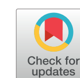
Electronic Journal of Biotechnology 46 (2020) 30–37

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Effect of dietary nutrition on tail fat deposition and evaluation of tail-related genes in fat-tailed sheep



Jie Zeng [a], Shiwei Zhou [a], Yuxin Yang [a], Junli Du [b], Danju Kang [c], Xiaolong Wang [a], Yulin Chen [a,*]

[a] College of Animal Science and Technology, Northwest A&F University, Yangling 712100, People's Republic of China
[b] College of Science, Northwest A&F University, Yangling 712100, People's Republic of China
[c] Department of Veterinary Sciences, Guangdong Ocean University, Zhanjiang, 524088, People's Republic of China

ARTICLE INFO

Article history:
Received 27 November 2019
Accepted 20 May 2020
Available online 23 May 2020

Keywords:
Adipogenesis
Dietary nutrition
Fat deposition
Fat-tailed sheep
Hedgehog signaling pathway
Lipogenic genes
Nutrition
OXCT1
Production performance
Sheep
Tail

ABSTRACT

Background: The effects of dietary nutrition on tail fat deposition and the correlation between production performance and the Hh signaling pathway and *OXCT1* were investigated in fat-tailed sheep. Tan sheep were fed different nutritional diets and the variances in tail length, width, thickness and tail weight as well as the mRNA expression of fat-related genes (*C/EBPα*, *FAS*, *LPL*, and *HSL*) were determined in the tail fat of sheep at three different growth stages based on their body weight. Furthermore, the correlations between tail phenotypes and the Hedgehog (Hh) signaling pathway components (*IHH*, *PTCH1*, *SMO*, and *GLI1*) and *OXCT1* were investigated.

Results: *C/EBPα*, *FAS*, *LPL*, and *HSL* were expressed with differences in tail fat of sheep fed different nutritional diets at three different growth stages. The results of the two-way ANOVA showed the significant effect of nutrition, stage, and interaction on gene expression, except the between *C/EBPα* and growth stage. *C/EBPα*, *FAS*, and *LPL* were considerably correlated with the tail phenotypes. Furthermore, the results of the correlation analysis demonstrated a close relationship between the tail phenotypes and Hh signaling pathway and *OXCT1*.

Conclusions: The present study demonstrated the gene-level role of dietary nutrition in promoting tail fat deposition and related tail fat-related genes. It provides a molecular basis by which nutritional balance and tail fat formation can be investigated and additional genes can be identified. The findings of the present study may help improve the production efficiency of fat-tailed sheep and identify crucial genes associated with tail fat deposition.

How to cite: Zeng J, Zhou S, Yang Y, et al. Effect of dietary nutrition on tail fat deposition and evaluation of tail-related genes in fat-tailed sheep. Electron J Biotechnol 2020;46. https://doi.org/10.1016/j.ejbt.2020.05.004.

© 2020 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Fat-tailed sheep are widely distributed around the world, and Tan sheep are one of the most famous fat-tailed breeds in China. Tan sheep are well known for their soft and thin fur, and good quality meat. The fat that is accumulated in their tail can, to some extent, be decomposed into energy [1,2]. Fat deposition is affected by several factors, including breed, age, and nutrition [3,4,5,6]. Carcasses with higher amounts of fat were produced by long-fed lambs offered ad libitum access to whole shelled corn than those offered other energy source diets [7]. Higher amounts of intramuscular fat (IMF) were deposited in the longissimus muscle in cattle fed high-energy diets than in those fed low-energy diets [8]. The IMF of pigs have been shown to increase when the pigs were fed low protein diets, and the mRNA expression of lipogenic genes also increased under low-protein diet conditions [9]. Therefore, an advanced understanding of the balance between diet and tail fat deposition may provide key insights that can be used to improve the production efficiency of fat-tailed sheep.

There are several genes that function as regulators in the synthesis and metabolism of fat [10]. CCAAT enhancer-binding protein (*C/EBPα*), an important transcription factor, is mainly expressed in the adipose tissue. It plays an essential role in the formation of white fat [11]. Fatty acid synthase (*FAS*), lipoprotein lipase (*LPL*), and hormone-sensitive lipase (*HSL*) are all genes that function during lipogenesis. *FAS* is involved in de novo fatty acid synthesis [12]; *LPL* acts as a metabolic gate keeper for the absorption of fatty acids in adipose tissue [13], and *HSL* is involved in the fat-limiting step of lipolysis [14].

The Hedgehog (Hh) signaling pathway is a vital pathway that inhibits fat formation [3,15,16]. It functions via the interactions of the

* Corresponding author.
E-mail address: chenyulin@nwafu.edu.cn (Y. Chen).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2020.05.004
0717-3458/© 2020 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Hedgehog ligands (HH), Patched 1 (PTCH1) and Smoothened (SMO) receptors, and the Gli–Kruppel family of transcription factors (GLI1) to inhibit lipogenesis. Hedgehog signaling is activated by one of the three homologous ligands in mammals, Sonic Hedgehog (SHH), Indian Hedgehog (IHH), or Desert Hedgehog (DHH) [17]. Hh ligands bind to the PTCH1 receptor on stromal cells and release SMO from PTCH1 suppression. The freed SMO activates a complex signaling pathway, which results in transcriptional regulation of several downstream genes by the GLI family. The transcription factor *GLI1*, a reliable marker and a target gene of the Hh signaling pathway, operates to amplify the transcriptional response of Hh signaling [18,19]. OXCT1 is the rate-limiting enzyme of ketolysis, and another study has demonstrated its negative regulatory role in adipose differentiation [20]. However, it is unknown whether the Hh signaling pathway or *OXCT1* is closely related to the development of tail fat.

To explore the effect of dietary nutrition on tail-fat deposition and identify an optimal nutritional diet for fat-tailed sheep, we evaluated the lipogenic and lipolysis genes *C/EBPα*, *FAS*, *LPL*, and *HSL* in combination with data of four tail phenotypes (tail length, width, thickness, and weight) of Tan sheep fed different nutritional diets. We hypothesized that the Hh signaling pathway or *OXCT1* may be related to tail fat deposition in fat-tailed sheep, and the correlations between the tail phenotypes and Hh signaling pathway or *OXCT1* were analyzed to identify key genes and lay a theoretical foundation for further research.

2. Materials and methods

2.1. Animals and diets

All animal procedures were conducted in accordance with the guidelines of the China Council on Animal Care and the Ministry of Agriculture of the People's Republic of China. All animal experiments were approved by the Review Committee for the Use of Animal Subjects of Northwest A&F University.

