



➤ **Transgenerational effects of maternal methionine restriction on production performance and liver transcript levels in mule ducks**

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# Context & issues: foie gras production

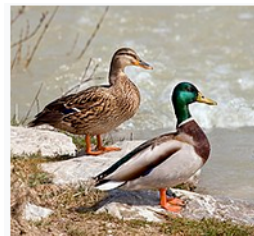
France accounts for 60% of the world's production of "foie gras".

97% of the French "foie gras" production comes from the mule ducks.

Male  
Muscovy duck  
*Cairina moschata*



Female  
Common Mallard duck  
*Anas platyrhynchos*

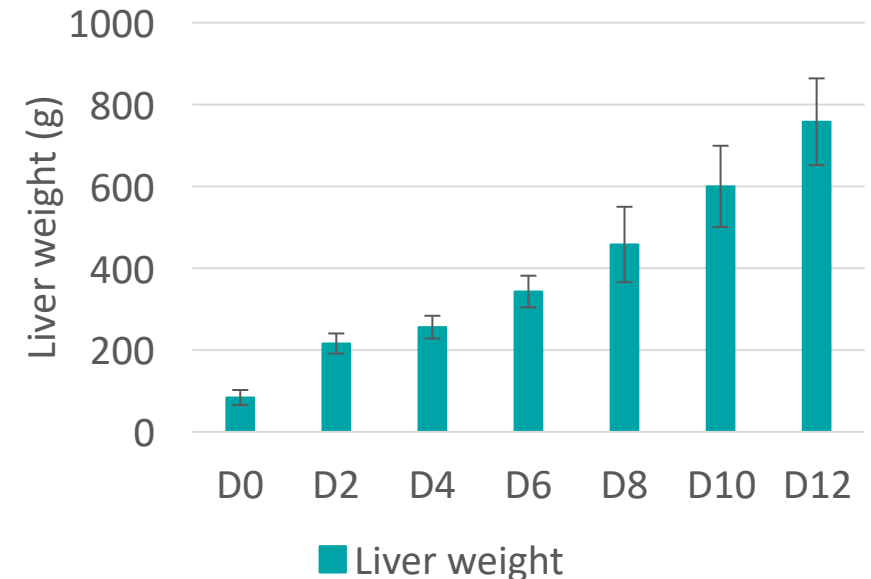


Mule ducks

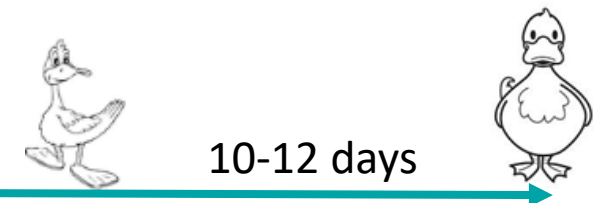


10-12 weeks

Liver evolution during overfeeding



Mozduri et al., Front Physiol. 2021

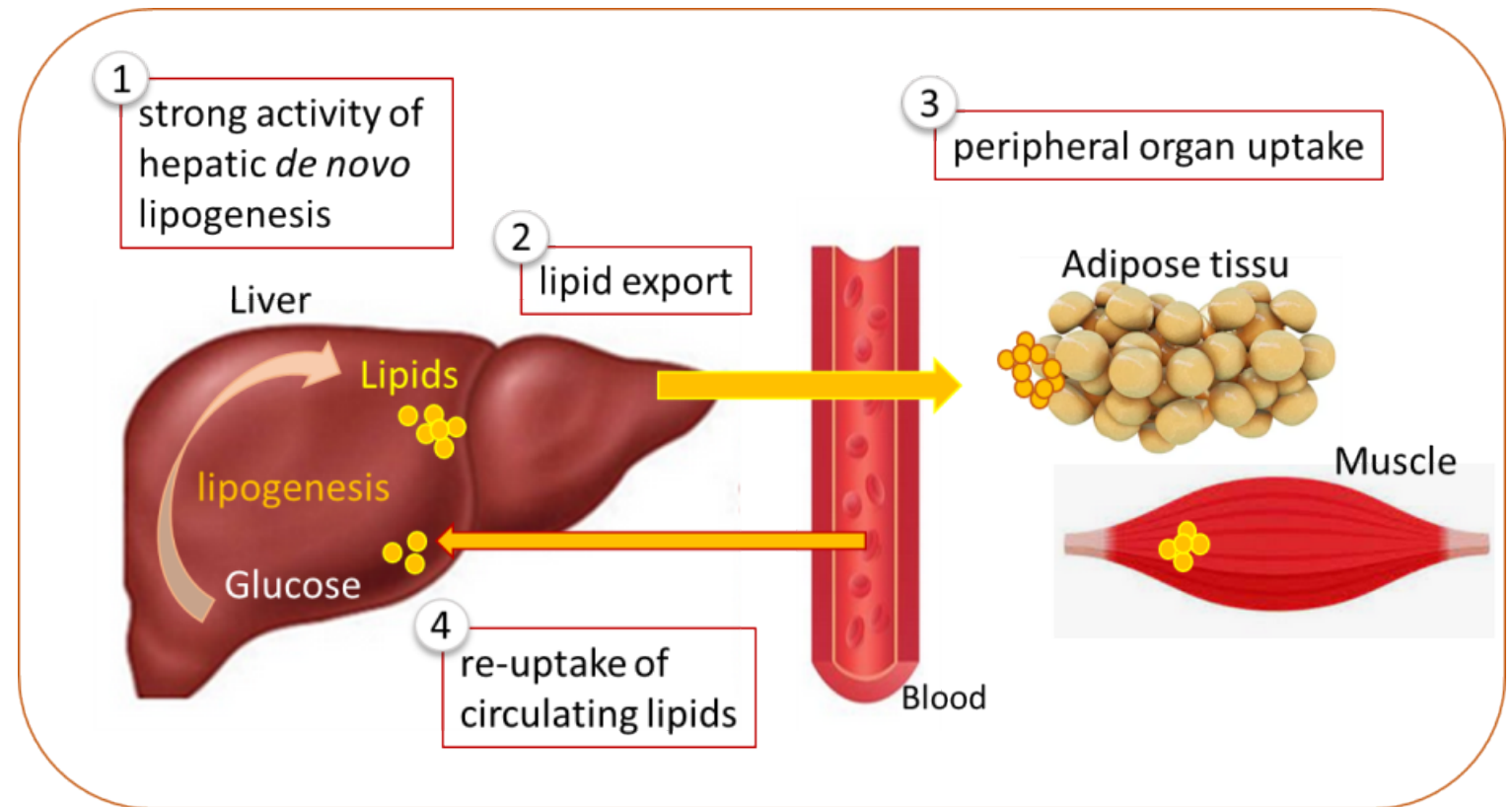


12.5-day period of overfeeding

# Context & issues: lipogenesis and lipid storage in liver

The liver is the main tissue for lipid synthesis in birds

In adult mule ducks, the capacity to accumulate and store lipids -mainly triglycerides- in liver, is enhanced by overfeeding that leads to hepatic steatosis for fatty liver production

