Liver damage and learning ability in female chicks

Laura Bona[1],

Nienke van Staaveren[1], Bishwo Pokharel[1], Marinus van Krimpen[2], Alexandra Harlander[1]

[1] Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada

[2] Wageningen UR Livestock Research, Wageningen, the Netherlands

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CCSAW
The Campbell Centre for the Study of Animal Welfare
Promoting animal welfare through research and education

Humans & diets

- People in industrialized societies eat what and how much they like because they have easy access to food (Tilman et. al. 2014)

- Chronic consumption of large amounts of energy-rich diets have been linked to negative effects on human health (Seo et. al. 2016)

Energy-rich diets & human liver health

- Intake greater than it's safe limits can exceed the ability of the liver to detoxify metabolites
  (Wu et al 2016)

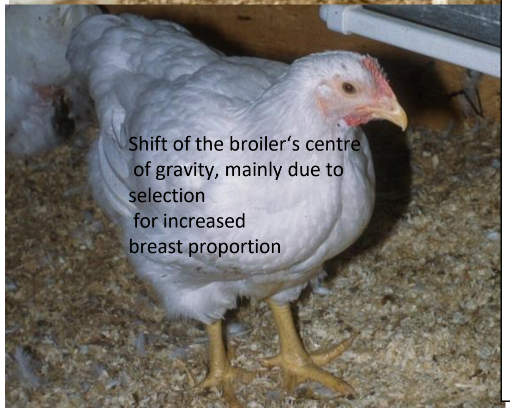
- This can lead to liver damage and fatigue, and includes disease such as obesity and non-alcoholic fatty liver disease
  (Santesso et. al. 2012)

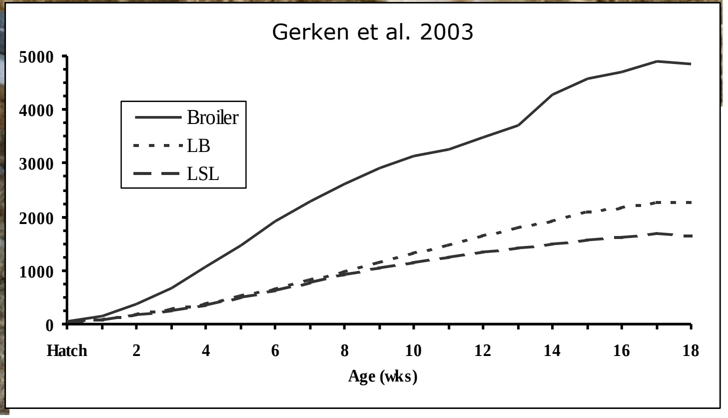
Meat-type chickens

- Chickens grow very fast due to genetic selection
- Diets are intended to produce high yield, pushing muscle fibers to their limits

  (Macrae et al. 2006)
- Producing overweight birds - obesity models

  (Ji et al. 2012)





