# Insect rearing: the Black Soldier Fly's potential as Bio converter

27/10/2023
POULTRYNSECT: FINAL SYMPOSIUM

David Deruytter (David.deruytter@inagro.be)
Inagro - Belgium

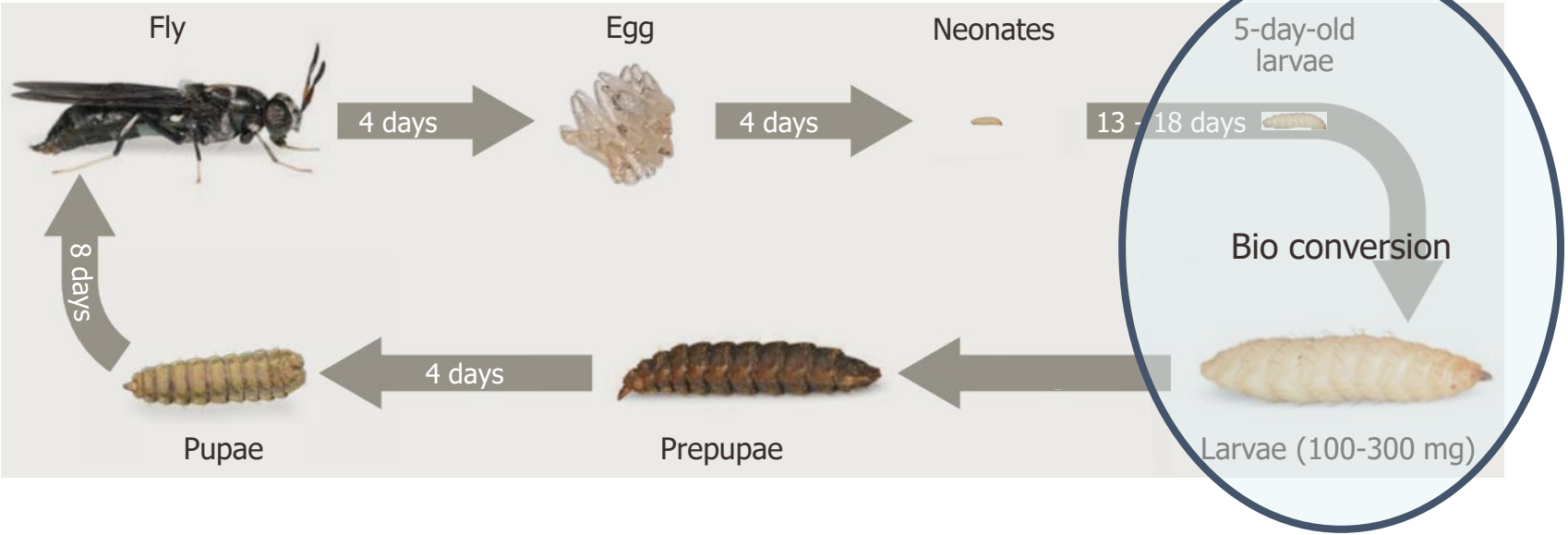
POULTRYNSECT

ondernemen in west-vlaanderen

inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

# BSF life cycle

- A lot of steps before we can start bio converting



# Wait…

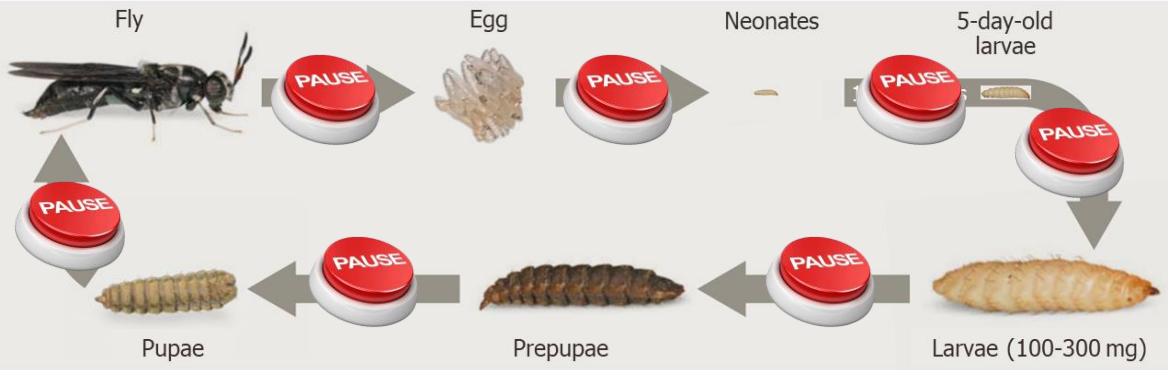
- BSF don’t follow a normal work week
- You know, weekends…

# Manipulating the life cycle

BUT we desire a specific number of larvae on a fixed day

Can we hit a pause button?

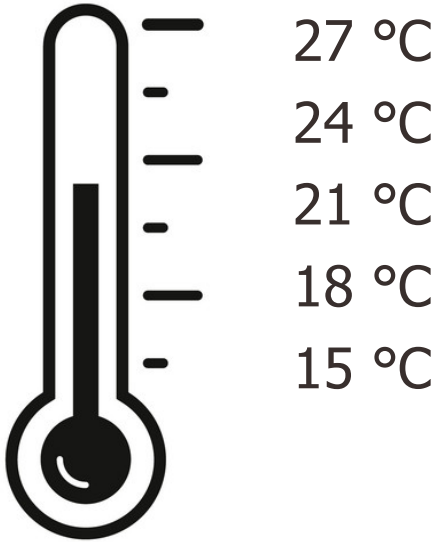
Focus on **delaying pupae eclosion**



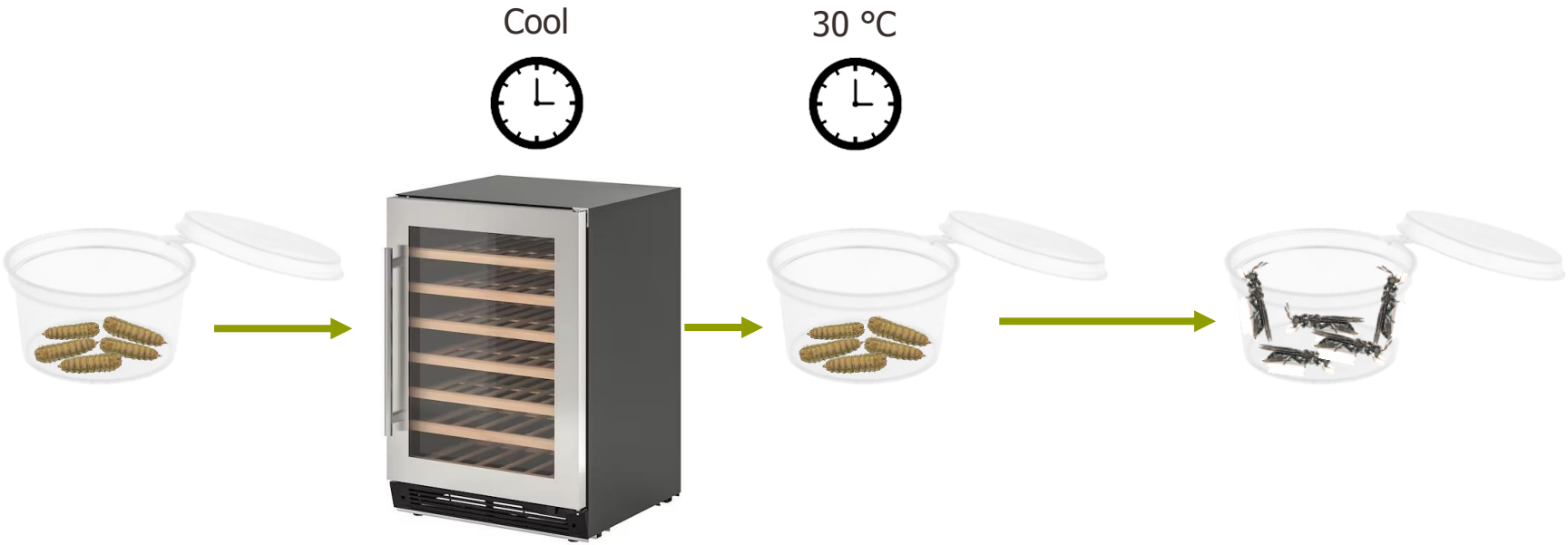
# Can we cool the pupae?