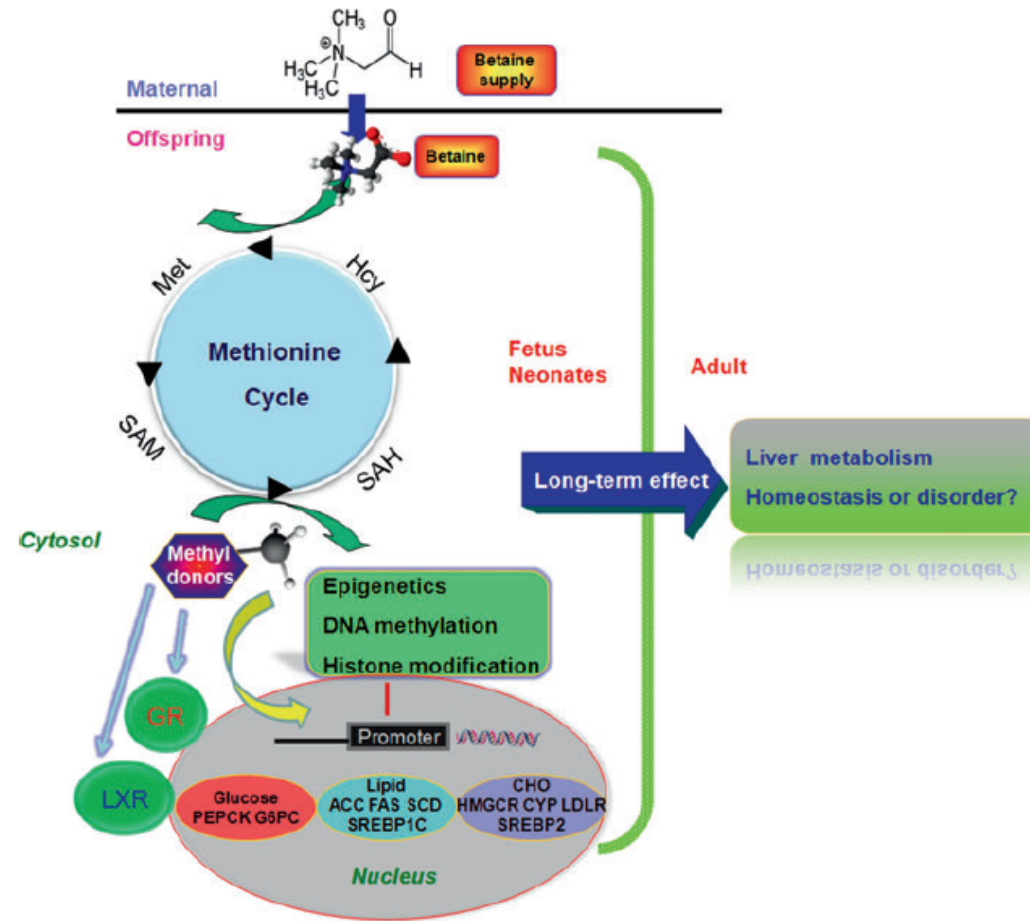


# Context & issues: impact of methyl donors

Liver metabolism is a major target of maternal availability in methyl donors

Maternal dietary intake of nutrients related to the methionine cycle affects methyl donor availability and neonatal liver metabolism via epigenetic mechanisms such as DNA and histone methylation.

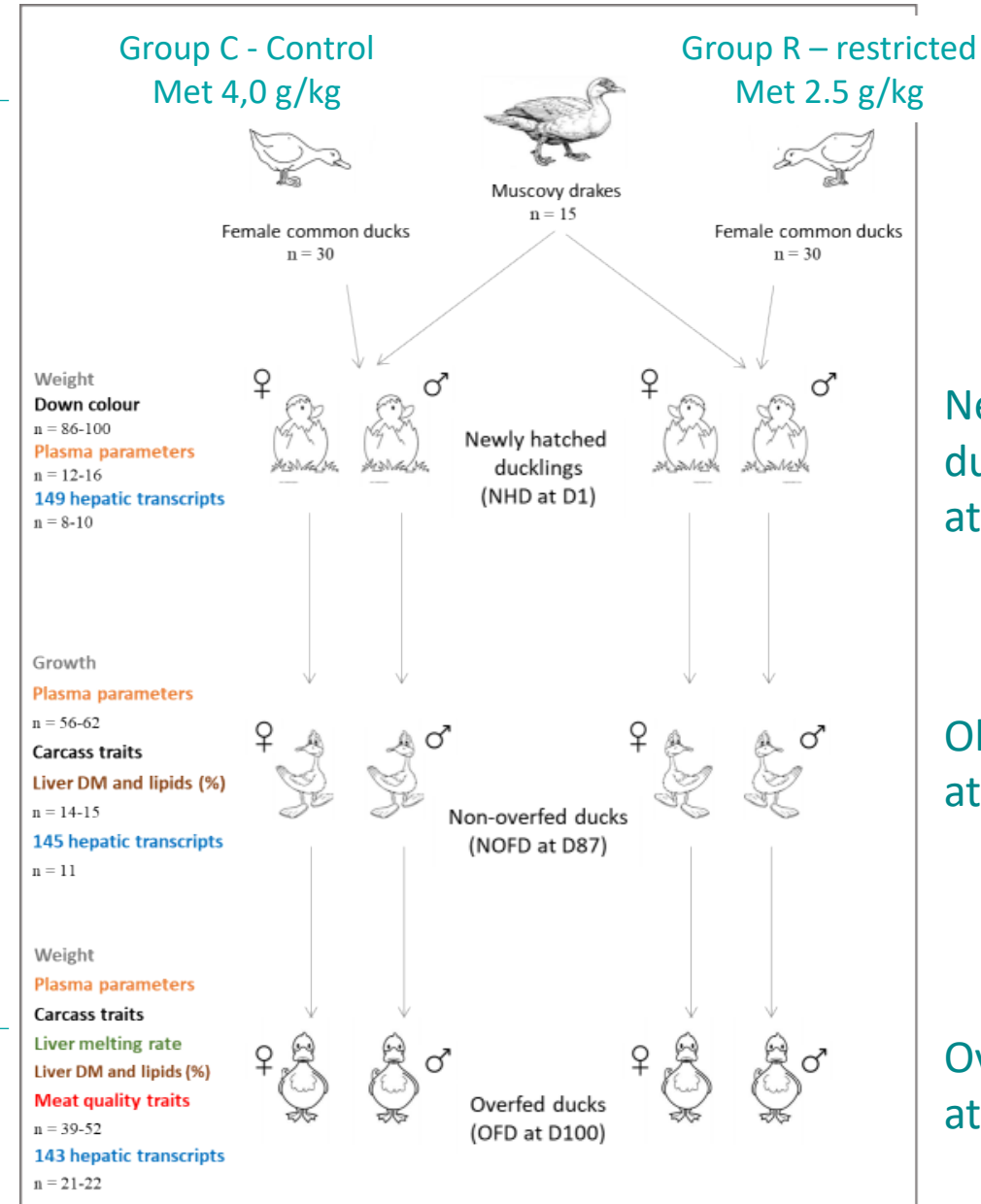
The status of hepatic lipid and glucose metabolism at birth can affect adult health.



