



# What a wonderful worm: live **black soldier fly** larvae effects on medium-growing chickens' welfare

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Background



# The «why?»

From the knowledge acquired...

## can the live black soldier fly larvae improve the welfare of medium laying chickens?



Consumers' empowerment in sustainable production  
✦ Better plumage condition of (Star et al., 2020)



Organic production  
Reduced broiler chickens' fear ensured welfare  
Increased foraging and activity behavior (et al., 2020ab; Biasato et al., 2022)



# Materials and Methods



# Materials and methods: **chicken reared**



Hubbard  
JA57 hybrid

Medium  
growing  
broiler

82d organic rearing cycle  
120 females + 120 males  
29-82d of age



# Materials and methods: **experimental design**

4 treatment groups, 6 replicates, 10 chicken/replicate (60 birds/treatment):

CM: control male



LM: larva male

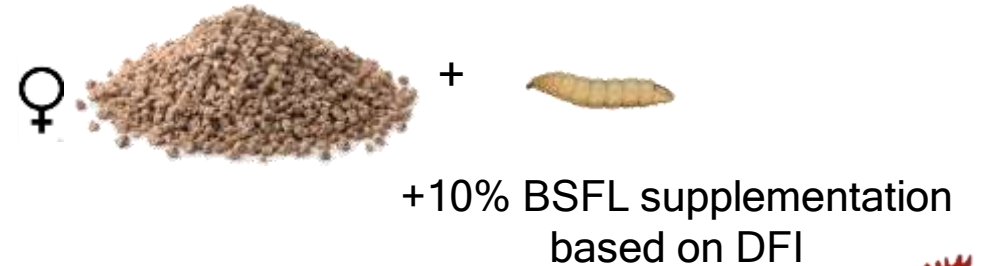


DFI: daily feed intake  
BSFL: black soldier fly larvae