Normally pupae develop at 30 °C

How low can we go?

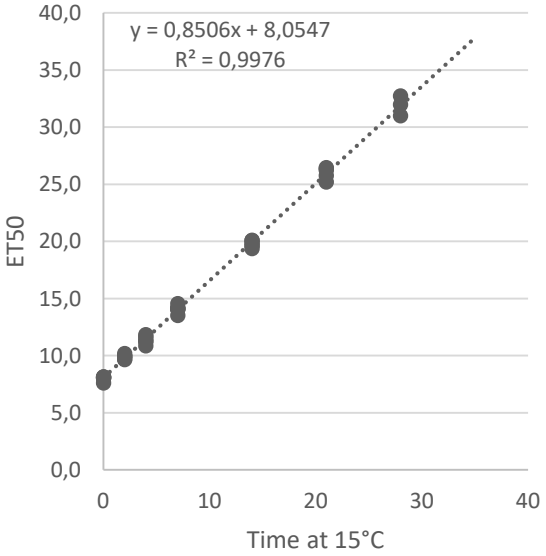


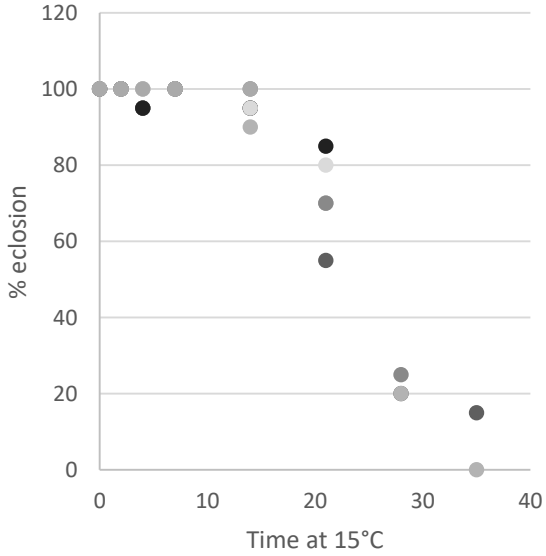
# How?



# Results: 15°C example

Linear delay in eclosion



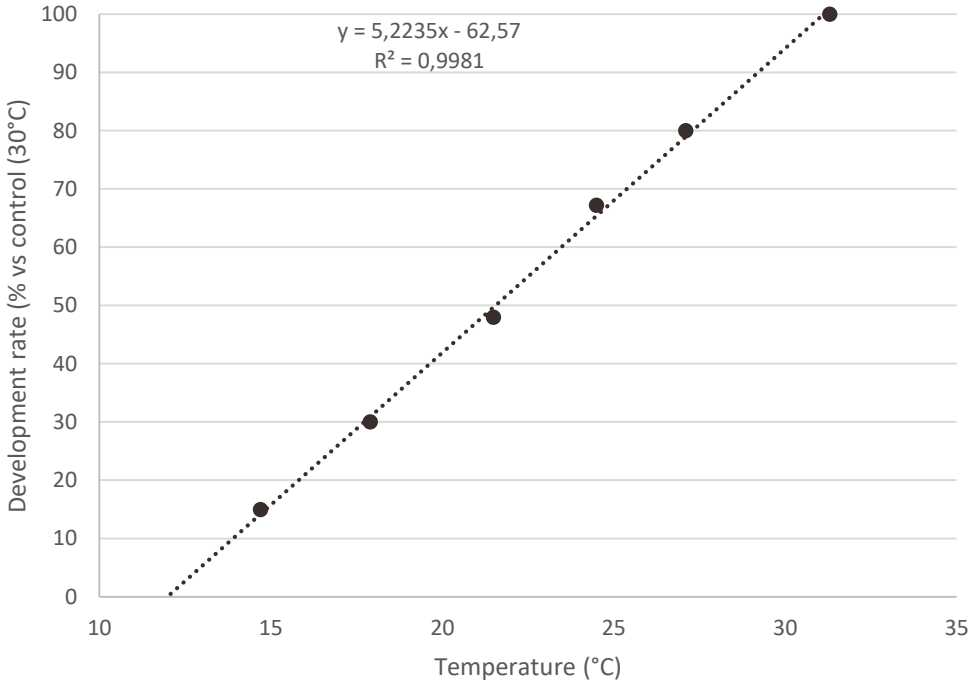




Prolonged storage may result in mortality

# Results

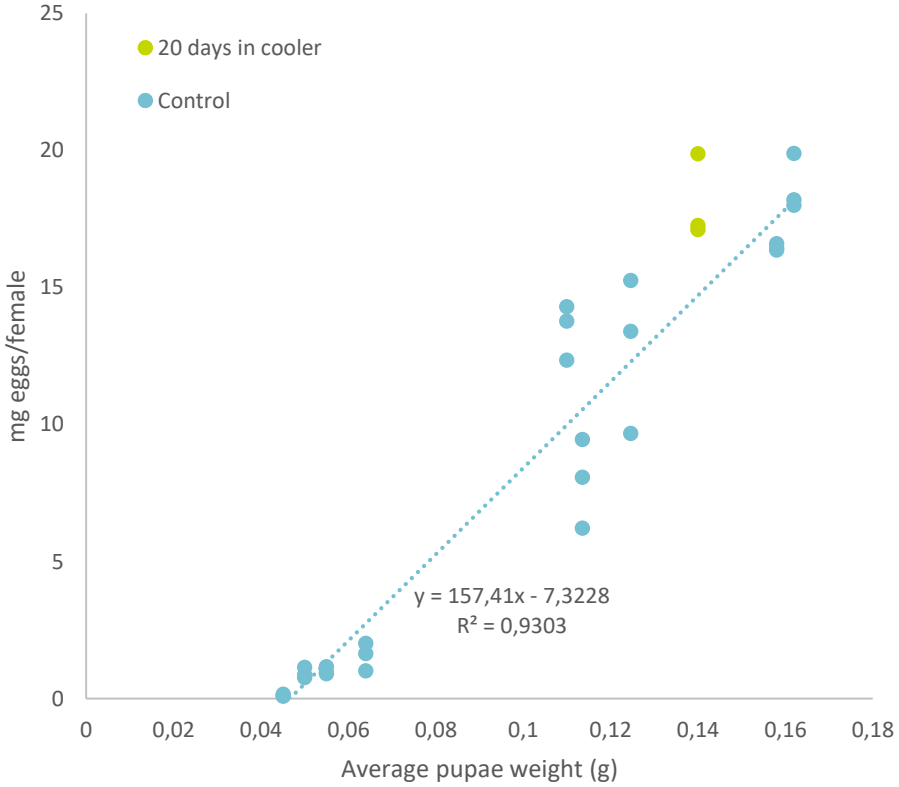
- Below 12 °C no net development
- One day at 30 °C is almost one week at 15 °C, BUT mortality!
- One day at 30 °C can be stretched to 3.3 days at 18 °C

POPULATION DIFFERRENCES!