The 112 healthy Tan sheep (n = 56 females, n = 56 males; age: 4 months) used in this study were obtained from Ningxia Tianyuan Tan Sheep Farm (Hongsibu, Ningxia, China). The animals were fed ad libitum during three growth stages, named stages 1, 2, and 3, according to the weight goals of 22–28, 29–35, and 36–40 kg, respectively (based on the requirements of the Feeding Standard of Meat-Producing Sheep and Goats (NY/T816-2004, China) [21]). The sheep were restricted feed for 12 h prior to each stage, and each stage lasted for 1 month. The animals were then randomly divided into four groups, each comprised of seven animals, with an average with four replicates per group. According to the Feeding Standard of Meat-Producing Sheep and Goats (NY/T816-2004, China) [21] and our previous studies, the required diet to maintain an average daily weight gain of 200 g was used as the reference for the standard diet for the three stages. In each stage, four different nutritional diets (84%, 96%, 108%, and 120% of the standard diets) were fed to the four groups of sheep, respectively. The energy–protein ratios of the different stages were maintained at ~0.88. The composition and nutrient levels of the diets are shown in Table 1.

At the end of each growth stage, four sheep that represented the average body weight were randomly selected from each group and were slaughtered to collect the adipose tissue from the tail. These samples were immediately wrapped in foil paper and frozen in liquid nitrogen after slaughter, and stored at −80°C until further use.

2.2. Quantitative PCR (qPCR)

The total RNA from the tail adipose tissue was extracted using the SV Total RNA Isolation System containing DNase (Promega, Shanghai, China). The concentration and purity of the total RNA were determined using Maestro Nanomicro-spectrophotometer (MaestroGEN, Las Vegas, NV), and the gel electropherogram of the total RNA from the tail adipose is shown in the Supplementary Files. The first strand cDNA was reverse-transcribed from the total RNA using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo, Carlsbad, America) and stored at −20°C until further analysis. The expression of genes was tested with real-time PCRs using SYBR Premix Ex Taq (TaKaRa, Dalian, China). The PCRs were conducted using a reaction mixture volume of 25 μL using the Bio-Rad CFX96 Touch PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). The reactions were carried out under the following cycling conditions: 95°C for 60 s, and 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Fluorescence was measured every 5 s from 65°C to 95°C with 0.5°C increments to construct the melting curve. The sequences of primers were designed based on the mRNA sequences from the NCBI (GenBank) and are shown in Table 2; β-actin was selected as the housekeeping gene.

Table 1
Composition and nutritional levels at different stages (DM basis).

	Stage 1				Stage 2				Stage 3			
	84%	96%	108%	120%	84%	96%	108%	120%	84%	96%	108%	120%
Ingredient, %												
Corn grain	23.41	26.24	34.53	34.97	23.21	24.85	32.60	32.18	18.41	22.97	27.24	35.89
Soybean oil	0.00	0.00	0.50	3.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.50
Soybean meal	0.00	8.97	6.90	13.86	0.00	2.89	7.40	6.45	0.00	0.03	12.76	4.84
Sunflower cake	21.59	9.79	3.08	0.00	16.86	12.26	0.00	0.00	16.62	17.00	0.00	0.00
Millet straw	17.57	50.00	0.00	0.00	0.00	55.00	25.68	0.00	6.00	43.73	49.00	0.00
Rice straw	32.43	0.00	0.00	0.00	54.93	0.00	0.00	0.00	53.97	11.27	6.00	0.00
Alfalfa	0.00	0.00	50.00	43.17	0.00	0.00	29.32	53.36	0.00	0.00	0.00	53.77
Premix	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Nutrient level, %												
Dry matter	88.96	89.42	88.28	88.57	88.80	89.52	88.82	88.62	89.05	89.38	89.49	88.26
Digestible energy/($MJ \cdot kg^{-1}$)	7.92	9.05	10.19	11.32	7.45	8.52	9.58	10.65	7.08	8.09	9.10	10.11
Crude protein	9.90	11.32	12.73	14.15	8.28	9.46	10.65	11.83	8.04	9.19	10.34	11.49
Ether extract	2.23	2.00	2.55	4.98	2.26	1.97	1.97	4.86	2.13	2.04	1.91	2.52
Crude fiber	18.32	19.18	23.19	19.97	17.05	20.98	21.97	23.96	18.65	20.81	18.52	24.11
Ash	6.77	5.47	4.85	4.63	7.87	5.61	5.24	4.91	8.17	6.09	5.92	4.89
Ca	0.93	0.91	1.51	1.44	0.93	0.89	1.24	1.54	0.93	0.90	0.90	1.54
P	0.42	0.36	0.51	0.50	0.39	0.34	0.40	0.49	0.37	0.37	0.31	0.49

The premix provides the following per kg diet: VA 7500 IU, VD 1050 IU, VE 10 IU, Fe 5500 mg, Cu 500 mg, Mn 5000 mg, Zn 4000 mg, Se 32.5 mg, I 100 mg, Co 32.5 mg. The nutrient levels in dry matter, digestible energy and crude protein content are analyzed values, ether extract, crude fiber, ash, Ca and P content are calculated values based on the obtained raw material composition (dry matter basis).

Table 2
Primer sequences used for real-time PCR in this study.

Gene	Sequence 5′-3′	Product size (bp)	Tm (°C)	Primer efficiency (%)	GenBank accession number
β-actin	F: TCTGGCACCACACCTTCTAC R: TCTTCTCACGGTTGGCCTTG	102	60	99.00	NM_001009784.2
C/EBPα	F: CAAGAACAGCAACGAATAC R: AGGCGGTCATTGTCACTGGT	135	61	97.95	NM_001308574.1
FAS	F: CTCGGTGCCCGTTGTCTA R: GGAGGTATGCCCGCTTTT	188	59	97.09	XM_015098375.1
LPL	F: CCCAGCAGCATTATCCAGTGT R: ATTCATCCGCCATCCAGTTC	87	60	98.00	NM_001009394.1
HSL	F: AGCACTACAAACGCAACGAG R: TCTGAATGATCCGCTCAAACT	117	59	97.79	NM_001128154.1
IHH	F: CCCAGCCACCAGCACATAC R: CCTCCTCTTCACGGCCAACA	100	62	96.98	XM_015092833.1
GLI1	F: AGCGCAGCCAATACAGAC R: AAGGCGGCGAAGAGTAGA	126	58	95.95	XM_015094784.1
PTCH1	F: CGGCATCATTAACCCTAG R: TTCAACGAAGTCCGAGGT	235	56	96.89	XM_012116602.2
SMO	F: TGTGAGCGGCATCTGTTT R: CAGGGTGGTTGCTCTTGAT	149	57	96.71	XM_015095115.1
OXCT1	F: TTCCAGGAGCCTCTTATT R: TTCCAGGTATCATCCAGTT	132	55	98.60	XM_004017014.4

2.3. Data analysis

The mRNA expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method. Each sample was tested in quadruplicate, and the average of their cycle threshold (CT) values represented the expression of target gene. The median CT value in each group was set to be the calibrator, and ΔΔCt values were calculated using the following formulas: $\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{calibrator}$, $\Delta Ct_{sample\ or\ calibrator} = CT_{Target} - CT_{\beta\text{-}actin}$ [22]. All data were statistically analyzed using SPSS Software (version 20.0, IBM, New York, USA) and are presented as means ± SDs. Statistical differences between groups were evaluated using a two-way ANOVA, and the correlations were analyzed using the Pearson correlation coefficient. Data were considered statistically significant at $P < 0.05$ and extremely significant at $P < 0.01$.