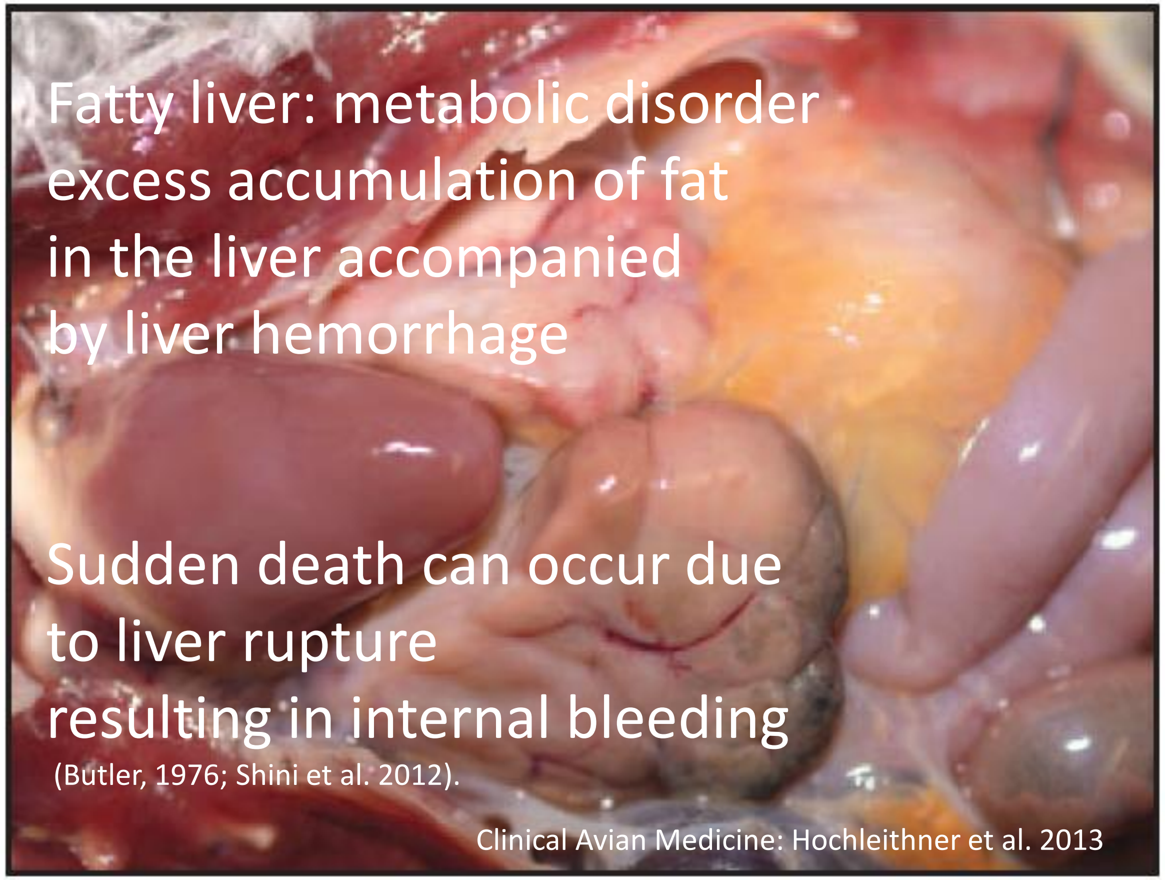
Overweight birds & liver health

- Combination of energy-rich diets & inactivity can result in non-alcoholic fatty liver diseases
- It can occur in caged layers, overweight backyard chickens and meat-type chickens

(Shini and Bryden 2012; Trott et al. 2014; Rozenboim et al. 2016)

- Non-alcoholic fatty liver disease leads to impaired liver function, an increase in plasma hepatic markers and changes in liver colour and texture

(Butler 1976; Hochleithner and Hochleithner 2006)



Non-alcoholic fatty livers & cognition

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- Close association between liver enzyme activity and cognitive function in **humans** with non-alcoholic fatty livers

  (Seo et al. 2016)

- Cognitive impairments in **humans/mice** due to liver diseases range from mild to severe cognitive changes, seen in memory and attention, and are associated with lower quality of life

  (Arguedas et al. 2003)

- No investigation whether impaired cognitive function is associated with liver diseases in meat-type chickens

Research hypothesis

We hypothesized that female chicks fed low-protein energy-rich diets will have

a higher risk of liver cell damage, indicated by increased plasma hepatic markers, and higher liver colour and hemorrhagic scores,

and will be **more inferior on learning a visual discrimination reversal task** compared to female chicks fed a standard control diet, as cognitive impairments have been associated with low-protein energy-rich diets in mammals (Davidson et al. 2012; Reyes-Castro et al. 2012)

Birds, housing and diets

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- 40 female Ross 308 meat-type chickens
  - 10 birds per floor pen 119 x 144 cm

- **Diets**
  corn, soybean/meat meal based; formulated by Dr. M. van Krimpen

Starter (CP 21%, EE 10.2)	Day 1-17	40 chicks
gradually introducing	Day 18-20	
Grower-finisher	Day 21-52	
Control (CP 19%, EE 12.6%)		20 chicks
LPHE (CP 17%, EE 13.7%)		20 chicks

- Bodyweight & feed intake (individually weighed/feed intake per pen)

Proc Glimmix (SAS V9.4); fixed effect diet; covariates (baseline measurements); repeated measures/pen as experimental unit