# Are there other risks to cooling?

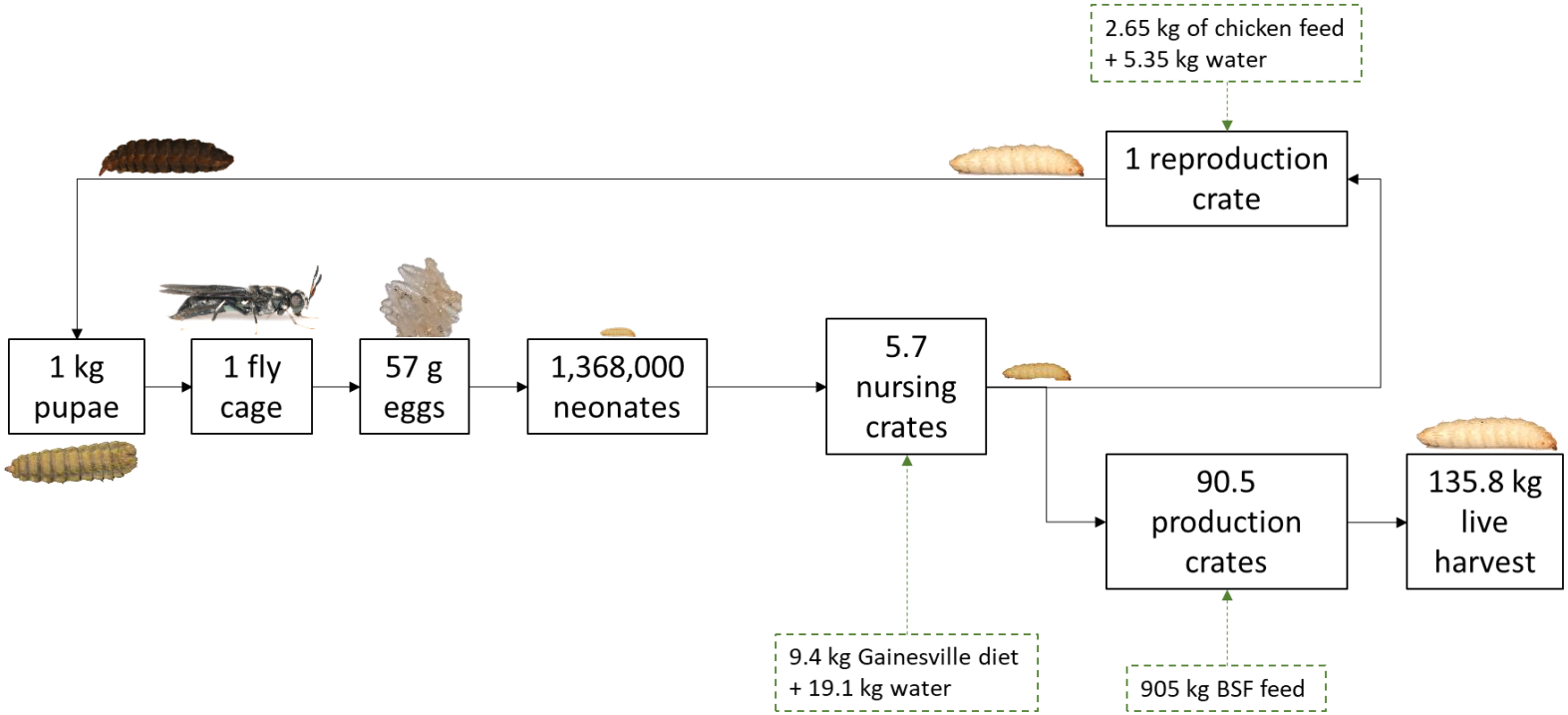
- No signs that the reproductive capacity of the flies is harmed



# Producing neonates

inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

# What is the output from a fly colony?

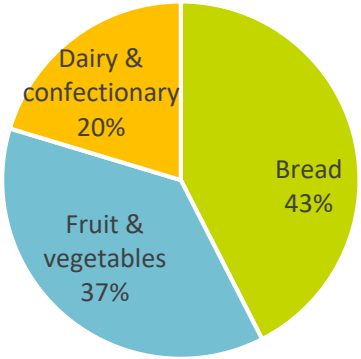


# Now let’s talk about bioconversion

inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

# An example, bio-converting pre-consumer waste

- 270 kg pre-consumer from 1 grocery store from 1 weekend




# BSF have requirements

**Macro nutritional**

- Dry matter: 25 - 45%
- DM < 25% → problems during harvest
- DM > 45% → no efficient use of the feed
- Protein: 12 - 20% DM ~ protein quality, too much protein → ammonia emissions!
- Fat: 2 - 5% DM
- Carbohydrates: 40% DM

**Physical**

- Fine particles, not bigger than 2 mm
- Structure → aeration!

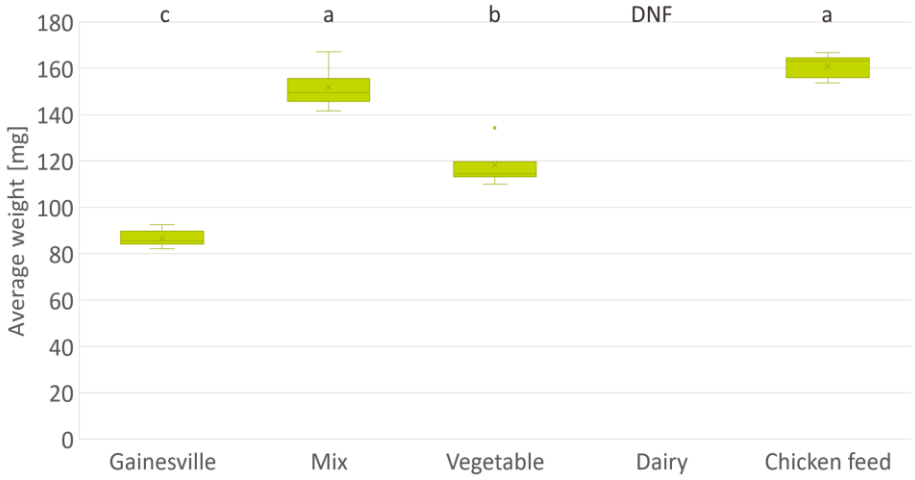
# Bio-converting pre-consumer waste

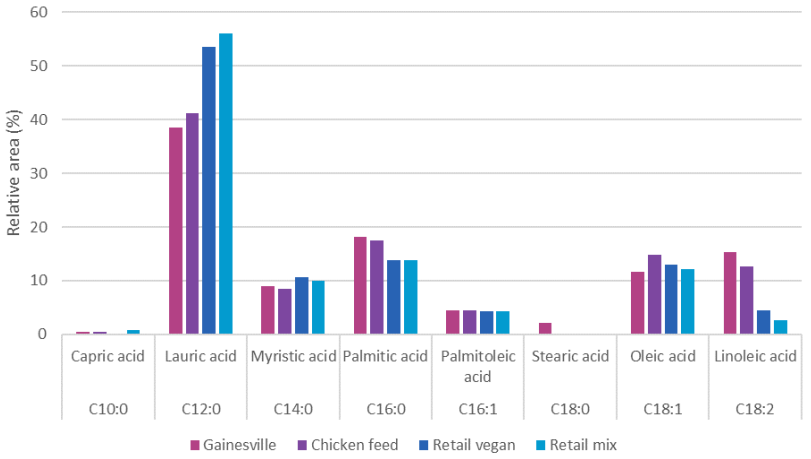
| | | Dry matter | Crude protein | Crude fat |
|---|---|---|---|---|
| Retailer waste | Bread | 75% | 13% | 5.3% |
| | Dairy & confectionary | 31% | 26% | 34% |
| | Fruit & vegetables | 13% | 9.0% | 2.0% |
| Feed mixtures | Gainesville | 30% | 16% | 4.1% |
| | Chicken feed | 30% | 20% | 4.3% |
| | Mix | 42% | 15% | 9.0% |
| | Vegetables + bread | 44% | 13% | 4.8% |
| | Dairy + bread | 41% | 21% | 21% |
| | **Desired range** | **25 – 45%** | **12 – 25%** | **1 – 5%** |

# Processed pre-consumer waste

# Larvae performance

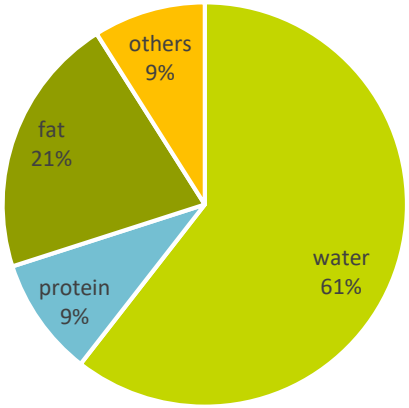
| Diet | Development time [days] | BCE [dry larvae/dry feed] | Crude fat larvae |
|---|---|---|---|
| **Gainesville** | 9 | 12 ± 0.78%[c] | 16.4% |
| **Mix** | 9 | 19 ± 1.0%[b] | 53.3% |
| **Vegetables** | 8 | 17 ± 2.0%[b] | 52.9% |
| **Dairy** | DNF | DNF | DNF |
| **Chicken feed** | 12 | 26 ± 1.0%[a] | 29.4% |