3. Results

3.1. Effect of dietary nutrition and growth stage on mRNA expression of C/EBPα, FAS, LPL, and HSL

The mRNA expression of *C/EBPα*, *FAS*, *LPL*, and *HSL* is shown in Fig. 1. The mRNA expression of *C/EBPα* was significantly different in all groups

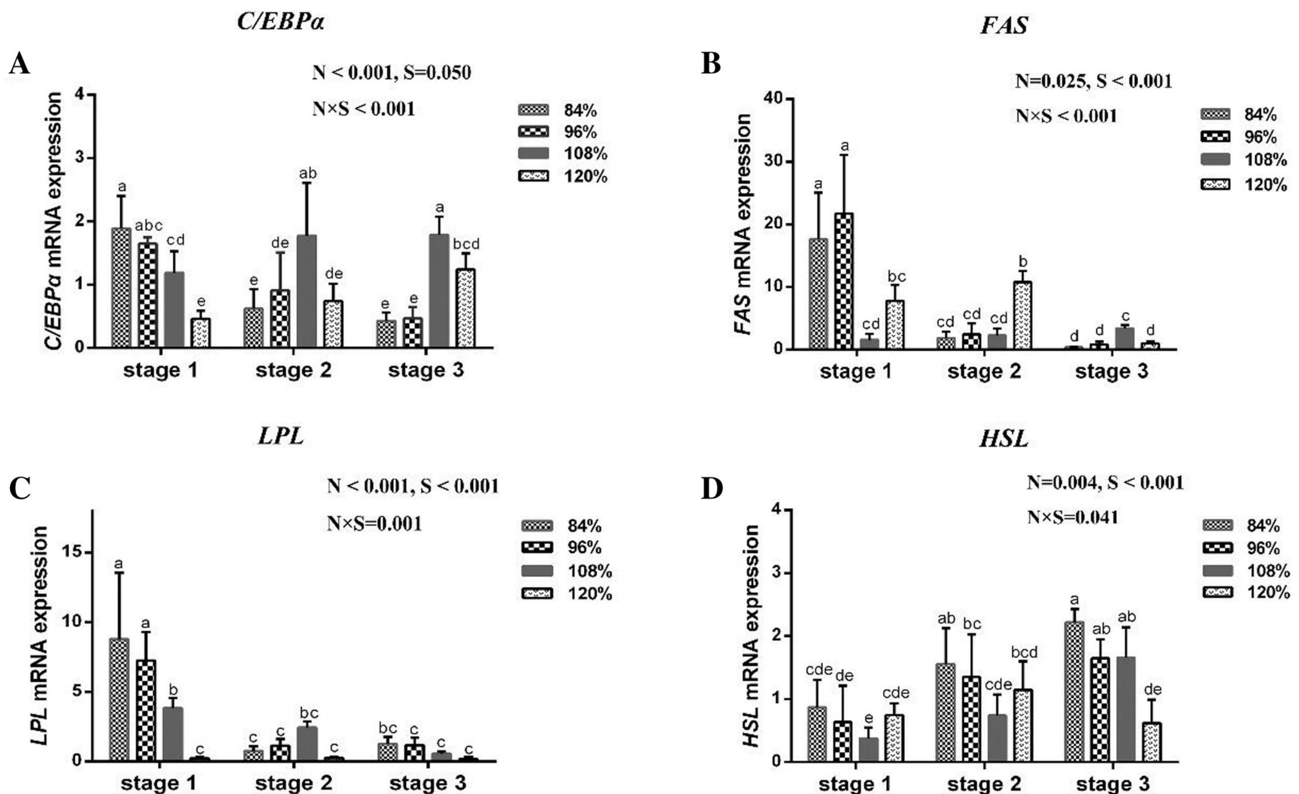


Fig. 1. Effect of dietary nutrition and growth stage on mRNA expression of *C/EBPα* (A), *FAS* (B), *LPL* (C), and *HSL* (D). Data were normalized to *β-actin* (housekeeping gene) mRNA expression levels. Bars represent means ± SEMs. Lowercase letter superscripts indicate values that are significantly different ($P < 0.05$). N, S, and N × S mean the *p*-value of nutrition level, growth stage, and their interaction in the two-way ANOVA, respectively.

($P < 0.05$) (Fig. 1A). In stage 1, *C/EBPα* mRNA expression was the highest in the 84% group and then decreased with increasing dietary nutrition, but its expression initially increased and then decreased in the 120% group in stages 2 and 3. The mRNA expression of *FAS* in stages 1 and 2 initially increased and then decreased in the 108% group before increasing again in the 120% group ($P < 0.05$) (Fig. 1B). In stage 3, *FAS* expression initially increased and then decreased with the highest in the 85% group ($P < 0.05$). The expression trend of *LPL* mRNA in stages 1 and 3 decreased with the increase in dietary nutrition, and in stage 2, initially increased and then decreased in the 120% group ($P < 0.05$) (Fig. 1C). No significant difference in *HSL* mRNA expression was observed in stages 1 and 2 ($P > 0.05$), but a considerably high expression was observed in stage 3 in the 84% group ($P < 0.05$) (Fig. 1D). *HSL* mRNA expression decreased with the increase in dietary nutrition. According to the results of the two-way ANOVA, dietary nutritional level significantly affected the mRNA expression of *C/EBPα*, and the effect of dietary nutrition and growth stage on *C/EBPα* expression was interactive ($P < 0.001$). The dietary nutrition, growth stage, and their interaction had significant effects on the mRNA expression of *FAS*, *LPL*, and *HSL* ($P < 0.05$).

3.2. Correlations between the tail phenotypes and C/EBPα, FAS, LPL, and HSL

The data of the four phenotypes, variances in tail length, width, and thickness per stage, and tail weight at the end of growth stages, were obtained from articles published previously by our team [23,24]. A two-way ANOVA was used to re-analyze the effect of dietary nutrition and growth stage on the deposition of tail fat, and the results are shown in Fig. 2. The variance in tail length was not significantly different among the groups ($P > 0.05$) (Fig. 2A). In stages 2 and 3, the variance in tail width increased with the increase in dietary nutrition ($P < 0.05$), but there was no significant difference in stage 1 ($P > 0.05$) (Fig. 2B). The variance in tail thickness increased with the increase in dietary nutrition in all three stages ($P < 0.05$) (Fig. 2C). Tail weights in stages 2 and 3 were significantly different and increased with the increase in dietary nutrition ($P < 0.05$) (Fig. 2D). There were no significant differences among other groups in stage 1 ($P > 0.05$). According to the results of the two-way ANOVA, growth stages significantly affected the variances in tail length ($P < 0.01$). The variances in tail width and thickness were affected by dietary nutrition and the growth stages ($P < 0.001$). However, dietary nutrition, growth stages, and their interaction had significant effects on tail weight ($P < 0.001$). Considering there was no significant effect of the interaction between dietary nutrition and growth stages on the variances in tail length, width, and thickness, an analysis of main effect was performed on the groups at $P < 0.05$. In Fig. 3A, the variances in tail length were significantly decreased with the increase in growth stages ($P < 0.01$), but the variances in tail width and thickness were conversed ($P < 0.001$). Dietary nutrition significantly affected the variances in tail width and thickness, with increasing trends as the nutritional levels increased ($P < 0.001$) (Fig. 3B).