Plasma hepatic markers and post-mortem inspections

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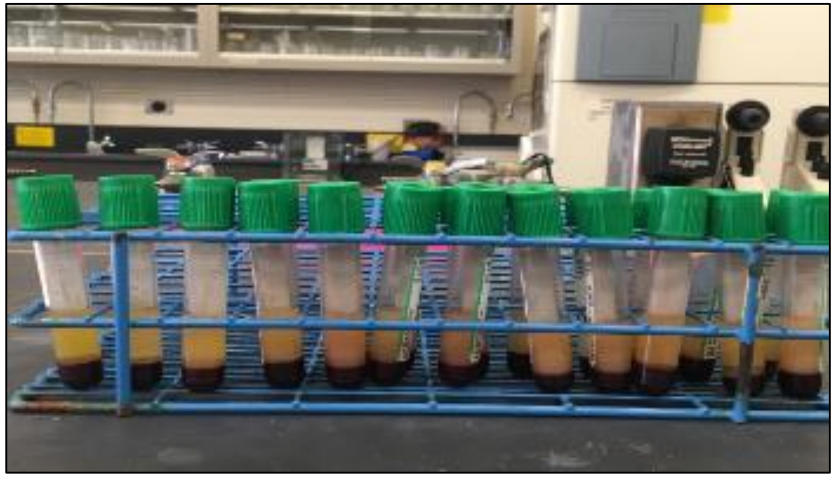
<u>Blood sampling occurred on day 18 and day 46 (wing vein 9:00-9:30am)</u>

- Alanine aminotransferase (**ALT**):
  non-specific cell damage (Hochleithner et al. 1994); Roche Cobas C ALTL kit ID 0-49
- Aspartate aminotransferase (**AST**):
  indicator for liver and muscle damage (Lumeij 1994); Roche Cobas C ASTL kit ID 0-494
- Gamma-glutamyl transferase (**GGT**):
  avian liver and biliary compromises (Harr 2005); Roche Cobas C GGT-2 kit



<u>Post-mortem inspections on day 52</u>

- Liver color: 1 = normal deep red color
  5 = yellow colored liver (Choi et al 2012)
- Liver hemorrhage: 0 = no hemorrhaging
  1 = less than 10 hemorrhages
  2 = more than 10 hemorrhages
  3-5 = severe hemorrhaging (Shini 2014)

Visual discrimination and reversal visual discrimination task

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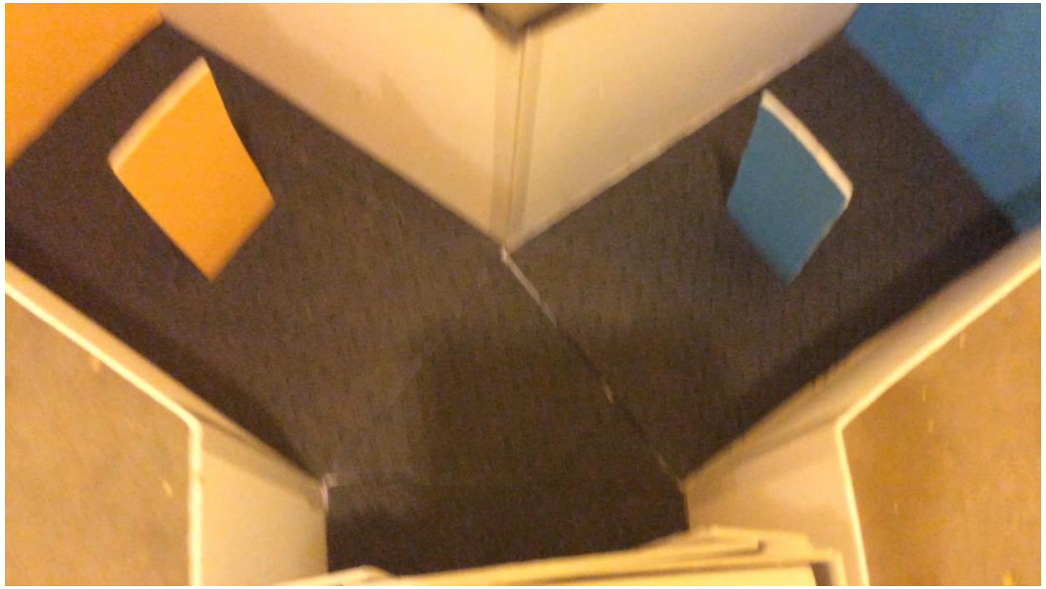
Visual discrimination task