# Objectives and experimental design

## Nutritional programming of hepatic metabolism in mule duck

We wanted to find out whether a 37% reduction in dietary methionine applied to female ducks could impact the production performance of their mule offspring.



Newly hatched ducklings at D1

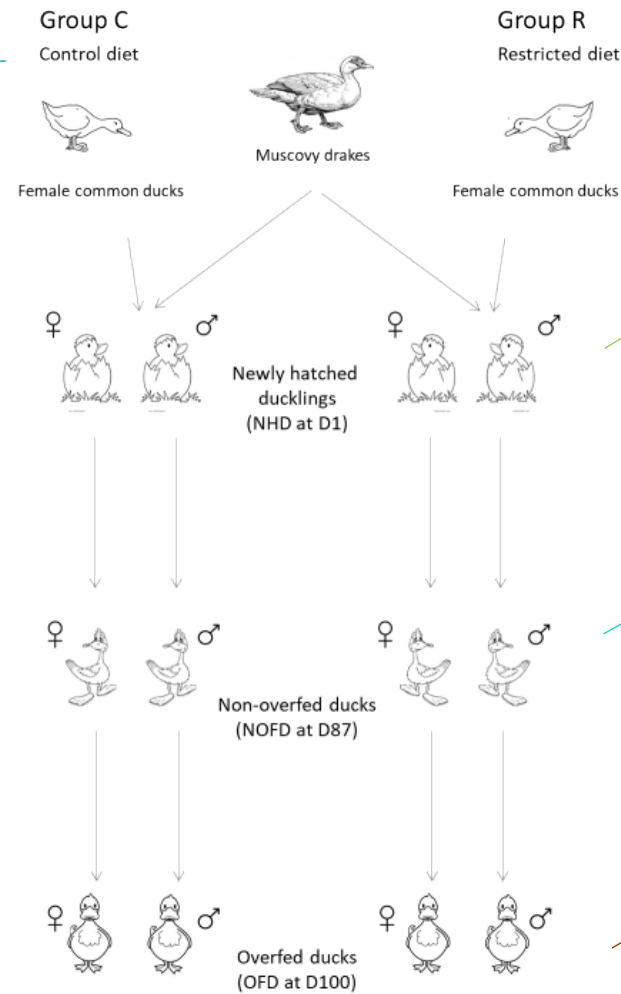
Older ducks at D87

Overfed ducks at D100

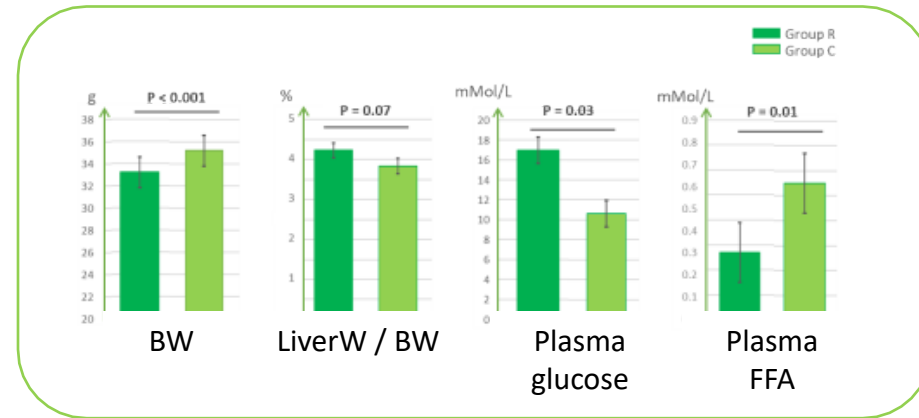
# Zootechnical results

**Maternal methionine restriction had long term effects on offspring phenotypic traits**

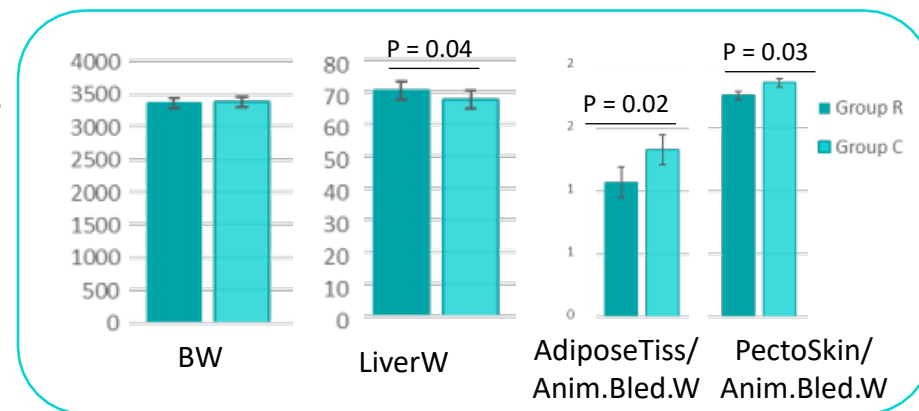
The effects of maternal methionine restriction were still present at D100 after the 12.5-day overfeeding period.