CF: control female

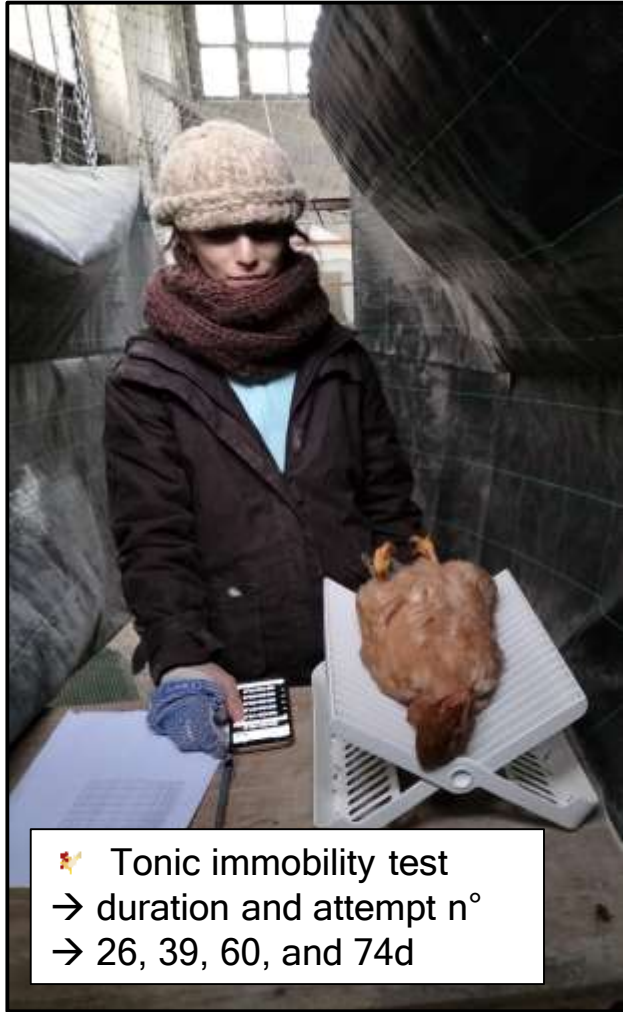


LF: larva female



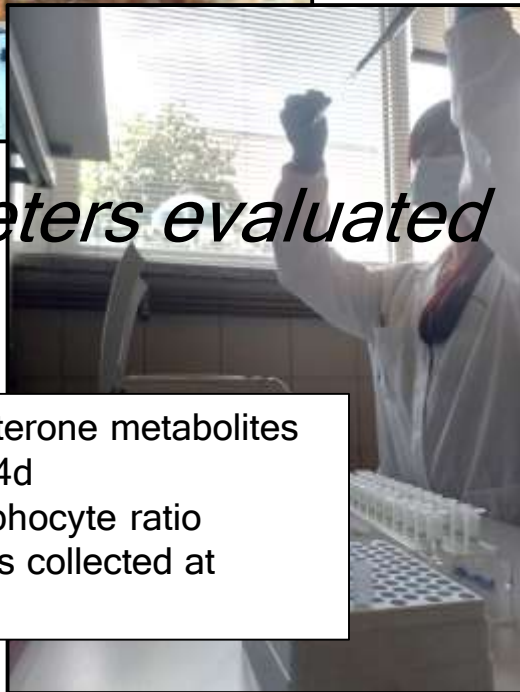


# Materials and methods: ethological tests and animal-based welfare measurements



🌟 Tonic immobility test  
→ duration and attempt n°  
→ 26, 39, 60, and 74d

🌟 Modified avoidance distance test →  
27, 41, 62, and 76d



*Parameters evaluated*

🌟 Excreta corticosterone metabolites  
→ 26, 39, and 74d  
🌟 Heterophile/lymphocyte ratio  
→ blood samples collected at  
slaughter (82d)

🌟 Plumage damage and cleanliness,  
hock burn, footpad dermatitis, and  
skin lesions  
→ score (0-4)  
→ 28, 49, 63, and 77d



→ 29d = start live BSFL provision

Days of age



T0 25-28      T1 39-41      T2 49      T3 61-63      T4 76-77

# Materials and methods: behavioral observations



- 🌟 Video recordings → morning (9.00 a.m.), during the live BSFL provision (11.00 a.m.), and afternoon (4.00 p.m.), 5 min/time slot, at 25, 61, and 75d
- 🌟 N° observations for each behavior recorded (frequency)