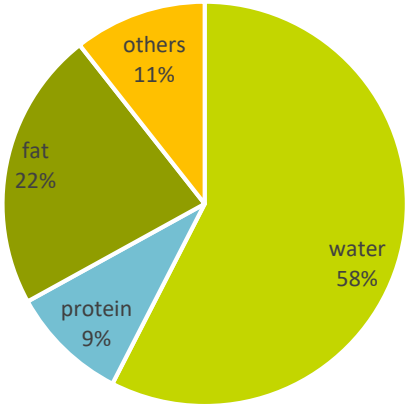
# Potential of pre-consumer waste: 1kg

- Mix scenario
- 207 g live larvae




- 261 g frass

- Vegetable + bread scenario
- 179 g live larvae

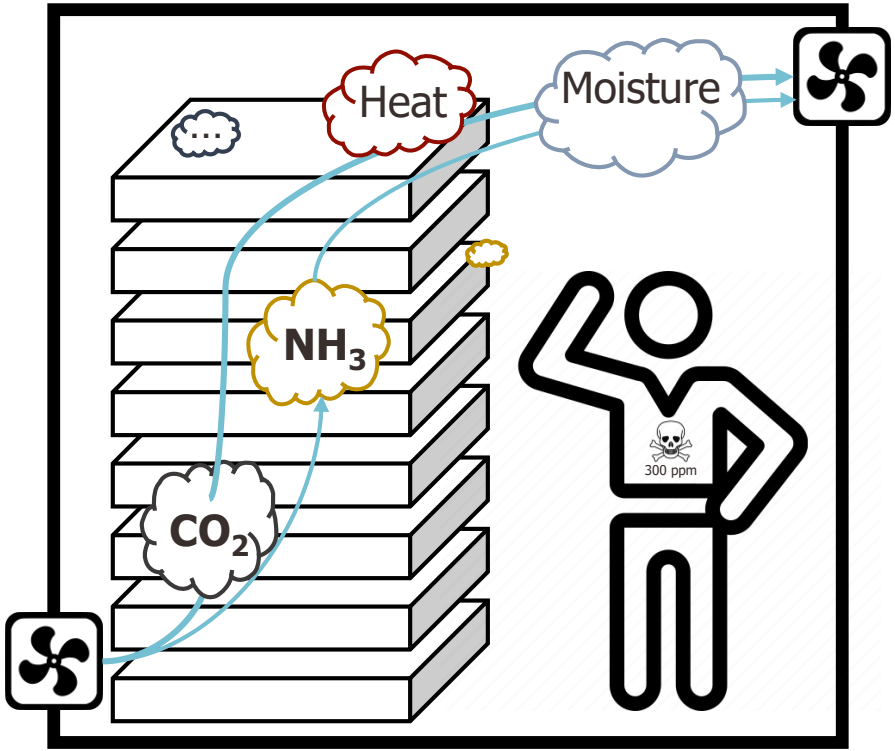



- 239 g frass

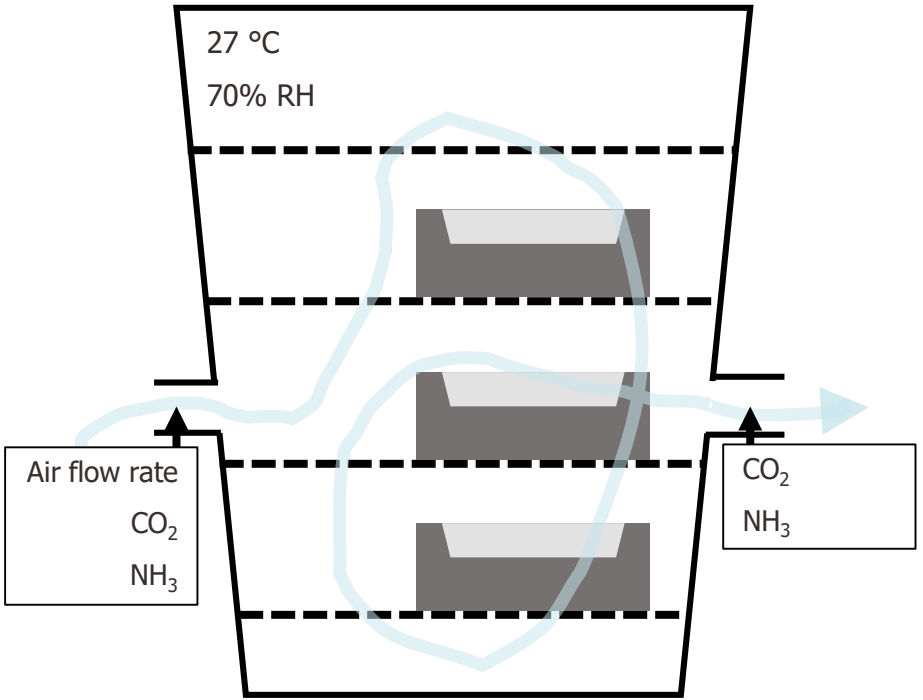
# Some hazards

inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

# Emissions



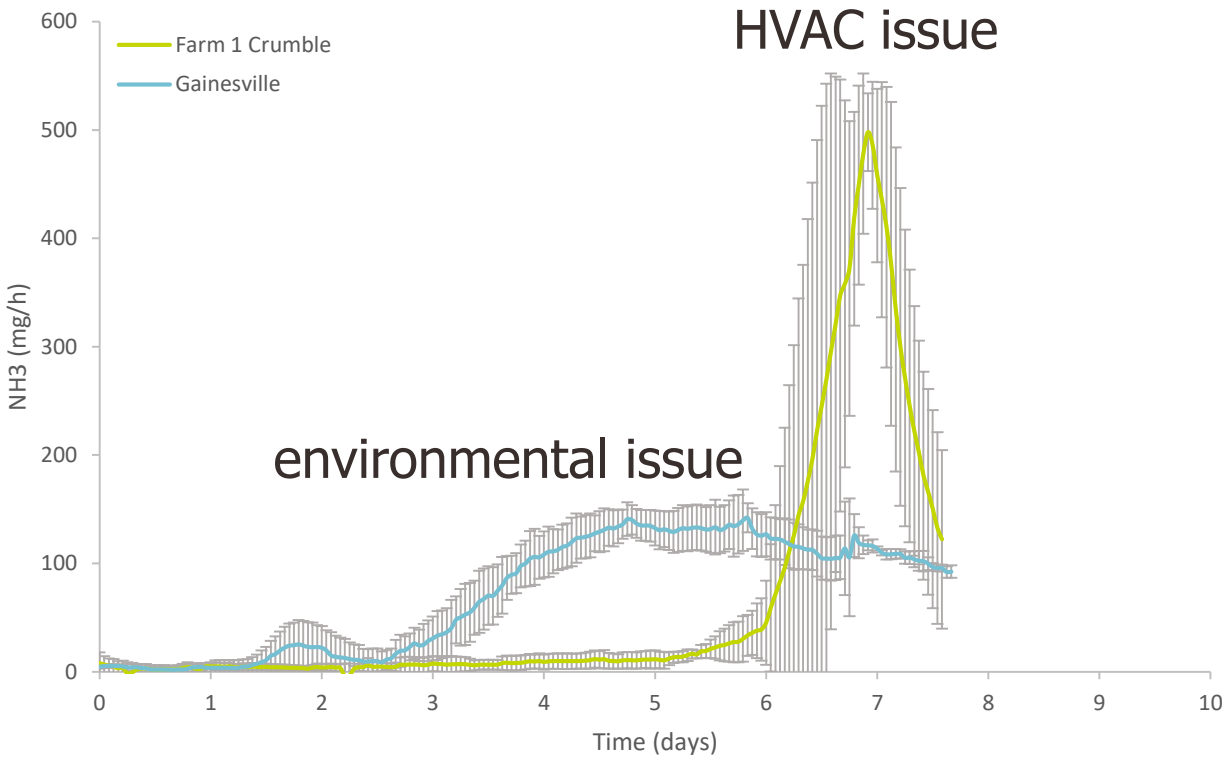
# How to determine emissions



# Ammonia emissions

1 crate with 10 kg of initial wet feed and 15,000 larvae

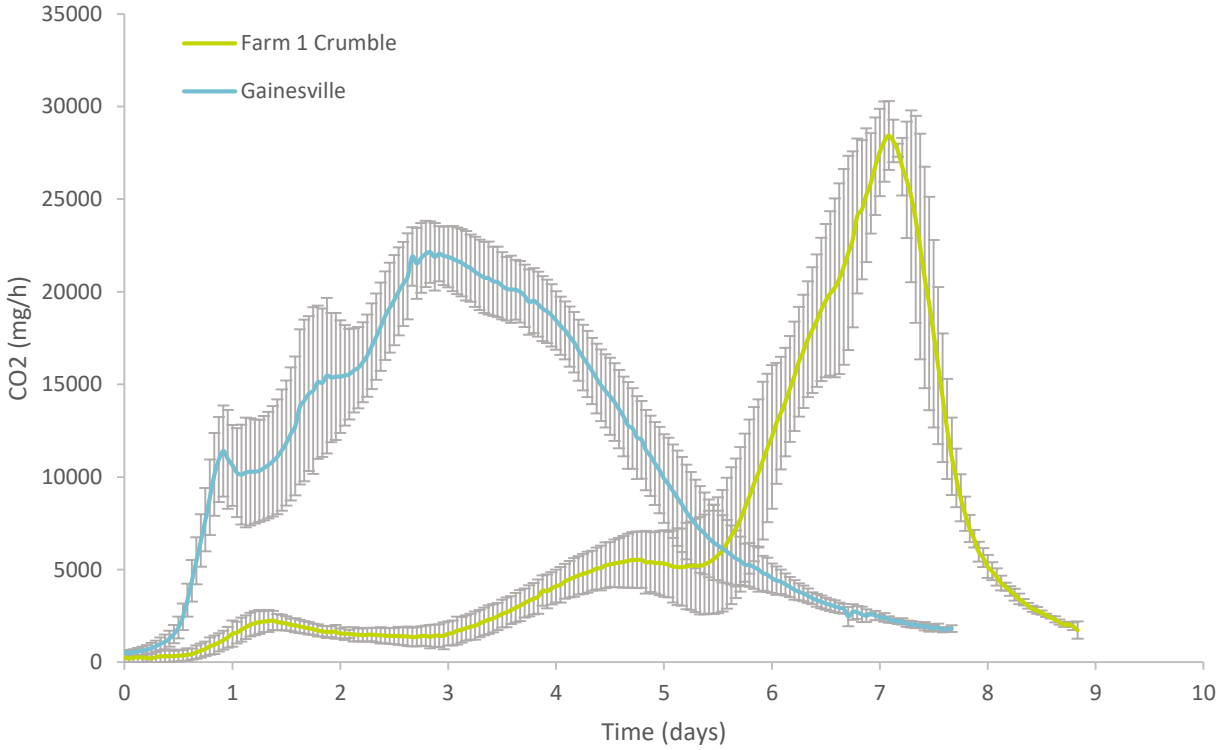
4 replicates



# Respiration gasses

1 crate with 10 kg of initial wet feed and 15,000 larvae

4 replicates



# How do larvae compare to broilers?

| | Farm 1 Crumble | Gainesville | Broilers |
|---|---|---|---|
| Live yield / crate | 2155 ± 172 g | 1443 ± 62 g | |
| NH3 / crate | 11.3 ± 2.1 g | 12.4 ± 2.3 g | |
| NH3 / kg live animal(s) | 5.20 ± 0.56 g | 8.63 ± 1.71 g | 11 – 16 g[1] |
| CO2 / crate | 1253 ± 202 g | 1948 ± 202 g | |