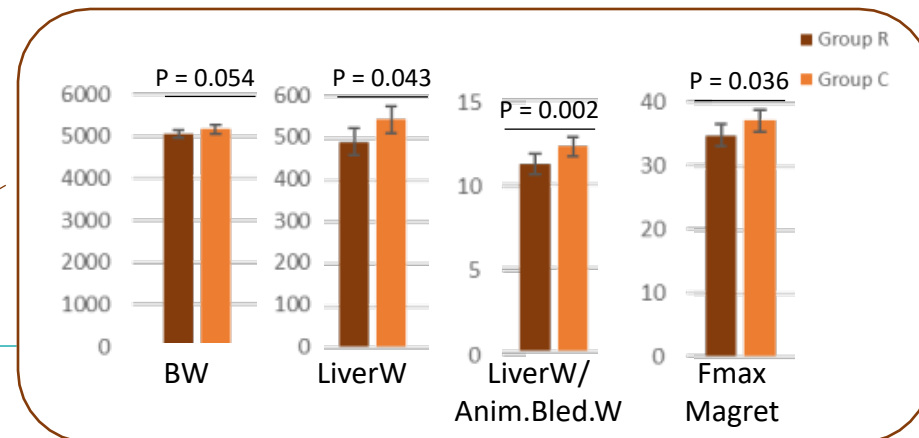
D1



D87



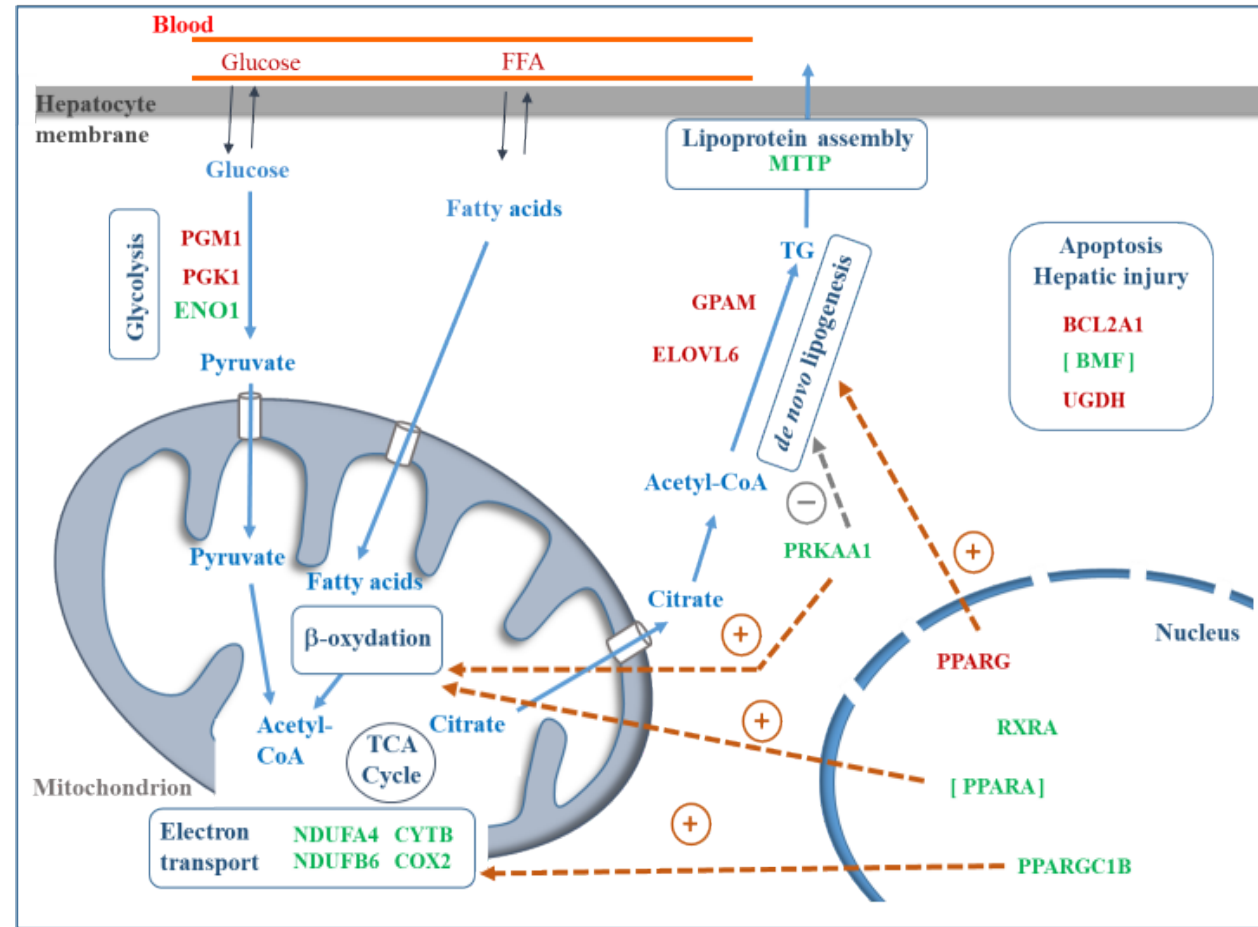
D100





# Differentially expressed genes in the liver of newly hatched ducklings

18 DEGs related to energy metabolism (87 genes studied)



Down-regulated genes in group R  
Up-regulated genes in group R



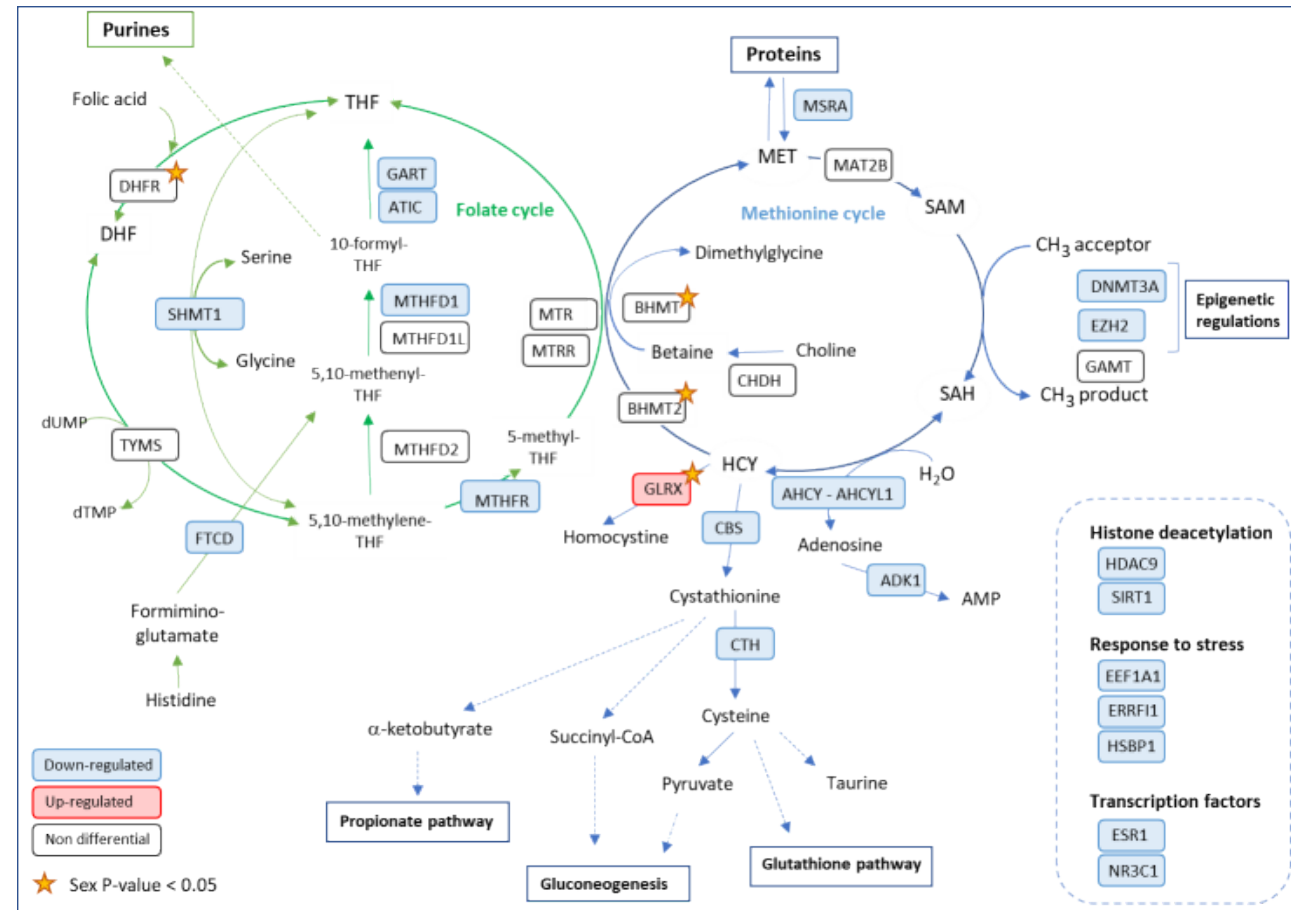


# Differentially expressed genes in the liver of newly hatched ducklings

22 DEGs related to one-carbon and epigenetic mechanisms (62 genes studied)

Most of the DEGs were down regulated in the offspring of the methionine restricted group.

Proteomic data and enzymatic activities should be studied to confirm the impacts on the early establishment of metabolic pathways.