Days of age

→ 29d = start live BSFL provision

T0  
25-28

T1  
39-41

T2  
49

T3  
61-63

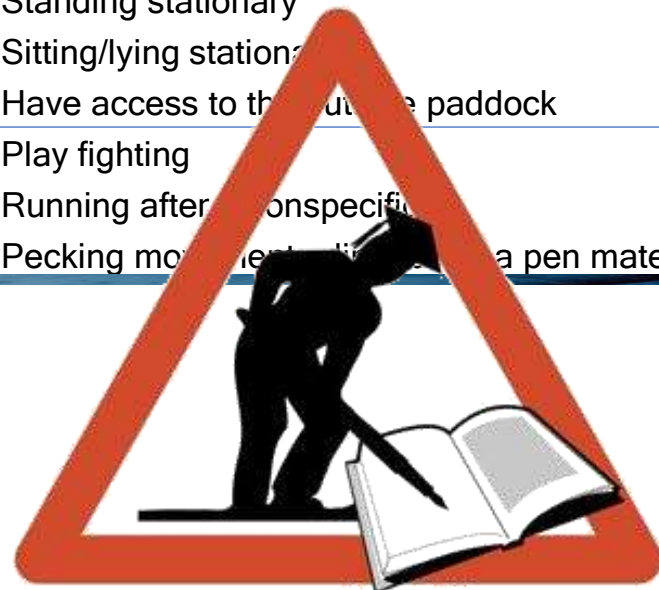
T4  
76-77





# Materials and methods: ethogram of specific behavior repertoire and activity of chickens

Clas	Denomination	Description	References
<ul style="list-style-type: none"> <li>Feeding and foraging related behaviors</li> </ul>	Eating larvae	Eating larvae	(Veldkamp and van Niekerk, 2019)
	Ground pecking	Pecking at the ground	(Ipema et al., 2020a)
	Object pecking	Pecking	(Veldkamp and van Niekerk, 2019)
	Scratching	Move the litter backwards by claws	(Biasato et al., 2022)
<ul style="list-style-type: none"> <li>Activity behaviors</li> </ul>	Walking	Walking/running	(Biasato et al., 2022)
	Standing	Standing stationary	(Veldkamp and van Niekerk, 2019)