(40 chicks; Starter diet; d 5-10)

Half of the birds assigned to blue, other half assigned to yellow coloured panel

6 trials/day = 1 session

Learning criteria = 5/6 trials in 2 consecutive days



Reversal visual discrimination task

(20 Control/20 LPHE chicks , 3 wks after diet introduction; d 38-46)

Using the same paradigm as before

Reward/stimulus (initial colour yellow/reversal colour blue) were reversed for each chick

Effect of reducing protein/increasing energy on body weight/feed intake

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- Birds did not differ in their initial body weight on day 1
- Final body weight/ADG of LPER diet fed birds were significantly lower

Table 1 Average body weight (BW) at the beginning (1d) and end (51d) of the study, average daily gain (ADG), average daily intake (ADI), and feed conversion ratio (FCR) for each treatment group. Values presented as mean ± standard error of the mean.

Variable	LPER	*n*	Control	*n*	Pr > F
BW (g; 1d)	42.6 ± 0.49	20	42.8 ± 0.49	20	0.8606
BW (g; 51d)	3240.3 ± 44.02	19	3451.0 ± 43.95	19	0.0045
ADG (g/day/bird)	63.9 ± 0.88	19	68.1 ± 0.87	19	0.0045
ADI (g/day/bird)	110.3 ± 1.43	19	106.6 ± 1.43	19	0.0798
FCR	1.7 ± 0.03	19	1.6 ± 0.03	19	0.0852

LPER = low-protein energy rich diet. Significant between treatment groups ($P < 0.05$).

Effect of reducing protein/increasing energy on liver function

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- ALT, AST, GGT activity levels did not differ between treatment groups
- AST activity levels increased with age
- **AST where above 230 U/L** = indicative of hepatic damage in birds! (Hochleithner 1994)
- Liver colour scores & hemorrhagic scores did not differ between treatment groups
- **All birds had hemorrhages** on the livers (score ≥ 1) indicative of mild degree of liver damage (Shini 2014)
- Standard control fed bird tended to have higher hemorrhagic scores

Table 2 Average concentration of liver enzymes (U/L) at the end of the visual discrimination experiment/start of the diet treatment (18d) and at the end of the reversal (46d) experiment, along with liver hemorrhagic and color score values, and liver to body weight ratio for birds receiving a control or low protein energy-rich (LPER) diet. Values presented as mean ± standard error of the mean.

Variable	LPER	Control	Pr > F
ALT, 18d	*3.9 ± 0.48 (n = 16)*	*2.8 ± 0.50 (n = 15)*	*0.1775*
	3.8 ± 0.41 (n = 18)	3.0 ± 0.43 (n = 19)	0.2206
ALT, 46d	*2.3 ± 0.57 (n = 16)*	*3.0 ± 0.65 (n = 16)*	*0.5406*
	2.5 ± 0.34 (n = 18)	2.7 ± 0.38 (n = 17)	0.7261
AST, 18d	*163.4 ± 5.23 (n = 16)*	*152.3 ± 5.40 (n = 15)*	*0.2022*
	159.7 ± 4.52 (n = 19)	153.8 ± 4.66 (n = 18)	0.3969
AST, 46d	*511.4 ± 60.28 (n = 13)*	*694.9 ± 67.00 (n = 13)*	*0.1191*
	570.1 ± 72.00 (n = 19)	630.8 ± 78.94 (n = 16)	0.6081
GGT, 18d	*9.2 ± 0.58 (n = 16)*	*8.4 ± 0.60 (n = 15)*	*0.4339*
	9.0 ± 0.44 (n = 19)	8.7 ± 0.45 (n = 18)	0.6721
GGT, 46d	*10.9 ± 0.83 (n = 15)*	*11.3 ± 0.90 (n = 15)*	*0.8016*
	10.9 ± 0.71 (n = 19)	11.1 ± 0.77 (n = 16)	0.9120
AST:ALT, 18d	*52.5 ± 8.83 (n = 15)*	*56.1 ± 8.79 (n = 15)*	*0.8038*
	59.6 ± 5.89 (n = 18)	56.8 ± 5.85 (n = 18)	0.7622
AST:ALT, 46d	*214.8 ± 18.95 (n = 13)*	*297.35 ± 20.88 (n = 12)*	*0.0417*
	214.0 ± 19.38 (n = 18)	270.2 ± 20.32 (n = 17)	0.4203
Liver Color score	*1.8 ± 0.12 (n =14)*	*1.8 ± 0.13 (n = 13)*	*0.8112*
	1.9 ± 0.12 (n = 19)	1.8 ± 0.12 (n = 19)	0.8744
Liver hemorrhagic Score	*0.98 ± 0.08 (n =14)*	*1.29 ± 0.09 (n = 13)*	*0.0531*
	1.06 ± 0.06 (n = 19)	1.2 ± 0.06 (n = 19)	0.0856
Liver to BW ratio	*0.01 ± 0.0007 (n =14)*	*0.01 ± 0.0006 (n = 13)*	*0.5321*
	0.02 ± 0.0004 (n = 19)	0.02 ± 0.0004 (n = 19)	0.5078