Correlations between the expression of *C/EBPα*, *FAS*, *LPL*, and *HSL* and four tail phenotypes are presented in Table 3. The mRNA expression of *C/EBPα* was significantly positively correlated with the variance in tail length in stage 1 with 120% of the standard diet ($P < 0.01$), but significantly negatively correlated with the tail thickness variance and tail weight in the 96% and 120% groups in stage 2 ($P < 0.05$), respectively. In stage 1, *FAS* mRNA expression was negatively correlated with tail weight in sheep fed the 96% diet ($P < 0.05$). Furthermore, in stage 2, there were significant negative correlations between *FAS* expression and the variances in tail width in the 84%, 108%, and 120% groups ($P < 0.05$). Sheep in the 96% groups in stage 1 presented a positive correlation between *LPL* mRNA expression and tail thickness variance, but *LPL* expression was negatively correlated

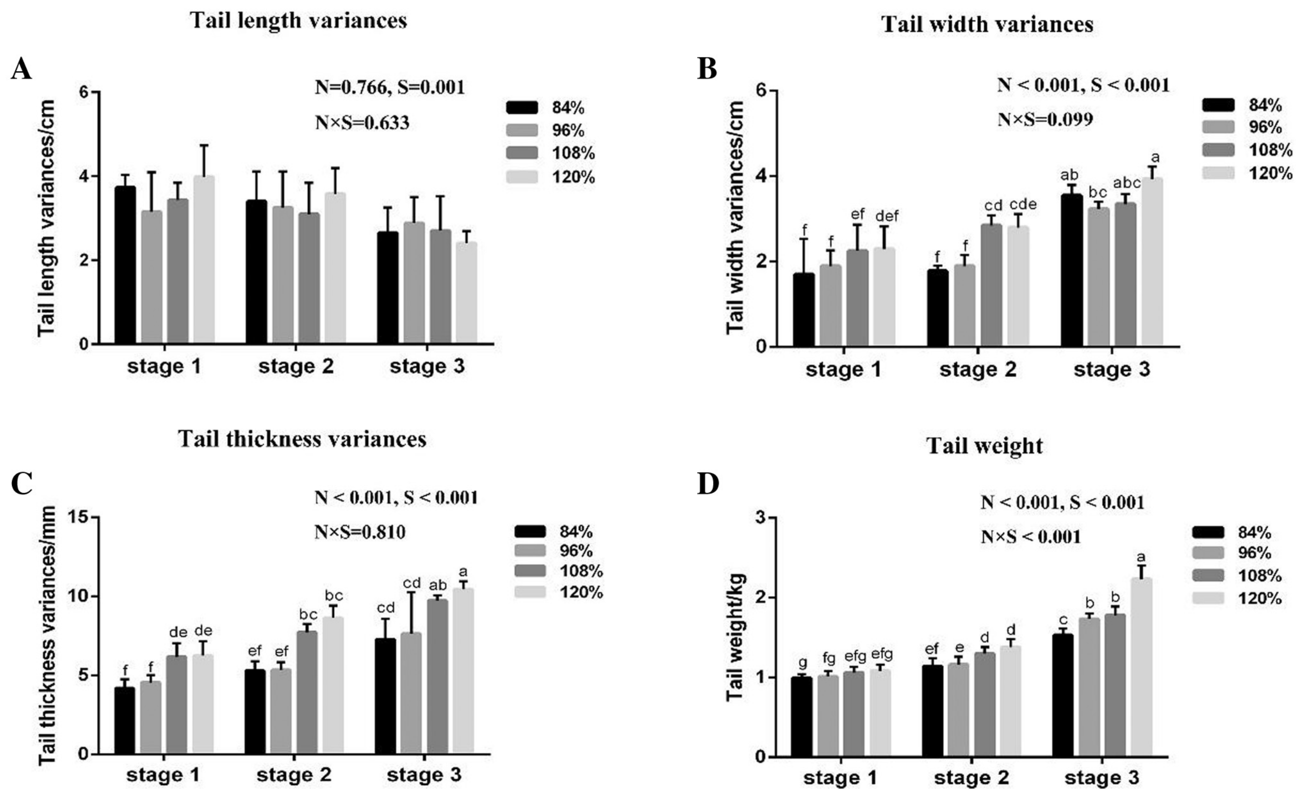


Fig. 2. Re-analysis of the effect of dietary nutrition and growth stage on tail length variances (A), tail width variances (B), tail thickness variances (C), and tail weight (D). The variances of tail length, width, and thickness mean their variances per stage, while tail weight represents the measured value at the end of each growth stage. Data were normalized to *β-actin* (housekeeping gene) mRNA expression levels. Bars represent means ± SEMs. Lowercase letter superscripts indicate values that are significantly different ($P < 0.05$). N, S, and N × S mean the *p*-value of nutrition level, growth stage, and their interaction in the two-way ANOVA, respectively.