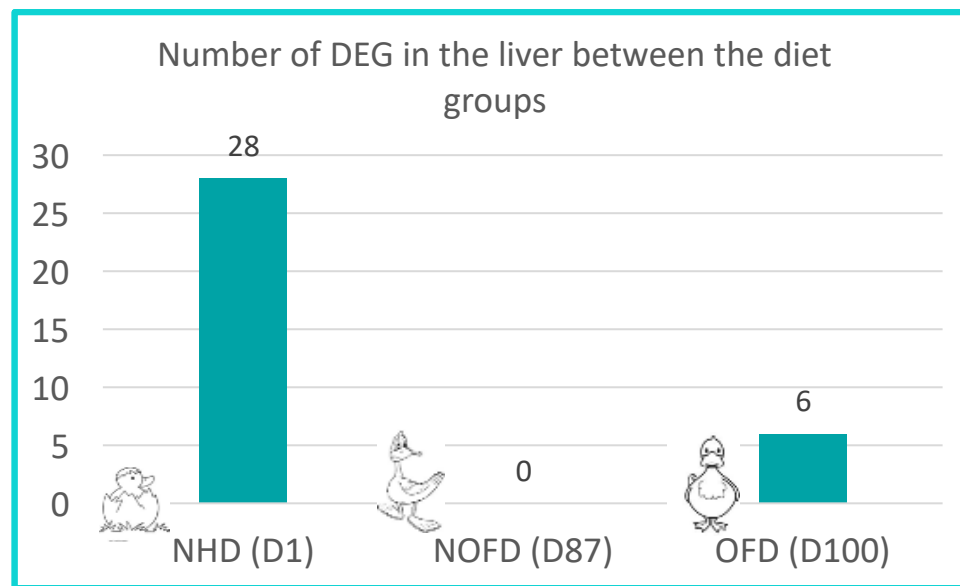


# Differentially expressed genes in the liver of ducks

At D87, no DEG in the liver for the diet effect

At D100, after 12.5 days of overfeeding, 6 DEG were identified.

They were all up-regulated in restricted group when compared to the control group.



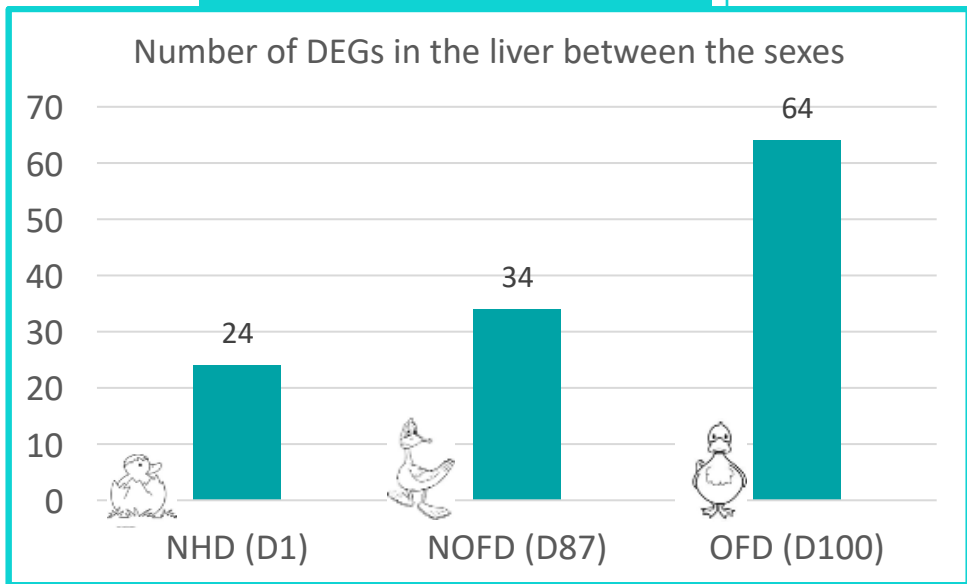
Number of studied genes: 143 – 149

ACADM,  
ACOX1,  
ADK,  
APOB,  
IL6ST,  
MTHFR



# Hepatic sex-biased gene expression

The number of differential genes is almost doubled by the 12.5 days of overfeeding



Number of studied genes: 143 – 149

Transgenerational effects of maternal dietary

EAAP/ August 2023 / Cécile Bonnefont et al.

