gestation. Animal 9:1996–2005. https://doi.org/10.1017/S1751731115001202

Picard B, Gagnière H, Robelin J, Geay Y (1995) Comparison of the foetal development of muscle in normal and double-muscled cattle.

J Muscle Res Cell Motil 16:629–639. https://doi.org/10.1007/BF00130244

Radunz AE, Fluharty FL, Zerby HN, Loerch SC (2011) Winter-feeding systems for gestating sheep I. Effects on pre- and postpartum ewe performance and lamb progeny preweaning performance1. J Anim Sci 89:467–477. https://doi.org/10.2527/jas.2010-3035

Radunz AE, Fluharty FL, Relling AE et al (2012) Prepartum dietary energy source fed to beef cows: II. Effects on progeny postnatal growth, glucose tolerance, and carcass composition1. J Anim Sci 90:4962–4974. https://doi.org/10.2527/jas.2012-5098

Rehfeldt C, Kuhn G (2006) Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J Anim Sci 84(Suppl):E113–E123. https://doi.org/10.2527/2006.8413_supple113x

Ritchie ME, Phipson B, Wu D et al (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47. https://doi.org/10.1093/nar/gkv007

Sanglard LP, Nascimento M, Moriel P et al (2018) Impact of energy restriction during late gestation on the muscle and blood transcriptome of beef calves after preconditioning 11 Medical and Health Sciences 1114 Paediatrics and Reproductive Medicine. BMC Genomics 19:702. https://doi.org/10.1186/s12864-018-5089-8

Sohel MMH (2020) Macronutrient modulation of mRNA and microRNA function in animals: a review. Anim Nutr. https://doi.org/10.1016/j.aninu.2020.06.002

Sohel MMH, Konca Y, Ulas Cinar M (2018) Impacts of macronutrients on gene expression: recent evidence to understand productive and reproductive performance of livestock. Turk J Agric Food Sci Technol. https://doi.org/10.24925/turjaf.v6i2.203-212.1573

Takanabe-Mori R, Ono K, Sowa N et al (2010) Lectin-like oxidized low-density lipoprotein receptor-1 is required for the adipose tissue expression of proinflammatory cytokines in high-fat diet-induced obese mice. Biochem Biophys Res Commun 398:576–580. https://doi.org/10.1016/j.bbrc.2010.06.123

Treacy O, Ryan AE, Heinzl T, et al (2012) Adenoviral transduction of mesenchymal stem cells: in vitro responses and in vivo immune responses after cell transplantation. PLoS ONE 7:e42662. https://doi.org/10.1371/journal.pone.0042662

Vazirinejad R, Ahmadi Z, Kazemi Arababadi M et al (2014) The biological functions, structure and sources of CXCL10 and its outstanding part in the pathophysiology of multiple sclerosis. NeuroImmunoModulation 21:322–330. https://doi.org/10.1159/000357780

Wallace JM, Aitken RP, Milne JS, Hay WW (2004) Nutritionally mediated placental growth restriction in the growing adolescent: consequences for the fetus1. Biol Reprod 71:1055–1062. https://doi.org/10.1095/biolreprod.104.030965

Wang X, Lan X, Radunz AE, Khatib H (2015) Maternal nutrition during pregnancy is associated with differential expression of imprinted genes and DNA methyltranfereases in muscle of beef cattle offspring1. J Anim Sci 93:35–40. https://doi.org/10.2527/jas.2014-8148

Webb MJ, Block JJ, Funston RN et al (2019) Influence of maternal protein restriction in primiparous heifers during mid- and/or late-gestation on meat quality and fatty acid profile of progeny. Meat Sci 152:31–37. https://doi.org/10.1016/j.meatsci.2019.02.006

Yan X, Zhu M-J, Dodson MV, Du M (2013) Developmental programming of fetal skeletal muscle and adipose tissue development. J Genomics 1:29–38. https://doi.org/10.7150/jgen.3930

Yung SC, Murphy PM (2012) Antimicrobial chemokines. Front Immunol 3:276

Zhang S, Heng J, Song H et al (2019) Role of maternal dietary protein and amino acids on fetal programming, early neonatal development, and lactation in Swine. Animals 9:19

Zhu M-J, Ford SP, Nathanielsz PW, Du M (2004) Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle1. Biol Reprod 71:1968–1973. https://doi.org/10.1095/biolreprod.104.034561

Zou T, He D, Yu B et al (2017) Moderate maternal energy restriction during gestation in pigs attenuates fetal skeletal muscle development through changing myogenic gene expression and myofiber characteristics. Reprod Sci 24:156–167. https://doi.org/10.1177/1933719116651151

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