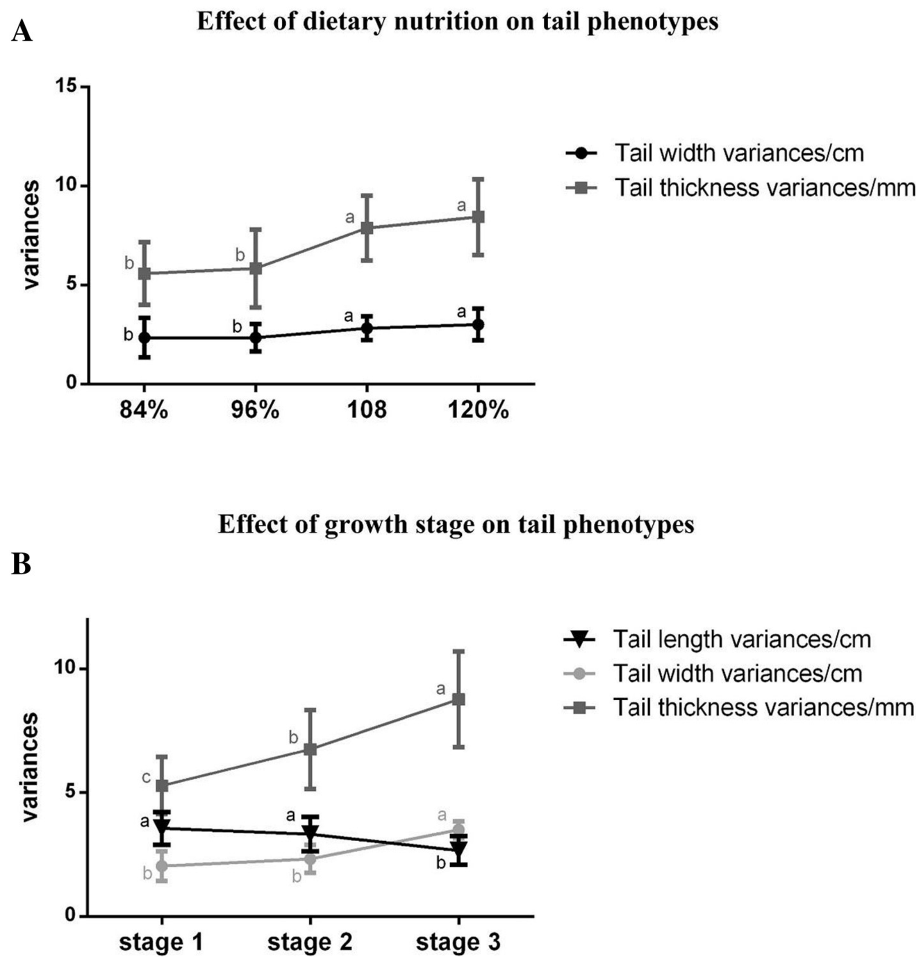


Fig. 3. Main effects analysis of the effect of dietary nutrition (A) and growth stage (B) on tail phenotypes. The variances of tail length, width, and thickness mean their variances per stage. Data were normalized to *β-actin* (housekeeping gene) mRNA expression levels. Points represent means ± SEMs. Lowercase letter superscripts indicate values that are significantly different ($P < 0.05$).

with tail weight and tail thickness variance in the 96% and 120% groups in stage 2 ($P < 0.05$), respectively. There were no significant correlations between the mRNA expression of *HSL* and the four phenotypes in all the groups ($P > 0.05$).

3.3. Role analysis of the Hh signaling pathway and OXCT1 in tail fat deposition combined with tail phenotypes

To determine whether the Hh signaling pathway and *OXCT1* were closely related to tail fat deposition, a correlation analysis was performed and the Hh signaling pathway components: *IHH*, *GLI1*, *SMO*, and *PTCH1*, were selected. The mRNA expression of the above genes by qPCR and the data of four tail phenotypes were used in this experiment. The results are shown in Table 4 and Table 5. In stage 2, *IHH* mRNA expression was significantly negatively correlated with the variance in tail length in the 96% group and tail width in the 108% group ($P < 0.01$). The expression of *IHH* in stage 3 was positively correlated with tail thickness variance in the 84% group, but negatively correlated in the 96% group ($P < 0.05$). The mRNA expression of *GLI1* in stage 1 showed a significant positive correlation with tail weight in the 96% group, but was significantly negatively correlated with the variance in tail width in the 108% group ($P < 0.01$). In stage 3, *GLI1* expression was negatively correlated with the variance in tail length in the 84% group and tail thickness in the 96% group ($P < 0.05$). The tail weight in stage 2 presented a positive correlation with the mRNA expression of *SMO* in the 96% group, but in stage 3, it was negatively correlated in the 108% group ($P < 0.05$). Another positive correlation was detected between the *SMO* expression and tail length variance in the 96% group in stage 3 ($P < 0.05$). The mRNA expression of *PTCH1* only showed a positive correlation with tail length variance in the 120% group in stage 1 ($P < 0.05$).

Significant correlations between *OXCT1* mRNA expression and phenotypes were observed in stage 1 (Table 5). There was a positive correlation between *OXCT1* expression and tail length variance in the 96% group ($P < 0.05$). Furthermore, the *OXCT1* expression was positively correlated with the variances in tail width and thickness in the 120% group ($P < 0.05$).

4. Discussion

In general, animals store excess energy by fat deposition to maintain a balance. In fat-tailed sheep, the tail is used to store fat to aid the resistance to cold stress during winter. The utilization and improvement of deposition regularity are efficient ways to improve the productivity of fat-tailed sheep. In sheep with docked tails, the tail fat is re-directed to the muscle tissue, and thus tail docking can improve meat quality [25,26]. In recent years, gene-level research has become a hot topic in the understanding of tail fat deposition. In the gene expression profile of Tan sheep, 1058 differentially expressed genes and 237 involved pathways were identified in the subcutaneous, visceral, and tail fats [10]. Proliferator-activated receptor (*PPARγ*), *FAS*, and *HSL* in the longissimus dorsi muscle, and subcutaneous and tail fat have been shown to differ significantly between Tan sheep and Shaanbei fine wool sheep [27]. The differential expression of genes depending on

Table 3
Pearson correlation between tail phenotypes and the mRNA expression of *C/EBPα*, *FAS*, *LPL*, and *HSL*.

Stage 1	84%				96%				108%				120%			
	C/EBPα	*FAS*	*LPL*	*HSL*	*C/EBPα*	*FAS*	*LPL*	*HSL*	*C/EBPα*	*FAS*	*LPL*	*HSL*	*C/EBPα*	*FAS*	*LPL*	*HSL*
Tail length (cm)	0.972	0.551	−0.014	0.070	−0.938	−0.887	0.064	0.955	0.832	0.578	−0.167	−0.681	1.000**	−0.697	0.749	−0.219
Tail width (cm)	0.945	0.916	−0.554	−0.482	0.989	0.963	0.141	−0.875	−0.088	−0.440	−0.945	−0.969	0.050	0.674	0.707	0.961
Tail thickness (mm)	−0.058	0.637	−0.952	−0.974	0.310	0.426	1.000*	0.333	−0.694	−0.389	0.374	0.822	−0.011	0.717	0.663	0.976
Tail weight (kg)	−0.985	−0.604	0.078	−0.006	−0.996	−0.999*	−0.375	0.733	−0.858	−0.617	0.119	0.645	0.470	0.295	0.941	0.752
Stage 2																
Tail length (cm)	−0.786	−0.086	−0.975	0.806	−0.153	0.903	0.811	−0.342	−0.985	−0.878	−0.07	−0.924	0.738	0.864	−0.995	−0.339
Tail width (cm)	0.556	−1.000**	0.148	−0.528	−0.293	0.833	0.886	−0.204	−0.813	−0.999*	−0.496	−0.665	−0.984	−0.999*	0.788	−0.215
Tail thickness (mm)	−0.720	−0.186	−0.947	0.743	−0.999*	−0.251	0.728	0.858	−0.845	−0.329	0.617	−0.942	0.677	0.817	−1.000**	−0.419
Tail weight (kg)	−0.676	0.987	−0.297	0.651	0.731	−0.444	−0.999*	−0.312	−0.874	−0.987	−0.396	−0.745	−1.000*	−0.972	0.648	−0.407
Stage 3																
Tail length (cm)	−0.950	−0.991	0.656	0.742	−0.931	−0.821	−0.472	0.785	−0.949	0.668	−0.759	0.941	−0.964	0.403	−0.906	−0.829
Tail width (cm)	0.815	0.695	0.219	0.100	0.976	0.725	0.600	−0.870	−0.717	0.276	−0.967	0.699	0.496	0.786	−0.185	−0.336
Tail thickness (mm)	0.924	0.837	−0.003	−0.124	0.989	0.424	0.850	−0.990	0.951	−0.979	0.227	−0.959	0.985	−0.315	0.862	0.772
Tail weight (kg)	0.996	0.961	−0.298	−0.411	−0.243	0.671	−0.814	0.515	0.236	0.278	0.954	−0.210	0.987	−0.302	0.855	0.764

Tail length, width, and thickness mean their variances per stage and tail weight represents the measured value at the end of growth stages. *$P < 0.05$, **$P < 0.01$. The same as below.