D1: 24 differential genes ( $p < 0.1$ ) of 149 studied genes

Gene	Females LsMeans $\pm$ SD	Males LsMeans $\pm$ SD	Sex effect P-value (BH)
ABCA1	-0.20 $\pm$ 0.07	0.09 $\pm$ 0.07	0.02
ACLY	0.87 $\pm$ 0.09	1.18 $\pm$ 0.09	0.08*
BHMT	-0.67 $\pm$ 0.24	0.56 $\pm$ 0.22	< 0.01
BHMT2	-0.57 $\pm$ 0.26	0.51 $\pm$ 0.25	< 0.01
CTSL2	0.92 $\pm$ 0.12	1.31 $\pm$ 0.11	0.08*
DHCR24	0.73 $\pm$ 0.09	1.10 $\pm$ 0.08	0.02
DHFR	-0.86 $\pm$ 0.17	0.70 $\pm$ 0.16	< 0.01
ELAVL1	0.81 $\pm$ 0.09	1.02 $\pm$ 0.03	0.00
ELOVL6	-0.93 $\pm$ 0.26	-0.09 $\pm$ 0.25	0.00
FASN	0.62 $\pm$ 0.19	1.13 $\pm$ 0.19	0.01
GLRX	-0.63 $\pm$ 0.19	0.52 $\pm$ 0.17	< 0.01
GPAM	-0.37 $\pm$ 0.08	0.12 $\pm$ 0.08	0.00
HMGCR	-0.82 $\pm$ 0.18	0.08 $\pm$ 0.17	0.00
HNF4A	-0.47 $\pm$ 0.24	0.36 $\pm$ 0.22	0.08*
LDHA	0.02 $\pm$ 0.14	-0.31 $\pm$ 0.13	0.02
MAPK1	-0.01 $\pm$ 0.04	0.13 $\pm$ 0.04	0.03
MAT2	-0.49 $\pm$ 0.24	0.37 $\pm$ 0.22	0.04
MEF2C	-0.37 $\pm$ 0.06	0.00 $\pm$ 0.05	0.00
PCK1	0.92 $\pm$ 0.10	0.58 $\pm$ 0.09	0.02
PSM1	0.73 $\pm$ 0.06	1.09 $\pm$ 0.06	0.00
PRKAA1	0.66 $\pm$ 0.10	1.18 $\pm$ 0.09	0.00
RBBP4	-0.43 $\pm$ 0.21	0.33 $\pm$ 0.19	0.08*
SCD1	0.36 $\pm$ 0.18	1.00 $\pm$ 0.17	0.00
TALDO1	-0.52 $\pm$ 0.08	1.11 $\pm$ 0.07	0.00

D87: 34 differential genes ( $p < 0.1$ ) of 145 studied genes

Gene	Females Means $\pm$ SD	Males Means $\pm$ SD	Sex effect P-value (BH)
ACSL1	-0.45 $\pm$ 0.20	0.46 $\pm$ 0.20	0.004
ACSL5	-0.61 $\pm$ 0.17	0.61 $\pm$ 0.17	0.000
ALDOB	-0.66 $\pm$ 0.16	0.66 $\pm$ 0.16	0.000
ARHGEF28	-0.40 $\pm$ 0.20	0.40 $\pm$ 0.20	0.021
BHMT	-0.64 $\pm$ 0.28	0.60 $\pm$ 0.28	0.000
BHMT2	-0.61 $\pm$ 0.30	0.59 $\pm$ 0.30	0.000
CB5	-0.34 $\pm$ 0.22	0.35 $\pm$ 0.22	0.065*
CD36	-0.39 $\pm$ 0.22	0.39 $\pm$ 0.22	0.031
CTSL2	-0.73 $\pm$ 0.15	0.73 $\pm$ 0.15	0.000
CYP2E1	-0.40 $\pm$ 0.20	0.40 $\pm$ 0.20	0.020
DHFR	-0.79 $\pm$ 0.18	0.78 $\pm$ 0.18	0.000
ELAVL1	0.51 $\pm$ 0.30	-0.40 $\pm$ 0.30	0.004
ERRF1	0.09 $\pm$ 0.39	-0.05 $\pm$ 0.39	0.091*
ESR1	-0.48 $\pm$ 0.18	0.48 $\pm$ 0.18	0.002
FAS	0.56 $\pm$ 0.34	-0.51 $\pm$ 0.34	0.000
GFPT2	0.49 $\pm$ 0.33	-0.62 $\pm$ 0.33	0.000
HMGCR	-0.73 $\pm$ 0.19	0.76 $\pm$ 0.19	0.000
IL6ST	-0.71 $\pm$ 0.16	0.73 $\pm$ 0.16	0.000
LDHA	0.58 $\pm$ 0.19	-0.58 $\pm$ 0.19	0.000
MEF2C	-0.68 $\pm$ 0.16	0.68 $\pm$ 0.16	0.000
MTHFD1L	-0.46 $\pm$ 0.27	0.42 $\pm$ 0.27	0.008
MTHFD2	0.49 $\pm$ 0.19	-0.49 $\pm$ 0.19	0.009
MTR	-0.33 $\pm$ 0.21	0.33 $\pm$ 0.21	0.091*
MTRR	0.34 $\pm$ 0.19	-0.34 $\pm$ 0.19	0.065*
MTPP	-0.52 $\pm$ 0.38	0.48 $\pm$ 0.38	0.000
NR1H4	0.36 $\pm$ 0.20	-0.36 $\pm$ 0.20	0.056*
PRKAA1	-0.76 $\pm$ 0.13	0.76 $\pm$ 0.13	0.000
PRKAB1	-0.33 $\pm$ 0.36	0.33 $\pm$ 0.36	0.054*
PRKAG2	-0.50 $\pm$ 0.22	0.52 $\pm$ 0.22	0.000
RAN	0.35 $\pm$ 0.28	-0.30 $\pm$ 0.27	0.086*
SHMT1	-0.37 $\pm$ 0.23	0.36 $\pm$ 0.23	0.034
TE71	0.34 $\pm$ 0.29	-0.40 $\pm$ 0.29	0.044
TP11	-0.33 $\pm$ 0.21	0.33 $\pm$ 0.21	0.091*
VLDLR	0.63 $\pm$ 0.20	-0.64 $\pm$ 0.19	0.000

Only 8 of the genes studied were consistently differentially expressed between the sexes at all 3 ages.  
BHMT, BHMT2, CTSL2, DHFR, ELAVL1, HMGCR, MEF2C, PRKAA1.

