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Effect of dietary *Sophora alopecuroides* supplementation on differential expression of intramuscular fat-related genes of Ningxia Tan sheep as determined by transcriptome sequencing

Guosheng Xin[1,2#], *Ju Guo*[3#], *Baojun Yu*[3], *Hao Li*[1,2], *Yaling Gu*[3],
Qiaoxian Gao[1,2], *Juan Zhang*[3*]

[1] *School of Life Science, Ningxia University, Yinchuan, P.R. China*
[2] *Ningxia Feed Engineering Technology Research Center, Ningxia University, Yinchuan, P.R. China*
[3] *School of Agriculture, Ningxia University, Yinchuan, P.R. China*

**Corresponding author: zhangjkathy@126.com*
#These authors contributed equally to this work

Electronic Supplementary Material (ESM)

Figure S1. Agarose gel electrophoresis of RNA samples from *m. longissimus dorsi* of sheep of the control group (NABB946, NABB950, NABB1361) and experimental group fed with 2% of *Sophora alopecuroides* (ABB1302, ABB1315, ABB1336)

Figure S2. RNA-Seq correlation between 2% *Sophora alopecuroides* and control group (A) and principal component analysis of RNA-Seq (B)

Table S1. Composition and nutrient levels of experimental diets (dry basis)

Table S2. Gene specific primers used for RT-qPCR

Table S3 (Not part of this document). Supplementary material for 66 genes significantly upregulated and 202 genes significantly down-regulated

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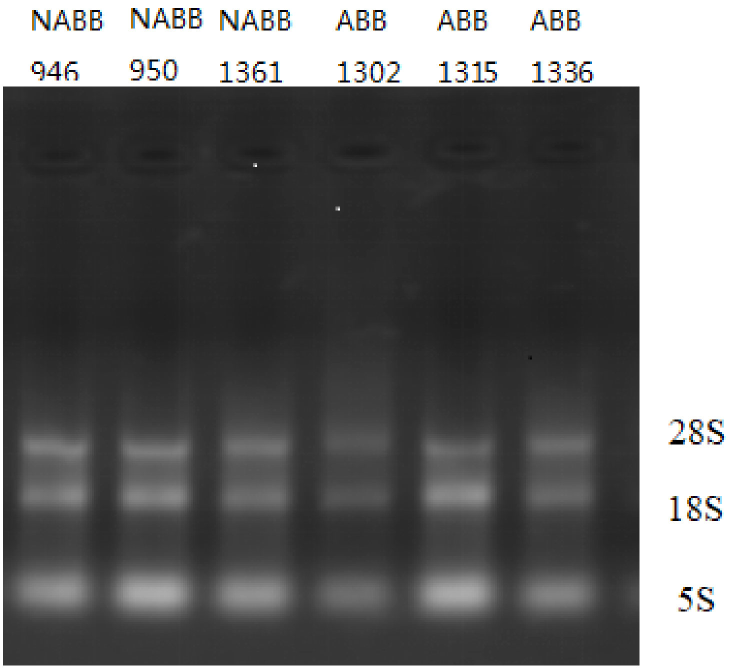

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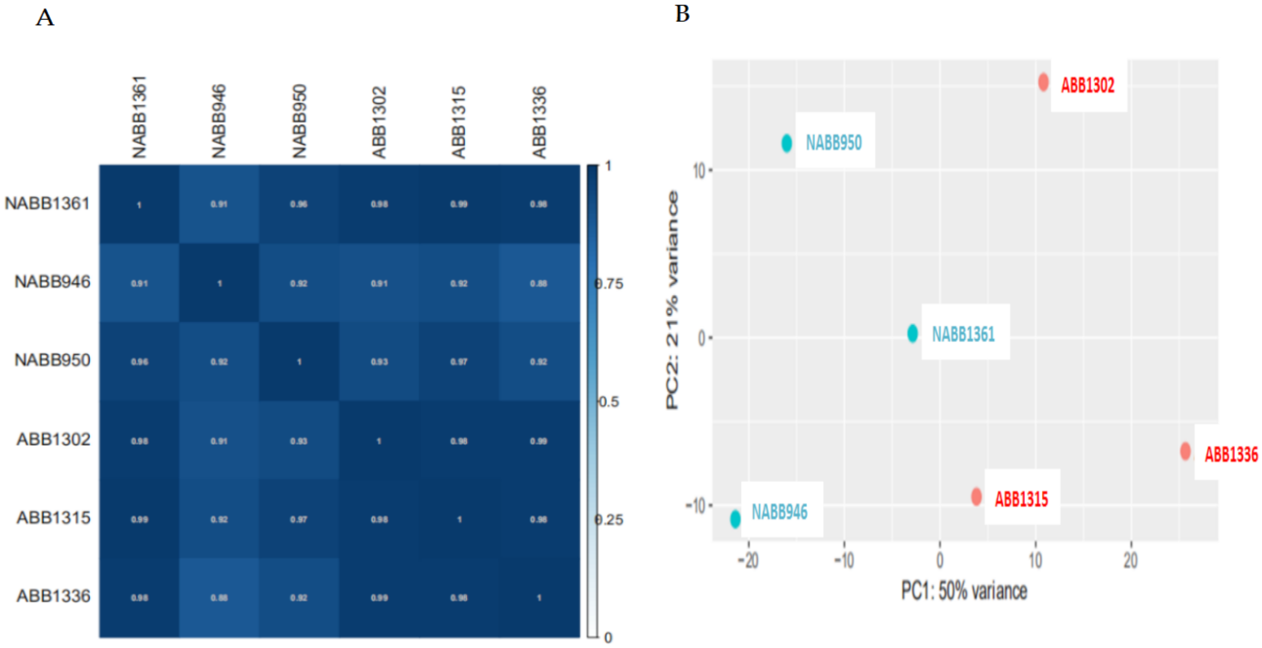