Table 4
Pearson correlation between tail phenotypes and Hedgehog signaling pathway.

Stage 1	*IHH*				*GLI1*				*SMO*				*PTCH1*			
	84%	96%	108%	120%	84%	96%	108%	120%	84%	96%	108%	120%	84%	96%	108%	120%
Tail length (cm)	−0.655	−0.854	0.606	−0.954	0.812	0.895	−0.491	0.164	−0.990	−0.874	−0.117	0.996	−0.948	−0.928	−0.243	0.997*
Tail width (cm)	−0.140	0.942	−0.407	−0.356	0.366	−0.967	−1.000**	0.995	−0.909	0.756	−0.928	−0.026	−0.969	0.832	−0.968	−0.012
Tail thickness (mm)	0.914	0.486	−0.422	−0.299	−0.796	−0.411	0.666	0.987	0.153	−0.52	0.327	−0.086	−0.028	−0.41	0.445	−0.073
Tail weight (kg)	0.605	−0.995	−0.644	−0.720	−0.773	1.000**	0.448	0.944	0.997	−0.577	0.068	0.402	0.966	−0.674	0.195	0.414
Stage 2																
Tail length (cm)	−0.411	−1.000**	−0.899	0.955	−0.973	−0.931	−0.207	−0.880	−0.787	−0.737	−0.952	−0.934	−0.696	−0.658	−0.996	0.211
Tail width (cm)	−0.941	−0.992	−1.000**	−0.650	0.156	−0.869	0.239	0.492	0.555	−0.826	−0.724	0.599	0.662	−0.544	−0.857	0.343
Tail thickness (mm)	−0.500	−0.202	−0.372	0.977	−0.945	0.183	−0.810	−0.918	−0.721	−0.803	−0.911	−0.961	−0.621	0.615	−0.800	0.294
Tail weight (kg)	0.878	0.794	−0.993	−0.484	−0.305	0.506	0.128	0.307	−0.676	0.997*	−0.797	0.426	−0.769	0.052	−0.910	0.524
Stage 3																
Tail length (cm)	−0.708	0.837	−0.912	−0.913	−0.999*	0.883	−0.869	0.261	0.833	0.999*	0.552	0.396	−0.674	0.408	−0.982	0.203
Tail width (cm)	0.988	−0.910	−0.640	−0.168	0.556	−0.944	−0.565	0.870	−0.939	−0.982	0.861	0.791	−0.195	−0.266	−0.966	0.898
Tail thickness (mm)	0.997*	−0.998*	0.978	0.871	0.727	−0.999*	0.993	−0.169	−0.992	−0.847	0.049	−0.308	0.028	0.104	0.680	−0.111
Tail weight (kg)	0.932	0.437	0.133	0.864	0.897	0.353	0.039	−0.156	−0.985	−0.163	−1.000*	−0.295	0.322	−0.957	0.68	−0.097

Table 5
Pearson correlation between tail phenotypes and *OXCT1*.

Items	Stage 1				Stage 2				Stage 3			
	84%	96%	108%	120%	84%	96%	108%	120%	84%	96%	108%	120%
Tail length (cm)	−0.894	1.000*	−0.978	0.021	−0.351	0.538	0.720	−0.249	0.852	−0.377	−0.971	−0.481
Tail width (cm)	−0.508	−0.983	−0.285	0.999*	−0.961	0.412	0.950	0.727	−0.927	0.513	−0.768	0.969
Tail thickness (mm)	0.690	0.018	0.910	1.000*	−0.443	−0.726	0.067	−0.165	−0.987	0.790	0.925	0.562
Tail weight (kg)	0.863	0.910	0.987	0.888	0.907	0.099	0.908	0.850	−0.990	−0.871	0.309	0.573

quantity, growth stage, and position reflects the physiological state of an animal to some extent. Thus, to effectively explore the growth, maturation, and senescence of an animal, the changes in gene expression should be investigated.

Dietary nutrition, including carbohydrates, fatty acids, and some trace elements, regulate gene transcription and translation [28]. C/EBPα is a member of the C/EBP transcription factor family and has been shown to promote adipogenesis by inducing the activity of PPARγ [29], which is required for fat formation [11]. The expression of *FAS* is positively correlated with the accumulation of triglyceride [12]. Inhibition of *FAS* has been shown to reduce food intake in rodents and lipid droplet formation in 3T3-L1 cells [30]. LPL and HSL are lipolytic enzymes that are mainly expressed in the adipose tissue [31,32]. *LPL* is expressed in the early differentiation of adipocytes and it mainly functions to hydrolyze triglycerides from chylomicrons and very low-density lipoproteins to provide fatty acids for storage or oxidation [13]. *LPL* expression and enzyme activity in the adipose tissue were reduced in fasting mice but rapidly increased to the fed level when food was provided [33]. HSL can mobilize triglycerides deposited in the tissues as free fatty acids to balance energy metabolism [14]. A high energy diet decreased the mRNA expression of *HSL* in the adipose tissue. The fold changes between different energy diets were found to be greater in the adipose tissue than in the muscle and heart tissue [34].

In this study, dietary nutrition significantly affected the expression of *C/EBPα*, *FAS*, *LPL*, and *HSL*. Overall, the lipogenic genes, *C/EBPα* and *FAS*, increased as the nutritional level increased when it was less than 108%. Considering the increase in tail phenotypes, that is, tail weight, with an increase in dietary nutrition, a possible reason for expression reduction in the 120% group observed in the present study can be attributed to the control of the excessive deposition rate of tail fat to maintain a certain growth pattern under excessive nutrition. The lipolytic genes, *LPL* and *HSL*, were negatively correlated with dietary nutrition. This finding may be because at low nutrient levels, Tan sheep mobilize the energy stored in their tails by upregulating the lipolytic enzyme genes and transporting the fat to other vital tissues to maintain growth and normal metabolism. This state is reversed with high-nutrient diets. Therefore, tail fat deposition increased with the increase in dietary nutrition. A similar finding was reported by another study on cattle, wherein a high-energy diet increased the *FAS* mRNA expression in the adipose tissue [6]. However, considering the gene expression pattern, we suspected that a certain limited diets may also be reasonable at some stages; a finding which is consistent with the results of our previous study on meat quality [23].

Animal fat deposition often gradually increases with growth stages. The size and weight of tail fat showed an increasing trend with the increase in age [35]. In male Kazak sheep, a short fat-tailed breed, the IMF increased significantly and continuously with growth [36]. The expression of *PPARγ*, *FAS*, and *HSL* was detected in Tan sheep aged 3–18 months and represented the rate of tail fat deposition, which was considerably slower during the early growth stages and considerably faster during the older stages [5]. A similar rate of fat deposition was demonstrated in Kazak and Xinjiang sheep [36]. We demonstrated that growth stages significantly affect the expression of *FAS*, *LPL* and *HSL*, but not *C/EBPα*. The expression of lipogenic genes, *C/EBPα* and *FAS*, decreased in the substandard nutritional treatment groups with growth stage. Furthermore, the expression of lipolytic genes, *LPL* and *HSL,* showed a conversed trend. It was similar to the rate of fat deposition described above. However, an opposite trend of these genes was detected with growth in the two high-nutritional diet groups, which may be attributed to the changes in dietary nutritional level. The results of the two-way ANOVA demonstrated that the interaction of nutritional level and growth stage significantly affected the expressions of *C/EBPα*, *FAS*, *LPL*, and *HSL*. It suggested that optimal feeding to improve efficiency of Tan sheep should consider both dietary nutrition and growth stage.