D100: 64 differential genes ( $p < 0.1$ ) of 143 studied genes

Gene	Females LsMeans $\pm$ SD	Males LsMeans $\pm$ SD	Sex effect P-value (BH)
ABCA1	-0.60 $\pm$ 0.18	0.59 $\pm$ 0.18	0.000
ACADS	-0.21 $\pm$ 0.34	0.19 $\pm$ 0.34	0.083*
ACAT1	-0.39 $\pm$ 0.13	0.39 $\pm$ 0.13	0.000
ACSL1	-0.29 $\pm$ 0.16	0.28 $\pm$ 0.16	0.017
ACSL5	-0.23 $\pm$ 0.15	0.22 $\pm$ 0.14	0.068*
AHCY	-0.23 $\pm$ 0.31	0.22 $\pm$ 0.31	0.024
ALDOB	-0.52 $\pm$ 0.17	0.52 $\pm$ 0.17	0.000
APOB	-0.35 $\pm$ 0.14	0.34 $\pm$ 0.14	0.002
ARHGEF28	-0.49 $\pm$ 0.14	0.48 $\pm$ 0.14	0.000
BCL2	0.26 $\pm$ 0.23	-0.27 $\pm$ 0.23	0.022
BHMT	-0.23 $\pm$ 0.31	0.24 $\pm$ 0.31	0.045
BHMT2	-0.20 $\pm$ 0.21	0.22 $\pm$ 0.21	0.097*
CD36	-0.28 $\pm$ 0.14	0.27 $\pm$ 0.14	0.022
CHDH	-0.18 $\pm$ 0.33	0.21 $\pm$ 0.33	0.086*
CPT1A	-0.32 $\pm$ 0.17	0.30 $\pm$ 0.16	0.009
CTSL2	-0.61 $\pm$ 0.14	0.59 $\pm$ 0.13	0.000
CYP2E1	-0.38 $\pm$ 0.14	0.37 $\pm$ 0.14	0.001
DGAT2	0.23 $\pm$ 0.21	-0.23 $\pm$ 0.20	0.040*
DHCR24	-0.28 $\pm$ 0.30	0.21 $\pm$ 0.29	0.025
DHFR	-0.80 $\pm$ 0.15	0.77 $\pm$ 0.15	0.000
DICER1	0.40 $\pm$ 0.26	-0.34 $\pm$ 0.26	0.001
DNM1T3A	0.27 $\pm$ 0.23	-0.26 $\pm$ 0.23	0.027
REO	0.31 $\pm$ 0.15	-0.31 $\pm$ 0.15	0.014
BHMHADH	-0.28 $\pm$ 0.22	0.28 $\pm$ 0.22	0.014
ELAVL1	0.32 $\pm$ 0.20	-0.30 $\pm$ 0.20	0.009
ELOVL6	-0.41 $\pm$ 0.22	0.41 $\pm$ 0.22	0.000
ERRF1	-0.30 $\pm$ 0.18	0.29 $\pm$ 0.17	0.015
FASN	-0.24 $\pm$ 0.22	0.23 $\pm$ 0.22	0.049
FTCD	-0.24 $\pm$ 0.18	0.23 $\pm$ 0.18	0.056*
GLP1R	0.34 $\pm$ 0.28	-0.31 $\pm$ 0.27	0.004
HDAC1	0.19 $\pm$ 0.33	-0.30 $\pm$ 0.33	0.021
HMGCR	-0.65 $\pm$ 0.12	0.63 $\pm$ 0.12	0.000
HNF4A	0.24 $\pm$ 0.24	-0.24 $\pm$ 0.23	0.056*
HSBP1	0.38 $\pm$ 0.18	-0.38 $\pm$ 0.18	0.001
IL6ST	-0.60 $\pm$ 0.14	0.58 $\pm$ 0.13	0.000
INSIG2	-0.22 $\pm$ 0.21	0.24 $\pm$ 0.20	0.056*
IYD	-0.22 $\pm$ 0.15	0.21 $\pm$ 0.15	0.089*
MEF2C	-0.58 $\pm$ 0.12	0.57 $\pm$ 0.12	0.000
MMP2	0.34 $\pm$ 0.30	-0.36 $\pm$ 0.30	0.001
MSRA	-0.46 $\pm$ 0.35	0.35 $\pm$ 0.35	0.000
MTHFD2	0.34 $\pm$ 0.19	-0.36 $\pm$ 0.19	0.002
MTRR	0.22 $\pm$ 0.16	-0.21 $\pm$ 0.16	0.097*
MTPP	-0.42 $\pm$ 0.17	0.41 $\pm$ 0.17	0.000
NR4A3	0.24 $\pm$ 0.17	-0.22 $\pm$ 0.16	0.063*
PARP1	0.32 $\pm$ 0.22	-0.34 $\pm$ 0.22	0.004
PCK1	-0.42 $\pm$ 0.20	0.40 $\pm$ 0.20	0.000
PPARG	0.31 $\pm$ 0.16	-0.31 $\pm$ 0.15	0.012
PPARGC1A	-0.24 $\pm$ 0.23	0.24 $\pm$ 0.23	0.046
PRKAA1	-0.74 $\pm$ 0.10	0.73 $\pm$ 0.10	0.000
PRKAB1	-0.42 $\pm$ 0.21	0.43 $\pm$ 0.21	0.000
RAN	0.16 $\pm$ 0.30	-0.21 $\pm$ 0.30	0.097*
RBBP4	0.46 $\pm$ 0.21	-0.43 $\pm$ 0.21	0.000
SDHA	-0.24 $\pm$ 0.26	0.17 $\pm$ 0.25	0.086*
SLC6A6	-0.37 $\pm$ 0.14	0.37 $\pm$ 0.14	0.001
SOD1	-0.31 $\pm$ 0.17	0.31 $\pm$ 0.17	0.007
TE71	0.29 $\pm$ 0.15	-0.29 $\pm$ 0.15	0.021
TE72	0.28 $\pm$ 0.15	-0.28 $\pm$ 0.15	0.025
TNFSF10	-0.33 $\pm$ 0.14	0.33 $\pm$ 0.14	0.004
TYMS	0.24 $\pm$ 0.29	-0.26 $\pm$ 0.29	0.024
UGDH	0.29 $\pm$ 0.15	-0.29 $\pm$ 0.15	0.021
UHRF1	0.24 $\pm$ 0.31	-0.32 $\pm$ 0.31	0.008
VLDLR	0.61 $\pm$ 0.17	-0.60 $\pm$ 0.17	0.000
WNT11	0.22 $\pm$ 0.22	-0.23 $\pm$ 0.21	0.071*
XPO5	0.20 $\pm$ 0.26	-0.26 $\pm$ 0.25	0.056

Work in progress: Analysis of the diet effect depending on the sex of the offspring.

# Conclusions and perspectives

- A 37% reduction in maternal dietary methionine induced modifications of egg composition that lead to embryonic development of offspring in a modified environment and affects the liver metabolism of the offspring at hatching and also in long term at D87 and at D100 after a strong challenge with the overfeeding period.
- **This mule duck experimental design is a nice model of transgenerational effects in the offspring in birds.**
- It led to hepatic differentially expressed genes accounting for 28 at hatching, 0 at D87 and 6 after overfeeding.
- It impacted both *foie gras* production and *magret* meat quality.
- **This nutritional programming model shows that in farmed birds, the diet of the female breeder has an impact on the early establishment of metabolic pathways modifying the phenotypes of the offspring, thus influencing production performance.**

=> The next step is to analyze the effect of diet depending on the sex of the offspring.