	Resting	Sitting/lying stationary	(Veldkamp and van Niekerk, 2019)
	Outside	Have access to the outside paddock	-
<ul style="list-style-type: none"> <li>Social behaviors</li> </ul>	Sparring	Play fighting	(Veldkamp and van Niekerk, 2019)
	Chasing	Running after conspecifics	(Biasato et al., 2022)
	Pecking conspecifics	Pecking more than once a pen mate	(McCowan et al., 2006)



**Research in Progress**



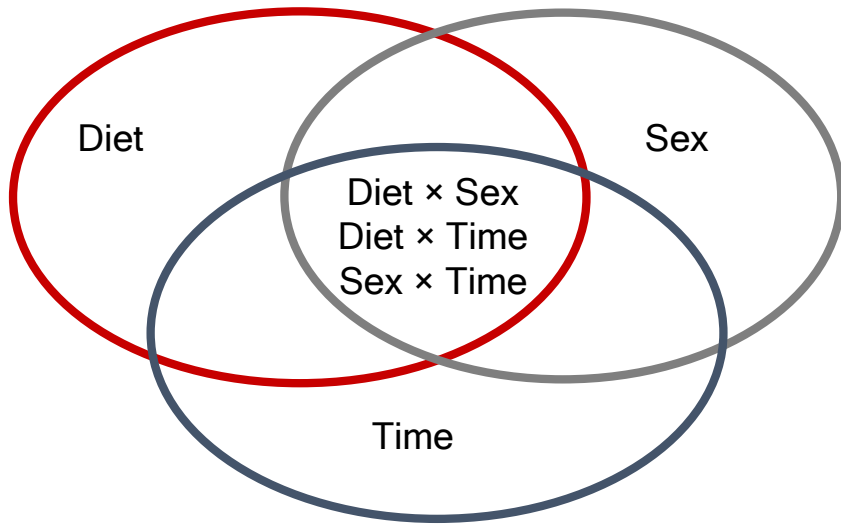
# Materials and methods: **statistical analyses**

## SPSS software

### General Linear Mixed Model (GLMM)



- Ethological test and animal-based welfare measurements
- excreta corticosterone metabolites



### Spearman correlation

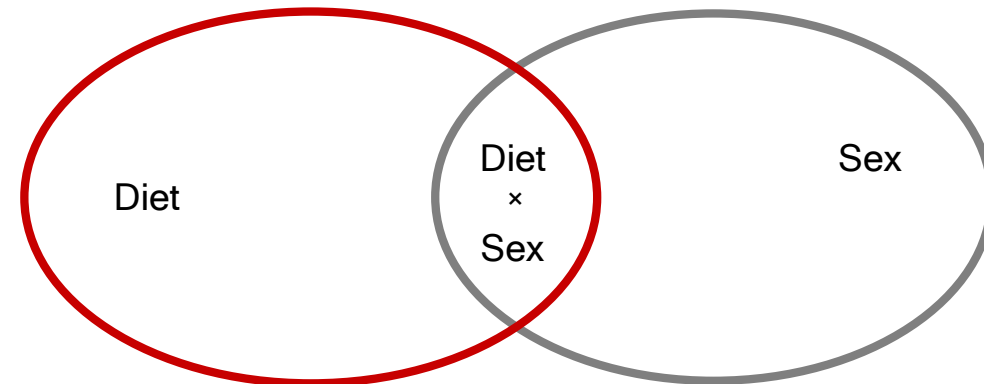


- excreta corticosterone metabolites
- tonic immobility duration.