In this study, tail weight was affected by dietary nutrition, growth stage, and their interaction, and showed considerable correlations with *C/EBPα*, *FAS*, and *LPL*. Therefore, we assume that diet and growth stage may function in tail fat deposition, especially in tail weight in Tan sheep under the control of these three genes. Interestingly, the gene that correlated with the variance in tail width was *FAS*, which may explain a close relationship between *FAS* and tail width. Additionally, tail thickness may be related to *C/EBPα* and *LPL*, and tail length to *C/EBPα*. These results revealed the possible interaction between genes and phenotypes, and provide a basis for the research of diet and production performance.

The Hh signaling pathway can be activated to improve diet-induced obesity [37]. The reduction in Hh signaling in mice fed a high-fat diet led to enhanced weight gain and expression of adipogenic genes [38]. Ketone bodies are metabolites of fatty acids to provide energy, and *OXCT1* plays a key role in ketolysis [39]. People with high body fat contents had a significant lower expression of *OXCT1* than those with low body fat content [40]. Another study demonstrated that *OXCT1* played a negative role in adipogenesis in the tail [20]. In this study, *IHH* may be related to tail length, width, and thickness; *GLI1* to tail length, width, thickness, and weight; *SMO* to tail length and weight; *PTCH1* to tail length; and *OXCT1* to tail length, width, and thickness. We suggest that the Hh signaling pathway and *OXCT1* could affect tail fat deposition in Tan sheep considering dietary nutrition and growth stage.

5. Conclusions

The expression of *C/EBPα*, *FAS*, *LPL*, and *HSL* was affected by dietary nutrition, growth stage, and their interaction. An elevation in dietary nutrition contributed to tail fat deposition in Tan sheep. Furthermore, considering gene expression, a certain limit diet was also reasonable at some stages. *C/EBPα*, *FAS*, and *LPL* were correlated with the tail phenotypes, which demonstrated that dietary nutrition may function under the regulation of genes. The correlation between the tail phenotypes and Hh signaling pathway and *OXCT1* suggested their importance to tail fat deposition, necessitating further research. In summary, tail fat deposition can, to some extent, be regulated by manipulating the diet. An advanced understanding of the balance between diet and tail fat deposition and identifying crucial genes may provide key insights that can be used to improve the production efficiency of fat-tailed sheep and figure out the mechanism of tail fat deposition.

Financial support

This study was supported by the Tan Sheep Breeding Project of Ningxia (NXTS201601), as well as the China Agriculture Research System (CARS-39-12).

Conflict of interest

The authors declare that they have no competing or financial interests this study.

Acknowledgments

The authors thank Ningxia Tan sheep breeding Centers for their kind help in feeding trial and sample collection and Lixia Dai, Chunli Tian, Xiaofang Wang, and Chongqi Tian for their help in this study.