| CO2 / kg live animal(s) | 579 ± 46 g | 1347 ± 83 g | 5200 g[2] |

[1]Coufal, C., Chavez, C., Niemeyer, P. and Carey, J. (2006). Nitrogen emissions from broilers measured by mass balance over eighteen consecutive flocks. Poultry science 85: 384-391.

[2] Knížatová, M., Mihina, Š., Broucek, J., Karandusovska, I., Sauter, G., & Macuhova, I. (2010). Effect of the age and season of fattening period on carbon dioxide emissions from broiler housing. Czech Journal of Animal Science, 55(10), 436-444.

# POULTRYNSECT

## The use of live insect larvae to improve sustainability and animal welfare of organic chickens production

**Prof. Achille Schiavone – University of Turin (Italy)**

Consiglio Nazionale delle Ricerche
(Coordinator)

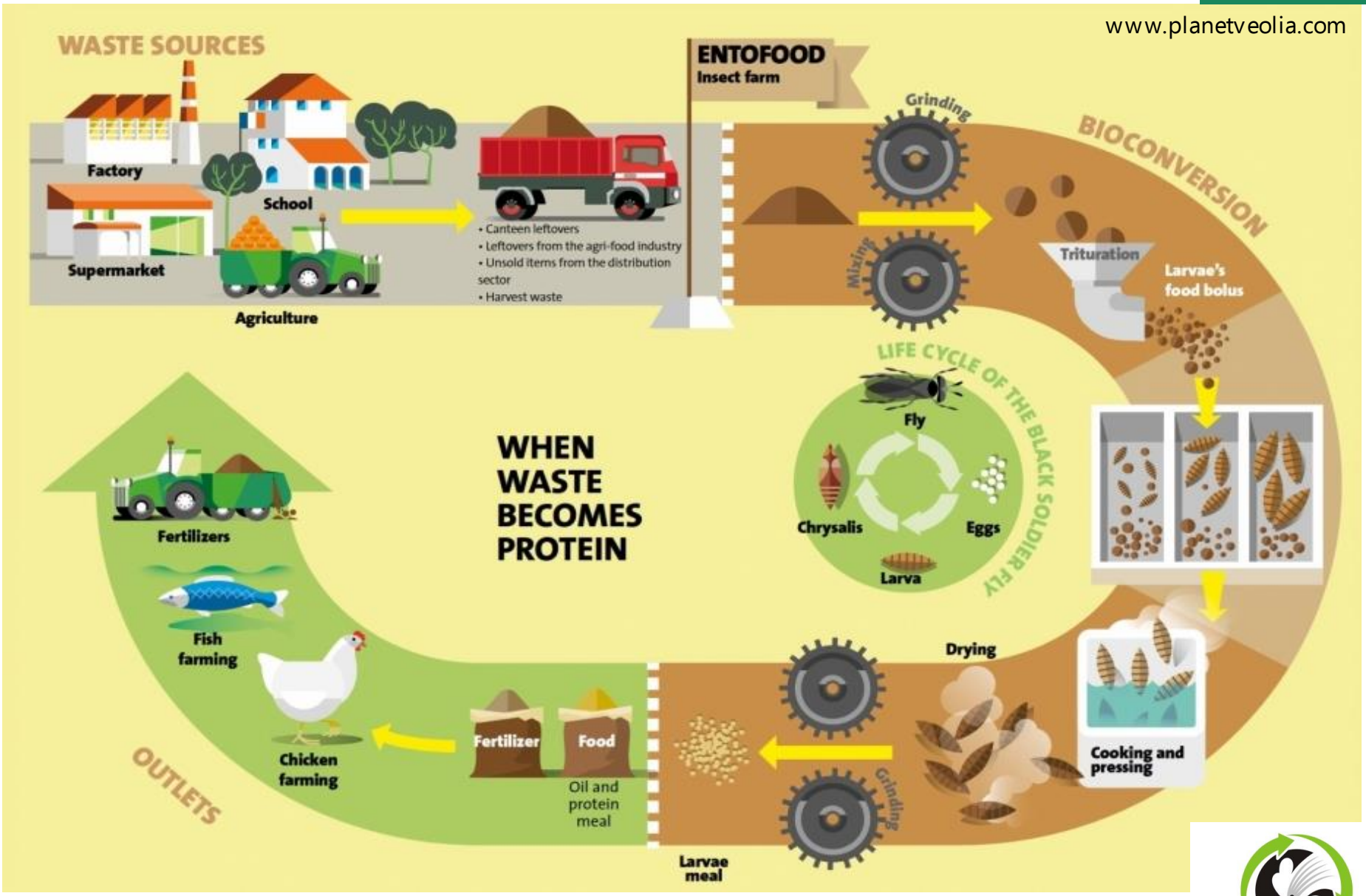

## Final Symposium
## Rome 27 October 2023

POULTRYNSECT FINAL MEETING
ROME 2023

# CONTENTS

www.planetveolia.com

Insects are proficient in converting agricultural and biological residues in high qualitative nutrients, reducing drastically gas emissions and waste mass