# Many thanks to...

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**INRAE**

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*Thank you for your attention!*



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# Sex dimorphisms at D87 and D100

## Offspring sex impacted *foie gras* production and *magret* meat quality

Traits D87	Females Means ± SD	Males Means ± SD	Sex effect P-value
<b>Weight_1 (D1)</b>	34.47 ± 1.12	33.82 ± 1.12	<b>0.02</b>
Weight_2 (D29)	1136.25 ± 40.51	1319.61 ± 40.45	<b>&lt;0.001</b>
Weight_3 (D57)	2523.83 ± 67.81	2781.84 ± 67.66	<b>&lt;0.001</b>
Weight_4 (D87)	3239.32 ± 83.1	3486.77 ± 82.82	<b>&lt;0.001</b>
ADG_D1-D87	37.27 ± 0.95	40.15 ± 0.94	<b>&lt;0.001</b>
Plasma_ALP_NOFD	-0.01 ± 0.05	0.06 ± 0.05	<b>0</b>
Plasma_Chol_NOFD	-0.05 ± 0.03	0.06 ± 0.03	<b>&lt;0.001</b>
Plasma_Gluc_NOFD	0 ± 0.01	0.02 ± 0.01	<b>0.05</b>
Plasma_Trigly_NOFD	0.05 ± 0.06	-0.07 ± 0.06	<b>0</b>
ThighW_NOFD	278.19 ± 6.59	279.17 ± 6.67	<b>&lt;0.001</b>
PectoMuscleW_NOFD	185.93 ± 3.76	211.13 ± 3.76	<b>&lt;0.001</b>
PectoSkinW_NOFD	48.12 ± 1.24	45.11 ± 1.22	<b>0</b>
AnimBledW_NOFD	2487.3 ± 45.02	2649.91 ± 44.28	<b>&lt;0.001</b>
<b>AATW/AnimBledW_NOFD</b>	1.58 ± 0.12	0.83 ± 0.12	<b>&lt;0.001</b>
PectoMuscleW/AnimBledW_NOFD	7.5 ± 0.11	7.97 ± 0.11	<b>0.01</b>
<b>PectoSkinW/AnimBledW_NOFD</b>	1.92 ± 0.03	1.69 ± 0.03	<b>&lt;0.001</b>
LiverW/AnimBledW_NOFD	2.78 ± 0.08	2.6 ± 0.08	<b>0.09*</b>

Traits D100	Females Means ± SD	Males Means ± SD	Sex effect P-value
Weight_D100	4883.44 ± 94.95	5328.02 ± 94.07	<b>&lt;0.001</b>
ADG_D87-D100	123.79 ± 3.33	138.81 ± 3.28	<b>&lt;0.001</b>
FeedIntake	9372.42 ± 73.12	9639.84 ± 71.58	<b>&lt;0.001</b>
Plasma_ALP	-0.16 ± 0.09	0.01 ± 0.09	<b>0.016</b>
Plasma_GlucPAP	0.76 ± 0.06	0.44 ± 0.06	<b>&lt;0.001</b>
Plasma_Trigly	1.11 ± 0.07	0.83 ± 0.07	<b>&lt;0.001</b>
Plasma_FFA	0.17 ± 0.06	0.45 ± 0.06	<b>0.002</b>
PectoMuscleW	209.90 ± 5.60	224.24 ± 5.56	<b>&lt;0.001</b>
PectoSkinW	113.23 ± 1.80	125.85 ± 1.70	<b>&lt;0.001</b>
AnimBledW	4217.77 ± 87.10	4597.49 ± 86.36	<b>&lt;0.001</b>
LiverW	510.64 ± 31.48	525.00 ± 31.29	0.325
Liver_MeltingRate	39.86 ± 4.60	26.31 ± 4.58	<b>&lt;0.001</b>
PectoMuscleW/AnimBledW	4.98 ± 0.11	4.88 ± 0.11	<b>0.037</b>
LiverW/AnimBledW	12.05 ± 0.59	11.38 ± 0.59	<b>0.031</b>
Pecto_pH_D1	5.73 ± 0.01	5.74 ± 0.01	<b>0.016</b>
Pecto_Face_L_D1	40.53 ± 0.53	40.89 ± 0.53	<b>0.005</b>
Pecto_Face_b_D1	7.56 ± 0.26	8.83 ± 0.25	<b>0.005</b>
Pecto_Face_L_D3	41.29 ± 0.33	40.81 ± 0.32	<b>0.001</b>
Pecto_Face_b_D3	10.32 ± 0.12	10.38 ± 0.11	<b>0.047</b>
Pecto_Slice_L_D1	40.17 ± 0.43	41.17 ± 0.42	<b>0.011</b>
Pecto_Slice_a_D1	21.34 ± 0.24	23.29 ± 0.24	<b>&lt;0.001</b>
Pecto_Slice_b_D1	6.63 ± 0.22	8.21 ± 0.22	<b>&lt;0.001</b>
Pecto_Slice_L_D3	39.87 ± 0.44	40.61 ± 0.42	<b>0.047</b>
Pecto_Slice_a_D3	21.77 ± 0.20	22.74 ± 0.19	<b>&lt;0.001</b>
Pecto_Slice_b_D3	7.00 ± 0.20	7.93 ± 0.19	<b>&lt;0.001</b>
Pecto_Cook_Face_b	10.36 ± 0.14	10.83 ± 0.13	<b>0.014</b>
Pecto_Cook_Slice_L	39.41 ± 0.59	40.51 ± 0.58	<b>0.004</b>
Pecto_Cook_Slice_b	8.41 ± 0.18	9.41 ± 0.17	<b>&lt;0.001</b>
Pecto_WaterWastD1_D3	1.23 ± 0.04	0.90 ± 0.04	<b>&lt;0.001</b>
Pecto_WaterWastD3_D6	1.40 ± 0.08	1.01 ± 0.07	<b>&lt;0.001</b>
Pecto_WaterW_Thaw	4.75 ± 0.23	3.56 ± 0.22	<b>&lt;0.001</b>
Pecto_WaterW_Cook	22.16 ± 0.54	20.04 ± 0.53	<b>&lt;0.001</b>
Pecto_Cook_Fmax	38.51 ± 1.80	33.82 ± 1.78	<b>&lt;0.001</b>
Pecto_Cook_Emax	147.90 ± 5.73	125.86 ± 5.62	<b>&lt;0.001</b>