### General Linear Model (GLM)



- heterophile/lymphocyte ratio



## Experimental unit

- Pen (n=6) → feather condition, leg health, skin lesion scores, AD test, TI test, excreta corticosterone metabolites
  - Bird (n=12) → heterophile/lymphocyte ratio



# Results and discussion





# Results and discussion: **ethological tests and animal-based welfare measurements**



*Feather condition, leg health, skin lesion scores*

## Prediction

The live BSFL provision can ameliorate the animal-based welfare parameters



## Result

Birds' feather, leg and foot condition, and skin damage frequency < 0.5 times on average



no statistical analyses applied

## Discussion

The live BSFL provision did not undermine welfare of birds

### Why?

research housing conditions  $\neq$  commercial housing conditions



Better leg health  
(Hall, 2001)



Enhanced feather condition  
(van Hierden, 2003)

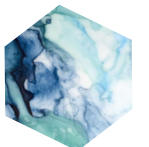


Diminished lameness  
(Dawkins et al., 2004)

### Commercial relevant scale



Sharper identification of the supplementation effects on the mentioned parameters



# Results and discussion: **ethological tests and animal-based welfare measurements**

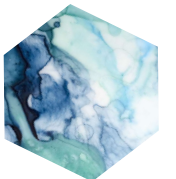
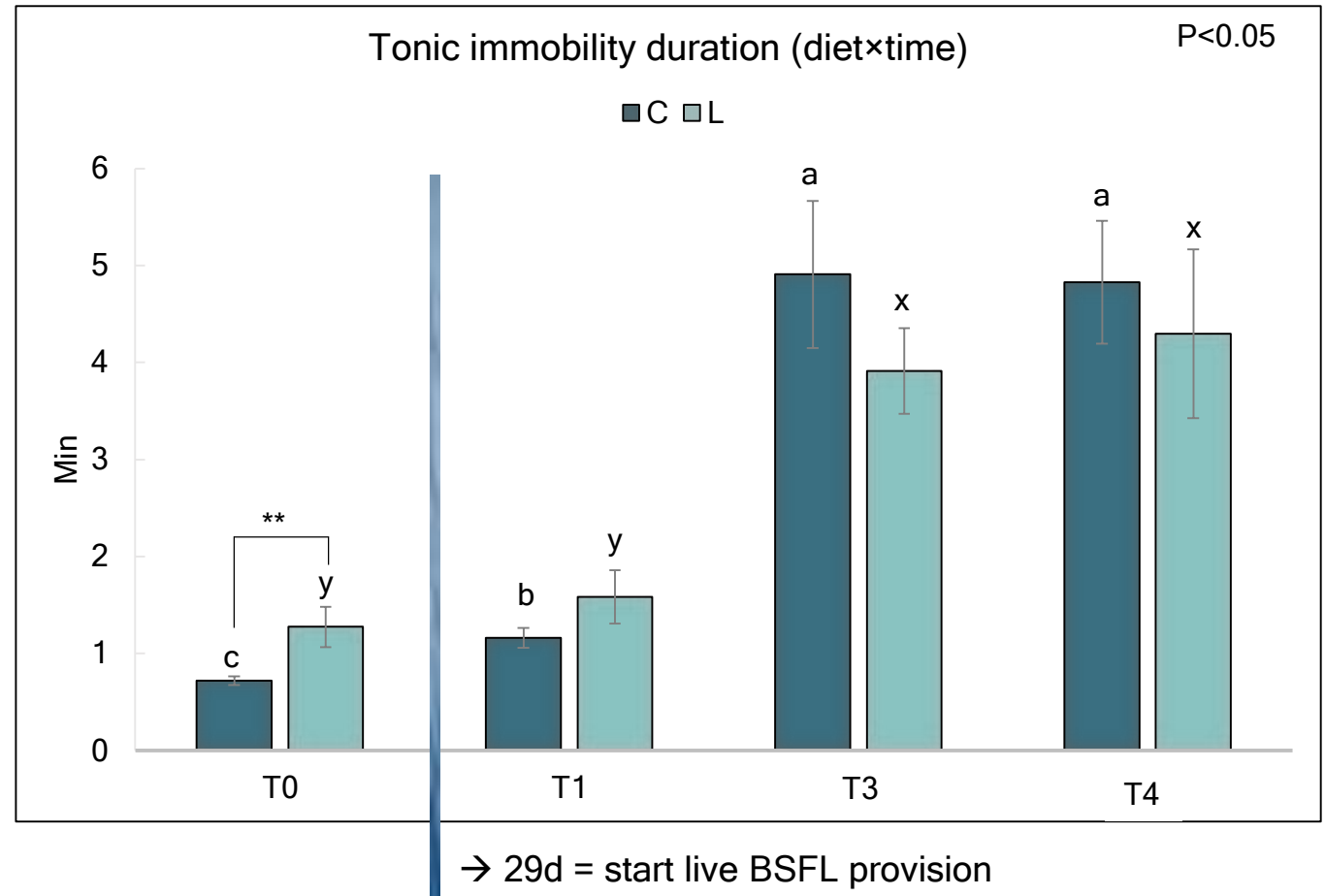
## *Tonic immobility and excreta corticosterone metabolites*

