

**The spatio-temporal features of chicken mitochondrial ND2 gene heteroplasmy
and the effects of nutrition factors on this gene**

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Table S1 *The ingredients and nutrient levels of energy restriction experimental diets**(air-dry basis) %*

Items	AL ^{a)}	ER ^{a)}
Ingredients		
Corn	57.94	48.74
Soybean oil	6.65	1.7
Soybean meal	31.1	44.23
CaCO ₃	1.33	1.63
CaHPO ₄	1.34	1.74
Choline Chloride	0.1	0.13
L-Lysine	0.08	
Methionine	0.11	0.15
Salt	0.35	0.43
Premix ^{b)}	1.00	1.25
Sum	100	100
Nutrient levels ^{c)}		
ME /(MJ /kg)	13.17	11.53
CP	19.00	23.75
Ca	0.90	1.13
TP	0.55	0.68
NPP	0.35	0.44
Lys	1.05	1.31
Met	0.40	0.50
CF	8.97	4.04

^{a)}AL for the ad libitum group and ER for the energy restriction group.

^{b)}Premix ,Vitamin and mineral mix per kilogram of feed: vitamin A, 12 000IU; vitamin D₃, 2 400 IU; vitamin K₃, 3 mg; vitamin B₁, 2.2 mg; vitamin B₂, 8 mg; vitamin B₆, 5 mg; vitamin B₁₂, 11µg; folic acid, 1.5 mg; biotin,150 µg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg.

^{c)}Calculated in accordance with nutrient requirements of broilers of NRC nutritional standards of chicken (1994).

Table S2 *The ingredients and nutrient levels of different types of dietary fats**experimental diets (air-dry basis) %*

Items	Aged from 1 to 3 weeks		Aged from 4 to 6 weeks	
	LO; SO; CO ^{a)}	LG ^{a)}	LO; SO; CO	LG
Ingredients				
Corn	51.15	50.65	57.35	56.94
Oil	5.00	5.00	5.00	5.00
Soybean meal	35.36	35.46	29.70	31.00
Fish meal	3.00	3.00	3.00	3.00
Corn gluten meal			0.90	
NaCl	0.35	0.35	0.35	0.35
Limestone	1.26	1.26	1.19	1.18
CaHPO 4	1.55	1.55	1.26	1.26
50% choline chloride	0.26	0.26	0.20	0.20
Lys	0.03	0.03		0.01
Met	0.14	0.14	0.05	0.06
Limestone	0.90	1.30		
Premix ^{b)}	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Nutrient levels ^{c)}				
ME /(MJ /kg)	12.56	12.56	12.98	12.98
CP	21.50	21.50	20.01	20.01
Ca	1.01	1.01	0.90	0.90
TP	0.68	0.68	0.62	0.62
AP	0.45	0.45	0.40	0.40
Lys	1.15	1.16	1.02	1.05
Met	0.50	0.50	0.40	0.41

^{a)}LO for the linseed oil group, SO for the sesame oil group, CO for the corn oil group and LG for the lard grease group.

^{b)}Premix provided the following per kg of diets: VA 0.45 mg, VD 3 0.005 mg, VE 10.00

mg, VK 0.50 mg, VB₁ 1.80 mg, VB₂ 3.60 mg, VB₆ 3.50 mg, VB₁₂ 0.002 5 mg, niacin acid 35.00 mg, folic acid 0.55 mg, biotin 0.20 mg, pantothenic acid 10.00 mg, Zn (ZnSO₄·7H₂O) 40.00 mg, Mn (MnSO₄·5H₂O) 60.00 mg, Fe (FeSO₄·7H₂O) 80.00 mg, Cu (CuSO₄·5H₂O), 10.00 mg, I (KI) 0.35 mg, Se (Na₂SeO₃·5H₂O) 0.15 mg.

^{c)}Calculated in accordance with the nutrient requirements of white-feathered broiler chickens (NY/T 33-2004).

Supplementary Method: Pyrophosphate sequencing technology

Biotin labeled primers were used to amplify polymerase chain reaction (PCR). Then the PCR product was purified and transformed into single chain. DNA polymerase, ATP sulfatase, luciferase and ATP diphosphatase were added to the mixture. A type of dNTP was added each time during the sequencing process. If the dNTP could complement the template chain, a series of reactions occurred under the action of four enzymes. Finally, the fluorescence signal was converted into electrical signal, showing peaks of different heights, the height of which was proportional to the number of bases. On the contrary, when dNTP could not bind to the template chain, it would be directly degraded by ATP-diphosphatase, accordingly, there would be no peak map. The figure below showed the principle of pyrophosphate sequencing technology.