	NABB1361	NABB946	NABB950	ABB1302	ABB1315	ABB1336
NABB1361	1	0.91	0.96	0.98	0.99	0.98
NABB946	0.91	1	0.92	0.91	0.92	0.88
NABB950	0.96	0.92	1	0.93	0.97	0.92
ABB1302	0.98	0.91	0.93	1	0.98	0.99
ABB1315	0.99	0.92	0.97	0.98	1	0.98
ABB1336	0.98	0.88	0.92	0.99	0.98	1

Figure S2. RNA-Seq correlation between 2% *Sophora alopecuroides* and control group (A) and principal component analysis of RNA-Seq (B)

Note: NABB946, NABB950 and NABB1361 belong to the control group (no *S. alopecuroides*); whereas ABB1302, ABB1315 and ABB1336 belong to the experimental group (2% *S. alopecuroides* added); (B) Principal component analysis of RNA-Seq in the test group (red dots) and control group (blue dots)

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Table S1. Composition and nutrient levels of experimental diets (dry basis)

Ingredients (%)	A Group	B Group	C Group	D Group	E Group
Sophora alopecuroides	0	0.25	0.50	1.00	2.00
DDGS	2.95	2.94	2.94	2.92	2.89
Til cake	2.90	2.89	2.89	2.87	2.84
Soybean meal	3.60	3.59	3.58	3.56	3.53
Soybean oil	0.20	0.20	0.20	0.20	0.20
Live yeast	0.50	0.50	0.50	0.50	0.50
Cotton pulp	3.40	3.39	3.38	3.37	3.33
Stone powder	0.90	0.90	0.90	0.90	0.90
NaCl	0.30	0.30	0.30	0.30	0.30
Baking soda	0.50	0.50	0.50	0.50	0.50
1% premix[1]	0.60	0.60	0.60	0.60	0.60
Wheat bran	12.00	11.96	11.92	11.85	11.70
Corn	32.05	31.97	31.89	31.73	31.41
Corn husk	10.00	9.98	9.95	9.90	9.80
Urea	0.10	0.10	0.10	0.10	0.10
Rice hull powder	24.00	23.94	23.88	23.76	23.52
Gourd seed shell	6.00	5.99	5.97	5.94	5.88
Total	100.00	100.00	100.00	100.00	100.00
Nutrient levels[2] (MJ/kg)	10.62	10.63	10.64	10.67	10.71
CP%	14.32	14.34	14.35	14.39	14.46
EE%	2.21	2.21	2.22	2.23	2.25
NDF%	50.47	50.42	50.37	50.27	50.08
ADF%	22.39	22.39	22.40	22.41	22.43
Ca%	0.76	0.76	0.76	0.75	0.75
P%	0.41	0.41	0.41	0.41	0.41

[1] Composition of premix (per kg of diets): Cu 25 mg, Fe 64 mg, Zn 40 mg, Mn 50 mg, Co 0.5 mg, I 1.5 mg, Se 0.25 mg, vitamin A 3200 IU, vitamin D1200 IU, vitamin E 30IU

[2] According to the amount of feed raw materials, each nutrient level was calculated based on air-dried mixed pellet feed, each nutrient component of group A was measured according to the national standard method, and the other groups were calculated according to the level of added *S. alopecuroides*. Nutrition level of *S. alopecuroides*: CP: 21.12%; EE: 4.07%; NDF; 40.80%; ADF: 24.28%; Ash: 3.08%; Ca: 0.22%; P: 0.32%

Table S2. Gene specific primers used for RT-qPCR

Gene	Gene name	Primer sequence (5'-3')	Accession number	Amplicon length (bp)
ACTB	Actin beta	F: CCACACCTTCTACAACGA R: GGTCATCTTCTCACGGTT	U39357.2	100
ACSL3	Acyl-CoA synthetase long chain family member 3	F: CCATCGCCATCTTCTGTGAAACC R: AATTGCTGGACCGCCTAGAGTAG	XM_027965239.1	113
PLIN2	Perilipin 2	F: TGTGACGACGACTGTGAC R: CCACTGACCACGGACTTG	XM_015092734.2	120
ABCA1	ATP binding cassette subfamily A member 1	F: GACCATCACGGACCTCTTC R: ACACAGGCAGCATCTTCTT	XM_027964161.1	101
ANGPTL4	Angiopoietin like 4	F: CCACCACCTTCTCGCCCAGT R: GCCAAACCACCAGCCACCAG	NM_001308589.1	111