- No significant effect

TI duration increased between T1 and T3 in both males and females and C and L groups

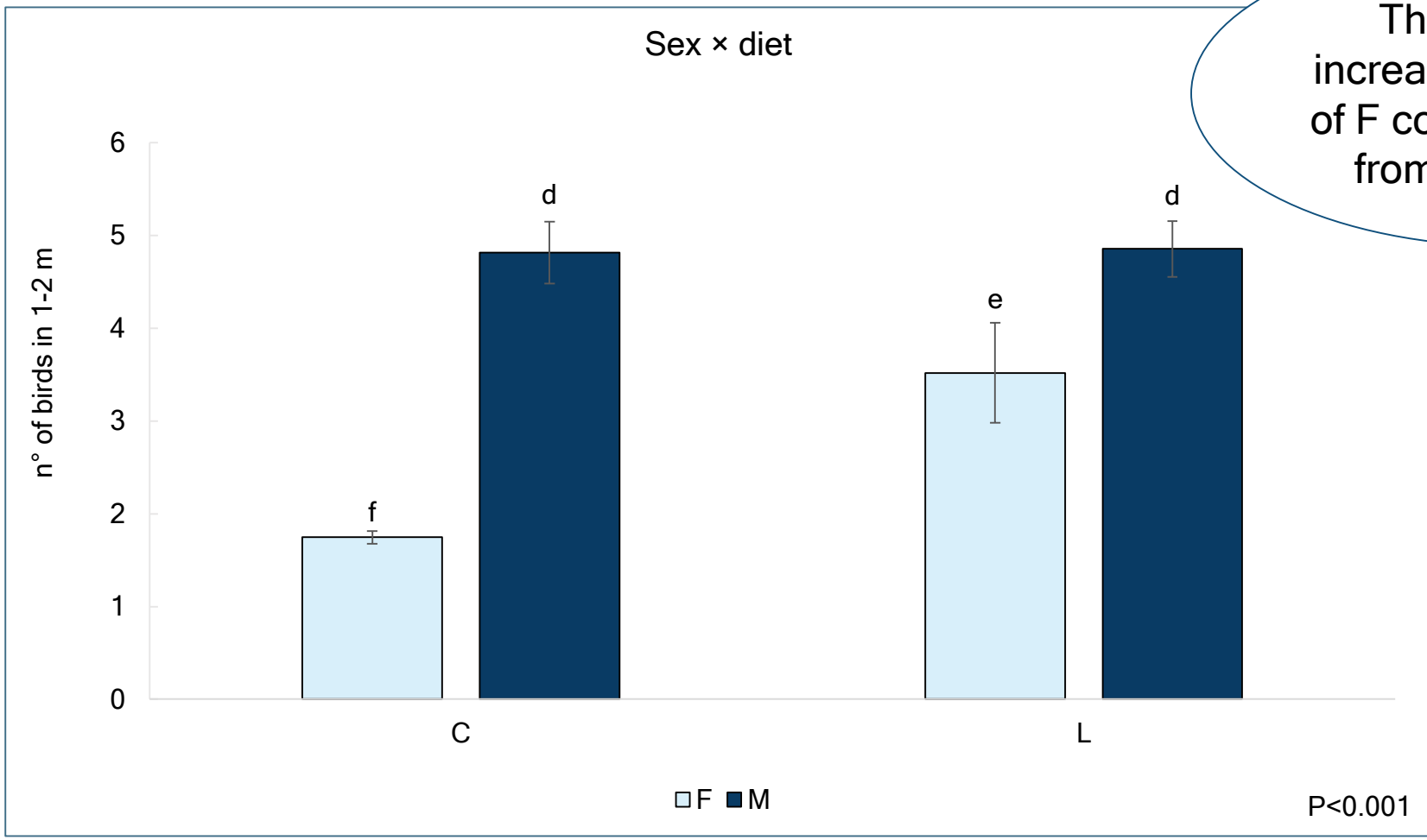
- NO sig. correlation

- Higher weight and the reduced activity level of adult birds

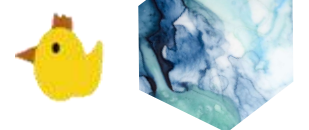


# Results and discussion: **ethological tests and animal-based welfare measurements**

## *Avoidance distance test*



The live larvae increased the number of F come within 1-2m from the operator





# Results and discussion: **ethological tests and animal-based welfare measurements**

## *Avoidance distance test*

### Prediction

The live BSFL provision can reduce the birds' fear towards humans



### Result

General reduction of F fear  
or  
F association of humans to a reward ?



(Rushen et al., 1999)

### Discussion



Reduction of F fear related to the larvae provision



What are you looking for and, first of all, why?



Generalized fear towards humans?



Capacity of the birds to associate humans to the larvae provision?