Avendaño et al. 2020 ; Veldkamp et al. 2022

**COMMISSION REGULATION (EU) 2017/893**

**of 24 May 2017**

**amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein**

APPROVED

**COMMISSION REGULATION (EU) 2021/1372**

**of 17 August 2021**

**amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals**

Black soldier fly

Mealworm

Housefly

INSECT LARVAE

FULL FAT MEAL

FATS

DEFATTED MEAL

NUTRACEUTICALS

CHITIN

ANTIM. PEPTIDES

FEED ADDITIVES

WHOLE LARVAE

LIVE

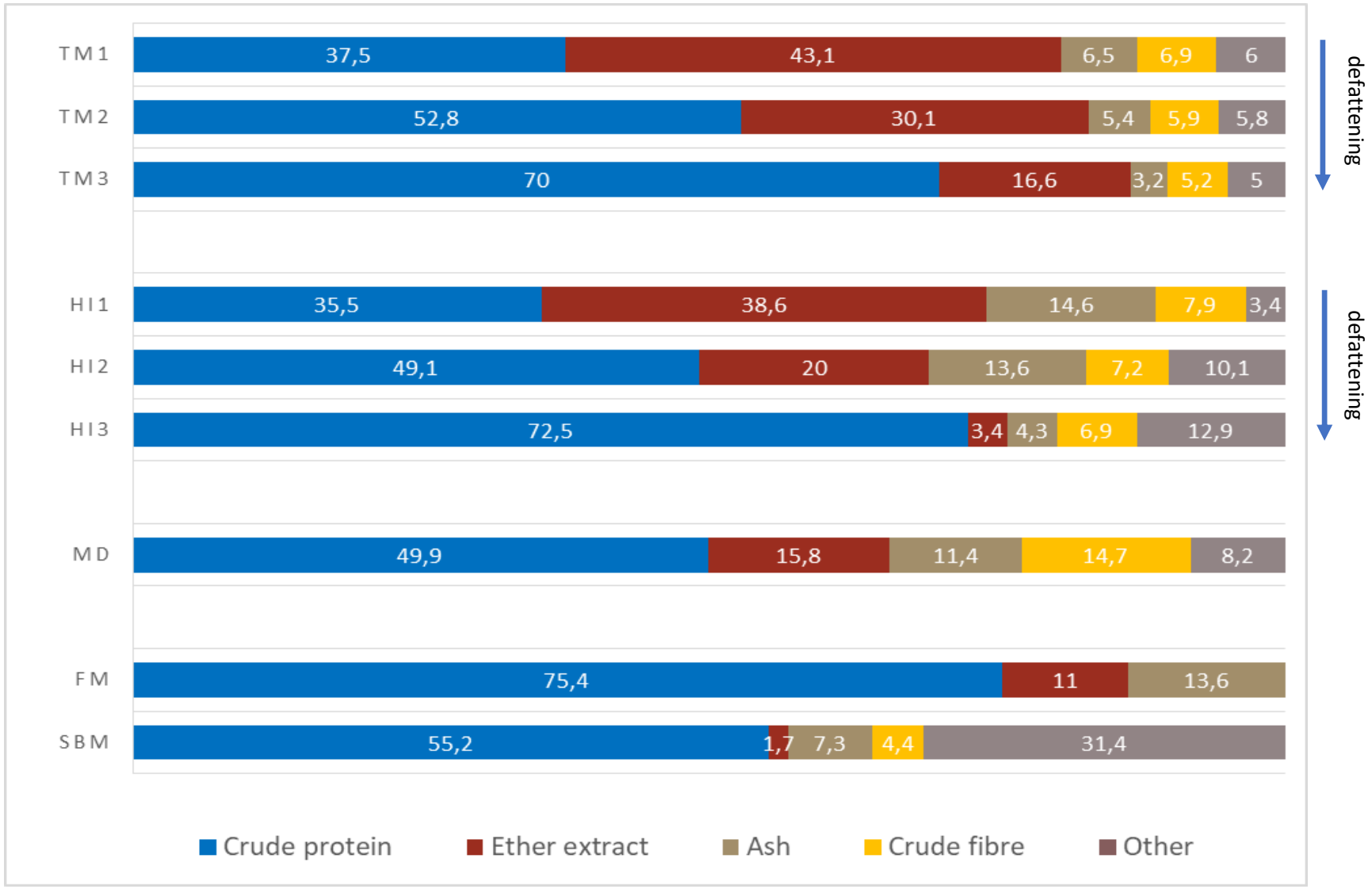
FROZEN

DEHYDRATED

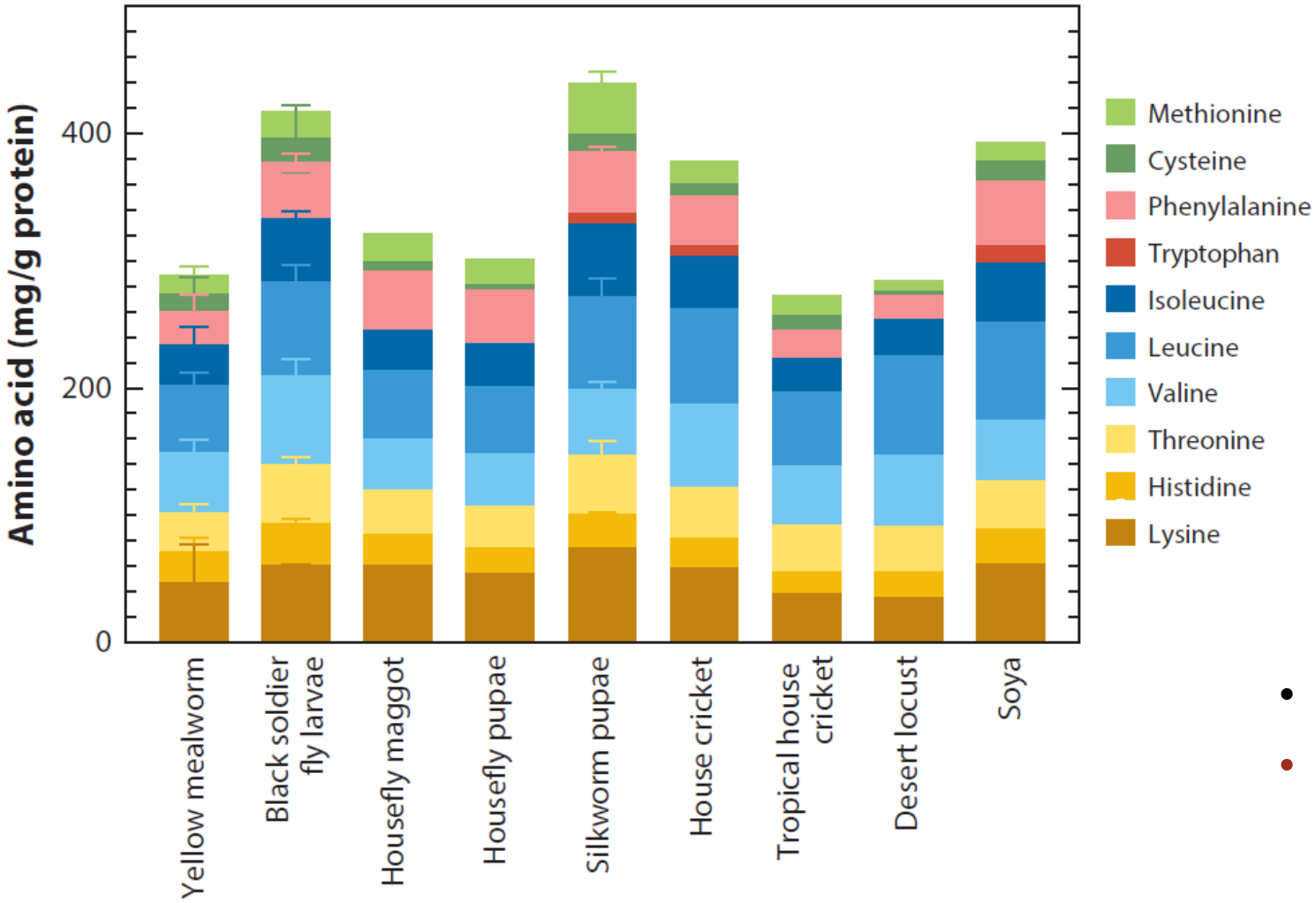
ENV. ENRICHMENT

ANIMAL NUTRITION

# Insect meals chemical composition *vs* FM & SBM (% DM)

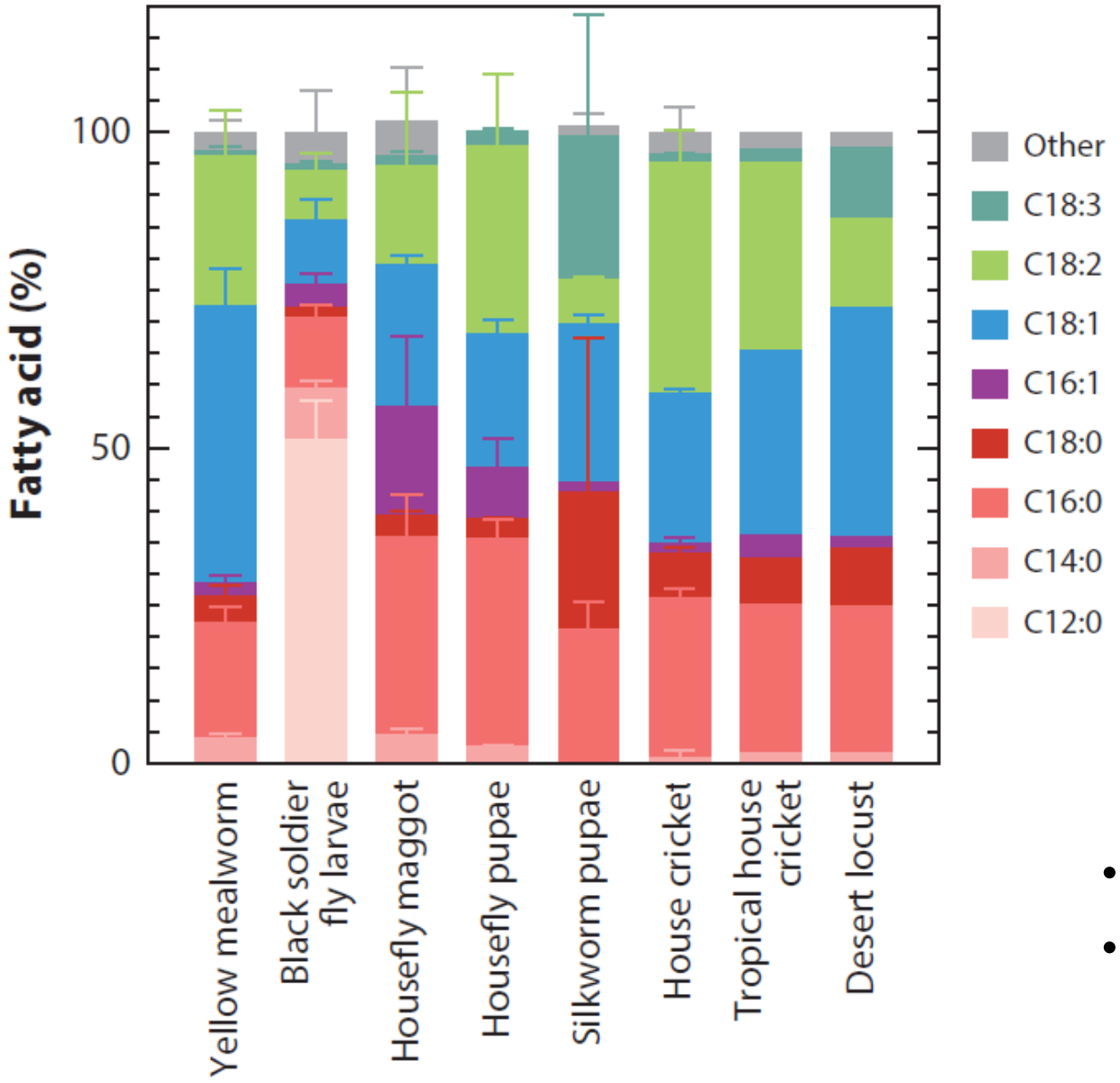



Gasco et al., 2020



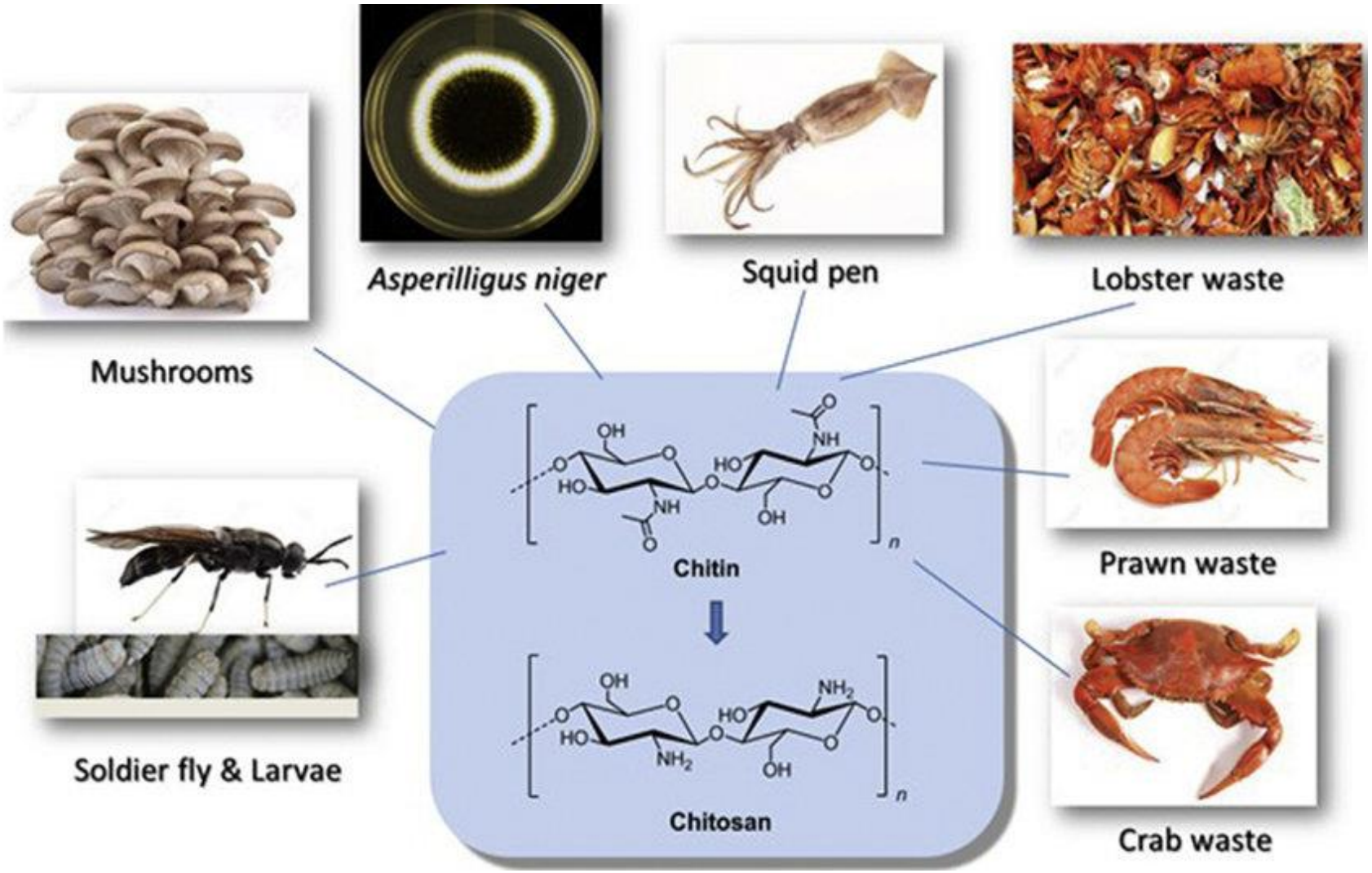

- good sources of EAAs
- **affected** by insect specie & stage

Hawkey et al., 2021




- Fat content & FA profile **affected** by **substrate**
- FA profile **affected** by **specie**

Hawkey et al., 2021.