Supplementary material

https://doi.org/10.1016/j.ejbt.2020.05.004

References

[1] Alizadeh A, Zare Shahneh A, Yousefi AR, et al. Determining the effect of the fat-tail and carcass weight on meat fatty acid composition of Iranian lambs. Small Ruminant Res. 2013;115(1–3):34–9. https://doi.org/10.1016/j.smallrumres.2013.06.004.
[2] Almeida AM. "By endurance we conquer": fat tailed sheep in the twenty-first century. Tropl Anim Health Prod. 2011;43(7):1233–5. https://doi.org/10.1007/s11250-011-9855-8 PMid: 21509457.
[3] Wang X, Zhou G, Xu X, et al. Transcriptome profile analysis of adipose tissues from fat and short-tailed sheep. Gene. 2014;549(2):252–7. https://doi.org/10.1016/j.gene.2014.07.072 PMid: 25088569.
[4] Bakhtiarizadeh MR, Moradi-Shahrbabak M, Ebrahimie E. Underlying functional genomics of fat deposition in adipose tissue. Gene. 2013;521(1):122–8. https://doi.org/10.1016/j.gene.2013.03.045 PMid: 23523858.
[5] Xu X, Wei X, Yang Y, et al. PPARγ, FAS, HSL mRNA and protein expression during tan sheep fat-tail development. Electron J Biotechnol. 2015;18(2):122–7. https://doi.org/10.1016/j.ejbt.2015.01.004.
[6] Zhang H, Zhang X, Wang Z, et al. Effects of dietary energy level on lipid metabolism-related gene expression in subcutaneous adipose tissue of yellow breed x Simmental cattle. Anim Sci J. 2015;86(4):392–400. https://doi.org/10.1111/asj.12316 PMid: 25440457.
[7] Jaborek JR, Zerby HN, Moeller SJ, et al. Effect of energy source and level, and sex on growth, performance, and carcass characteristics of long-fed lambs. Small Ruminant Res. 2018:16761–9. https://doi.org/10.1016/j.smallrumres.2018.08.005.
[8] Lingyan, Yuankui, Xianyou, et al. Effects of different dietary energy and protein levels and sex on growth performance,carcass characteristics and meat quality of F1 Angus x Chinese Xiangxi yellow cattle. J Anim Sci Biotechnol. 2014;5(4):21-21. https://doi.org/10.1186/2049-1891-5-21 PMid: 24739901.
[9] Guo X, Tang R, Wang W, et al. Effects of dietary protein/carbohydrate ratio on fat deposition and gene expression of peroxisome proliferator activated receptor γ and heart fatty acid-binding protein of finishing pigs. Livest Sci. 2011;140(1–3):111–6. https://doi.org/10.1016/j.livsci.2011.02.016.
[10] Kang D, Zhou G, Zhou S, et al. Comparative transcriptome analysis reveals potentially novel roles of Homeobox genes in adipose deposition in fat-tailed sheep. Sci Rep. 2017;7(1):14491. https://doi.org/10.1038/s41598-017-14967-9 PMid: 29101335.
[11] Cristancho AG, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol. 2011;12(11):722–34. https://doi.org/10.1038/nrm3198 PMid: 21952300.
[12] Semenkovich CF. Regulation of fatty acid synthase (FAS). Prog Lipid Res. 1997;36(1):43–53. https://doi.org/10.1016/S0163-7827(97)00003-9.
[13] Goldberg IJ, Merkel M. Lipoprotein lipase: physiology, biochemistry, and molecular biology. Front Biosci. 2001;6(3):D388. https://doi.org/10.2741/Goldberg PMid: 11229871.
[14] Langin D, Holm C, Lafontan M. Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. Proc Nutr Soc. 1996;55(1B):93–109. https://doi.org/10.1079/PNS19960013 PMid: 8832784.
[15] James AW, Pan A, Chiang M, et al. A new function of Nell-1 protein in repressing adipogenic differentiation. Biochem Biophys Res Commun. 2011;411(1):126–31. https://doi.org/10.1016/j.bbrc.2011.06.111 PMid: 21723263.
[16] James AW, Pang S, Askarinam A, et al. Additive effects of sonic hedgehog and Nell-1 signaling in osteogenic versus adipogenic differentiation of human adipose-derived stromal cells. Stem Cells Dev. 2012;21(12):2170–8. https://doi.org/10.1089/scd.2011.0461 PMid: 22264144.
[17] Braune J, Weyer U, Matz-Soja M, et al. Hedgehog signalling in myeloid cells impacts on body weight, adipose tissue inflammation and glucose metabolism. Diabetologia. 2017;60(5):889–99. https://doi.org/10.1007/s00125-017-4223-5 PMid: 28233033.
[18] Magno LD, Basile A, Coni S, et al. The energy sensor AMPK regulates hedgehog signaling in human cells through a unique Gli1 metabolic checkpoint. Oncotarget. 2016;7(8):9538–49. https://doi.org/10.18632/oncotarget.7070 PMid: 26843621.
[19] Hojo H, Ohba S, Yano F, et al. Gli1 protein participates in hedgehog-mediated specification of osteoblast lineage during endochondral ossification. J Biol Chem. 2012; 287(21):17860–9. https://doi.org/10.1074/jbc.M112.347716 PMid: 22493482.
[20] Zeng J, Zhou S-W, Zhao J, et al. Role of OXCT1 in ovine adipose and preadipocyte differentiation. Biochem Biophys Res Commun. 2019;512(4):779–85. https://doi.org/10.1016/j.bbrc.2019.03.128 PMid: 30928098.
[21] Feeding standard of meat-producing sheep and goats (NY/T816-2004), Hunan Feed. 2006(6):9–15. [In Chinese].
[22] Li X, Wang Y, Guo J, et al. Identification and expression patterns of adipokine genes during adipocyte differentiation in the Tibetan goat (Capra hircus). Gene. 2018: 64317–25. https://doi.org/10.1016/j.gene.2017.11.069 PMid: 29197590.
[23] D.L.-X., Zhou Shi-wei, Tian Chun-li, et al. Study on the phased fattening effect of 4 to 8 month-old tan sheep under the same energy-protein ratio. Chin J Anim Sci. 2016; 52(23):44–52. [In Chinese].
[24] Z.S.-w., Dai Li-xia, Tian Chong-qi, et al. Effects of different diets on tail-fat depositions and expressions of leptin and GLUT4 genes of tan sheep. J Domestic Anim Ecol. 2017;38(1):13–9. [In Chinese].
[25] Sutherland MA, Tucker CB. The long and short of it: a review of tail docking in farm animals. Appl Anim Behav Sci. 2011;135(3):179–91. https://doi.org/10.1016/j.applanim.2011.10.015.
[26] Wang YQ, Zhong RZ, Fang Y, et al. Influence of tail docking on carcass characteristics, meat quality and fatty acid composition of fat-tail lambs. Small Ruminant Res. 2018: 16217–21. https://doi.org/10.1016/j.smallrumres.2017.09.005.
[27] Xu X, Wei X, Yang Y, et al. mRNA transcription and protein expression of PPARγ, FAS, and HSL in different parts of the carcass between fat-tailed and thin-tailed sheep. Electron J Biotechnol. 2015;18(3):215–20. https://doi.org/10.1016/j.ejbt.2015.03.010.
[28] Clarke SD, Abraham S. Gene expression: nutrient control of pre- and posttranscriptional events. FASEB J. 1992;6(13):3146–52. https://doi.org/10.1096/fasebj.6.13.1397836 PMid: 1397836.
[29] Li D, Zhang F, Zhang X, et al. Distinct functions of PPARgamma isoforms in regulating adipocyte plasticity. Biochem Biophys Res Commun. 2016;481(1–2):132–8. https://doi.org/10.1016/j.bbrc.2016.10.152 PMid: 27818196.
[30] Schmid B, Rippmann JF, Tadayyon M, et al. Inhibition of fatty acid synthase prevents preadipocyte differentiation. Biochem Biophys Res Commun. 2005;328(4):1073–82. https://doi.org/10.1016/j.bbrc.2005.01.067 PMid: 15707987.
[31] Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab. 2009;297(2):E271. https://doi.org/10.1152/ajpendo.90920.2008 PMid: 19318514.
[32] Berraondo B, Martínez JA. Free fatty acids are involved in the inverse relationship between hormone-sensitive lipase (HSL) activity and expression in adipose tissue after high-fat feeding or beta3-adrenergic stimulation. Obes Res. 2012;8(3):255–61. https://doi.org/10.1038/oby.2000.30 PMid: 10832769.
[33] Ruge T, Wu G, Olivecrona T, et al. Nutritional regulation of lipoprotein lipase in mice. Int J Biochem Cell B. 2004;36(2):320–9. https://doi.org/10.1016/S1357-2725(03)00256-5.
[34] Zhang Y, Liu Y, Cheng S, et al. Effects of dietary energy level on the expression of the HSL gene in different tissues of sheep. J Integr Agric. 2012;11(7):1167–72. https://doi.org/10.1016/S2095-3119(12)60111-7.
[35] Hamouda MB, Atti N. Comparison of growth curves of lamb fat tail measurements and their relationship with body weight in Babarine sheep. Small Ruminant Res. 2011;95(2–3):120–7. https://doi.org/10.1016/j.smallrumres.2010.10.001.
[36] Qiao Y, Huang Z, Li Q, et al. Developmental changes of the FAS and HSL mRNA expression and their effects on the content of intramuscular fat in Kazak and Xinjiang sheep. J Genet Genomics. 2007;34(10):909–17. https://doi.org/10.1016/S1673-8527(07)60102-7.
[37] Shi Y, Long F. Hedgehog signaling via Gli2 prevents obesity induced by high-fat diet in adult mice. eLife. 2017;6:e31649. https://doi.org/10.7554/eLife.31649.
[38] Hye-Jin L, Shin-Bum J, Romer AI, et al. Overweight in mice and enhanced adipogenesis in vitro are associated with lack of the hedgehog coreceptor boc. Diabetes. 2015; 64(6):2092–103. https://doi.org/10.2337/db14-1017 PMid: 25576054.
[39] Cotter DG, Schugar RC, Wentz AE, et al. Successful adaptation to ketosis by mice with tissue-specific deficiency of ketone body oxidation. Am J Physiol Endocrinol Metab. 2013;304(4):E363–74. https://doi.org/10.1152/ajpendo.00547.2012 PMid: 23233542.
[40] Badoud F, Lam KP, DiBattista A, et al. Serum and adipose tissue amino acid homeostasis in the metabolically healthy obese. J Proteome Res. 2014;13(7):3455–66. https://doi.org/10.1021/pr500416v PMid: 24933025.