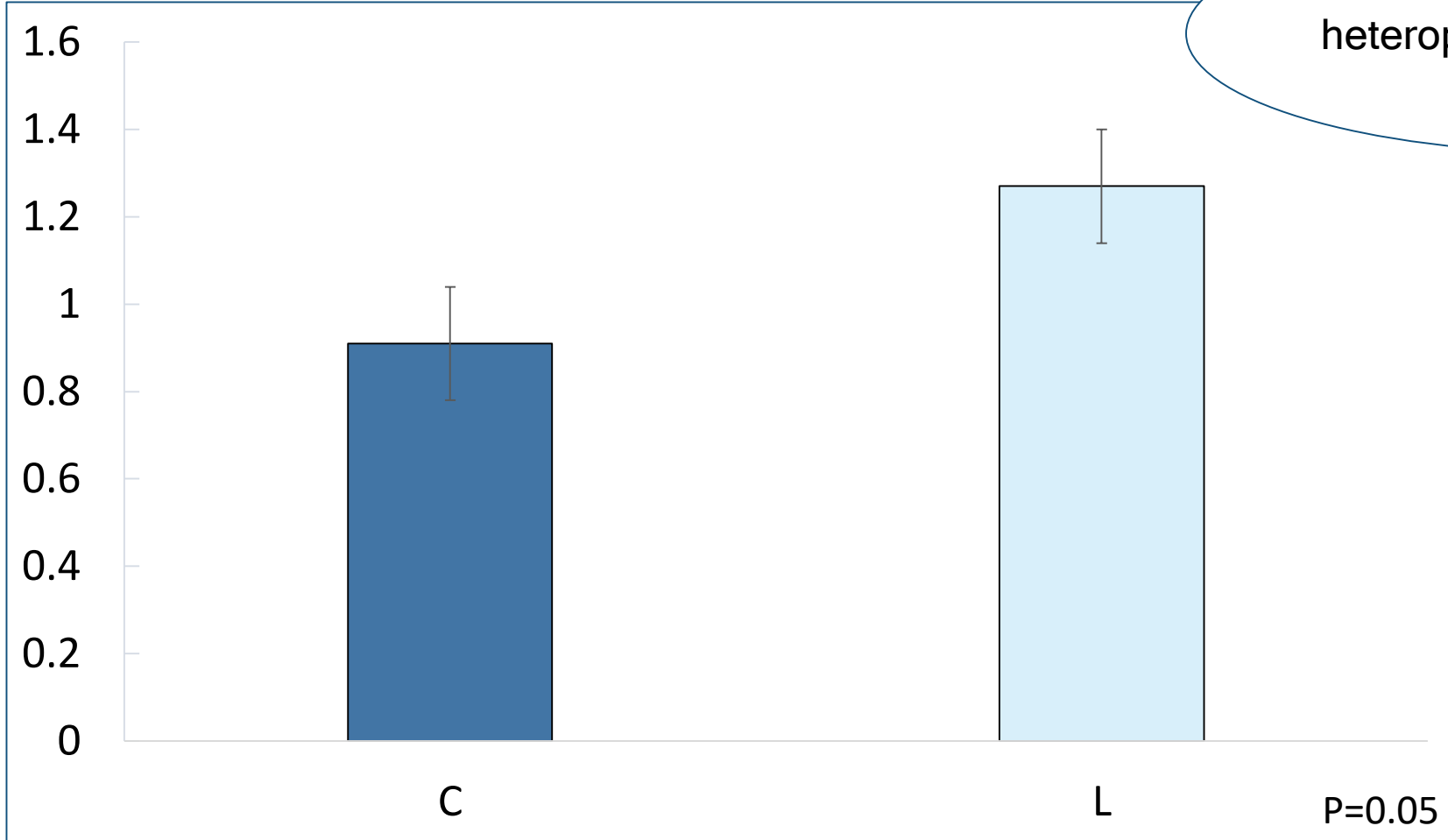


**Adaptation of the test based on my research question**



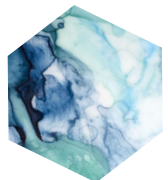
# Results and discussion: **ethological tests and animal-based welfare measurements**

 *Heterophile lymphocyte ratio*



The live BSFL increased the heterophile lymphocyte ratio

and  
and stress conditions  
↓  
While lymphocyte ratio variation among the larvae access?  
↓  
Resulted obtained might not be directly related to a negative bird experience



(McFarlane and Curtis 1988; Mahboub and Von Borell, 2010; Bellezza Oddon, 2021)



**Conclusions  
and  
considerations**







## Take home messages



- ✦ No negative implications related to birds' feather, leg and foot condition, and skin damage
- ✦ No significant effect on the tonic immobility and corticosterone level
- ✦ Advantages in fear reduction, denoted especially in F birds
- ✦ Higher heterophile lymphocyte ratio in L birds than C ones → competition based stress



Open question





Aknowledgments:

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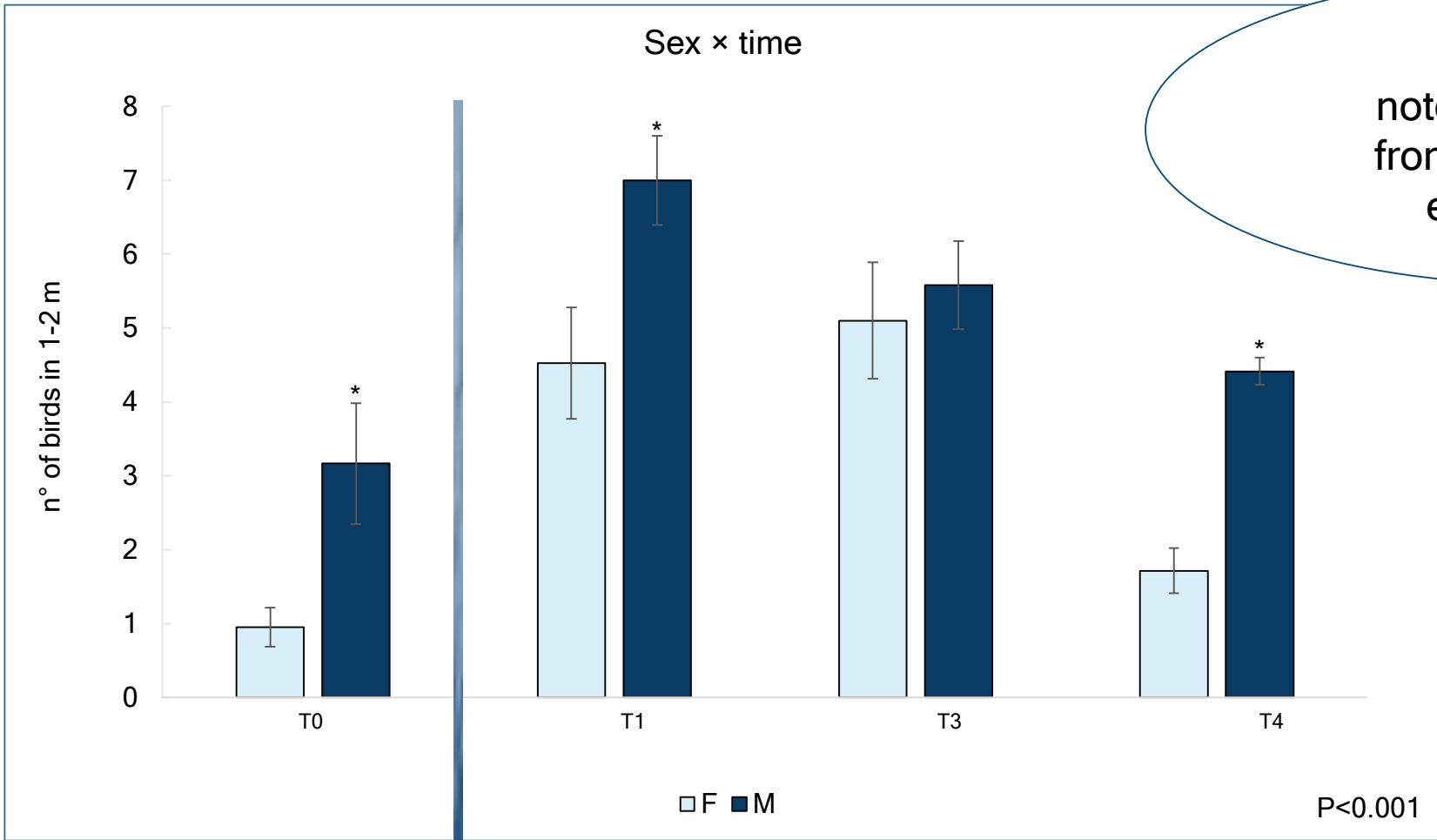
...

Maja Makagon and Joy A. Mench

**THANK YOU  
FOR YOUR  
ATTENTION** 

# Results and discussion: **ethological tests and welfare animal-based measurements**

## *Avoidance distance test*



M > F  
noted within 1-2m  
from the operator,  
except at T3

- Hens' responsibility for brooding and offspring protection
- ↓
- major prudence than males



→ 29d = start live BSFL provision



## STATISTICAL ANALYSIS: video recordings

### General linear mixed model (GLMM)

- Feather condition, leg health, skin lesion scores → negative binomial response probability distribution with a nonlinear link function -log;
- TI duration, TI induction frequency and FCM → gamma probability distribution and log-link function;
- AD test and video recordings → Poisson loglinear distribution;

The D, G, T, and their interactions D×G, G×T, and D×T were considered as fixed factors (assessed by pairwise comparisons) and the replicate was included in the model as indicator of the repeated measurements on the same pen.







### 5.1A.4.3 Good human–animal relationship

<i>Title</i>	<b>Avoidance Distance Test (ADT)</b>
<i>Scope</i>	Animal-based measure: Broiler chicken
<i>Sample size</i>	Sample size according to § 5.1A.5
<i>Method description</i>	<p>The assessor approaches a group of at least 3 birds in the litter area, squats for 10 seconds and then counts the number of birds at arm's length (i.e. within 1 m of the observer). Every attempt to approach a group of birds is considered as a trial, even if all birds from the group withdraw from the approaching or squatting assessor.</p> <p>Repeat the trial 21 times. Carry out the trial at a number of different locations around the house to avoid repeat scoring of birds. Record the number of birds within arm's length at each trial.</p>
<i>Classification</i>	<b>Individual level:</b> Total <b>number</b> of birds in reach (Tr)