ALT = Alanine aminotransferase.AST = Aspartate aminotransferase. GTT = Gamma-glytamyl transferase. NH4 = plasma ammonia. Significant between treatment groups ($P < 0.05$). Values in *italics* refer to the number of birds that successfully passed the reversal discrimination tasks; regular values refer to all of the birds that had data for all co-variates used in the models.

Effect of reducing protein/increasing energy on learning

- <u>Visual discrimination task:</u> The number of sessions to achieve the learning criterion was significantly higher in those birds assigned to the LPER, compared to birds assigned to Control diets.

(7.9 ± 0.55 vs. 5.7 ± 0.47, $F_{(1,8.65)}$ =8.31; p <0.05).

- <u>Reversal visual discrimination task:</u> The number of sessions required to reach the learning criterion in the reversal trial revealed no significant difference between LPER/Control treatments.

(6.2 ± 0.47 vs. 5.5 ± 0.49)

Table 3 Average liver enzymes (U/L) at the end of the reversal discrimination task, along with liver color and hemorrhagic scores, and liver to body weight ratio for birds that were able to learn the reversal task by reaching the learning criterion of 5 out of 6 successful sessions in two consecutive days (learners), and those that were not able to learn the reversal task (non-learners), independent of diet treatment. Mean ± standard error of means.

Variable	Learners (n = 28)	Non-learners (n = 12)	Pr > F
ALT	2.7 ± 0.25	2.4 ± 0.39	0.6000
AST	598.8 ± 47.61	644.3 ± 74.59	0.6100
GGT	11.7 ± 0.48	9.2 ± 0.76	0.0101
AST:ALT	252.3 ± 17.88	256.2 ± 28.01	0.9071
Liver color score	1.8 ± 0.08	2.0 ± 0.13	0.2979
Liver hemorrhagic score	1.1 ± 0.06	1.2 ± 0.10	0.4441
Liver to BW	0.01 ± 0.0004	0.01 ± 0.0006	0.6122

ALT = Alanine aminotransferase. AST = Aspartate aminotransferase. GGT = Gamma-glutamyl transferase. NH4 = plasma ammonia. Leaners = birds that were able to pass the reversal discrimination task (n = 19). Non-learners = birds that were unsuccessful in passing the reversal discrimination tasks (n = 19). Significant between learners and non-learners (*P* < 0.05).

Plasma hepatic markers/post-mortem scores were not related to achieving the learning criterion.

Summary

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- Current change in energy to protein ratio and duration of feeding (21 days) was not capable of inducing hypothesized differences in liver health and learning.

- **All chicks had post mortem signs of hepatic hemorrhage/increased liver colour scores** and **increased AST enzyme levels** above 230U/L, which serves as a sign of hepatic damage in birds! (Hochleithner 1994)

- The present study suggests that due to genetic selection, the potential for growth of meat-type chickens is so high that it is **overloading their liver** regardless of whether they were fed a Control or LPER diet.

- Welfare of modern meat-type chickens is compromised, further research is needed to improve avian liver health!

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Thank you!

- This research was done in partial fulfilment of the requirements for the completion of Laura Bona's MSc degree.

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