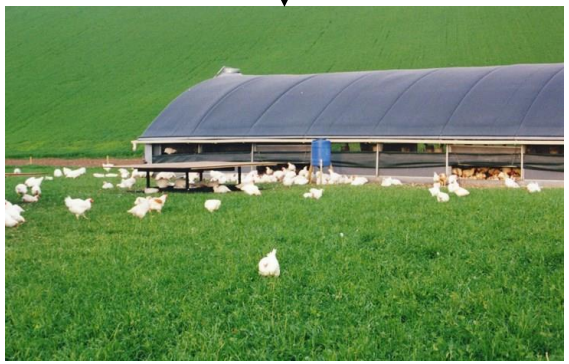



## CHITIN

- antioxidant effects
- immune system stimulation
- microbiota modulation

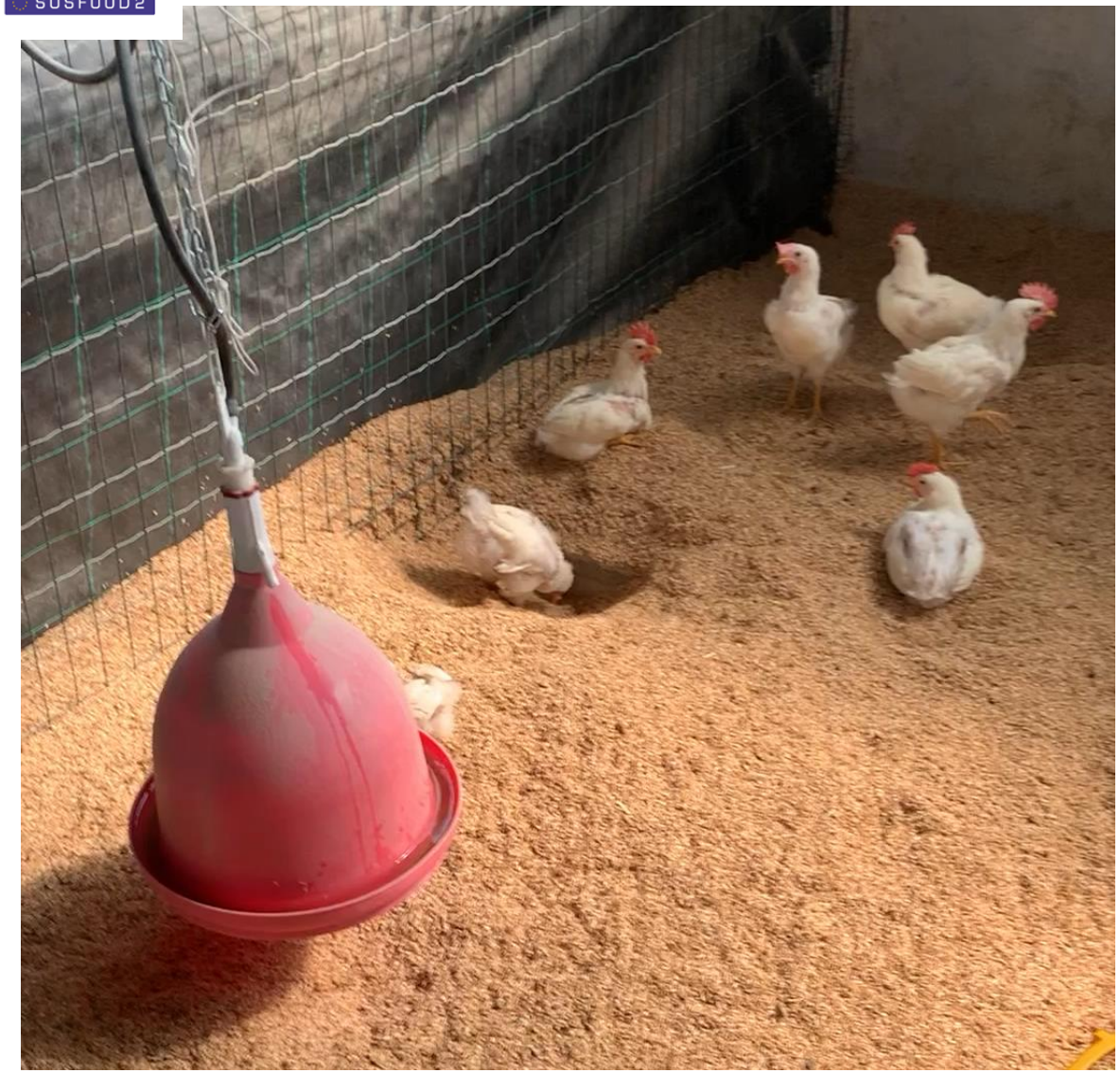
Hawkey et al., 2021; Gasco et al., 2019

**ENVIRONMENTAL ENRICHMENTS**



?

Riber et al., 2018



In free-range farming systems insects are part of the natural poultry diet

Great part of the day is spent by the bird foraging for feed. During this natural behavior, the bird pecks and scratches the ground, and eats.

Moreby et al., 2006; Mench 2009

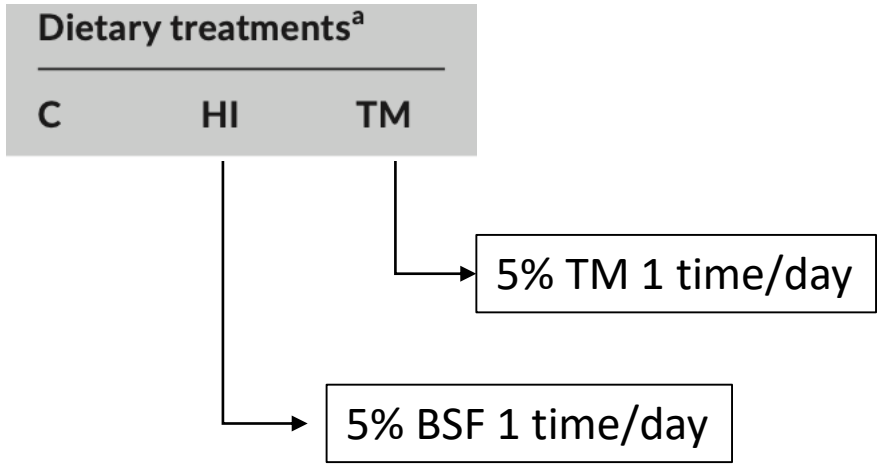
# 2. WHOLE INSECT LARVAE in BROILER CHICKENS

PLEMENT ARTICLE

Journal of Animal Physiology and Animal Nutrition WILEY J Anim Physiol Anim Nutr. 2021;105(Suppl.

# Black soldier fly and yellow mealworm live larvae for broiler chickens: Effects on bird performance and health status

Sara Bellezza Oddon[1] | Ilaria Biasato[1] | Arianna Imarisio[2] | Miha Pipan[3] | Dominik Dekleva[3] | Elena Colombino[2] | Maria Teresa Capucchio[2] | Marco Meneguz[1] | Bergagna Stefania[4] | Raffaella Barbero[4] | Marta Gariglio[2] | Sihem Dabbou[5,6] | Edoardo Fiorilla[2] | Laura Gasco[1] | Achille Schiavone[2]




Bellezza Oddon et al., 2021

# TABLE: chemical composition of live HI and TM larvae at two stages

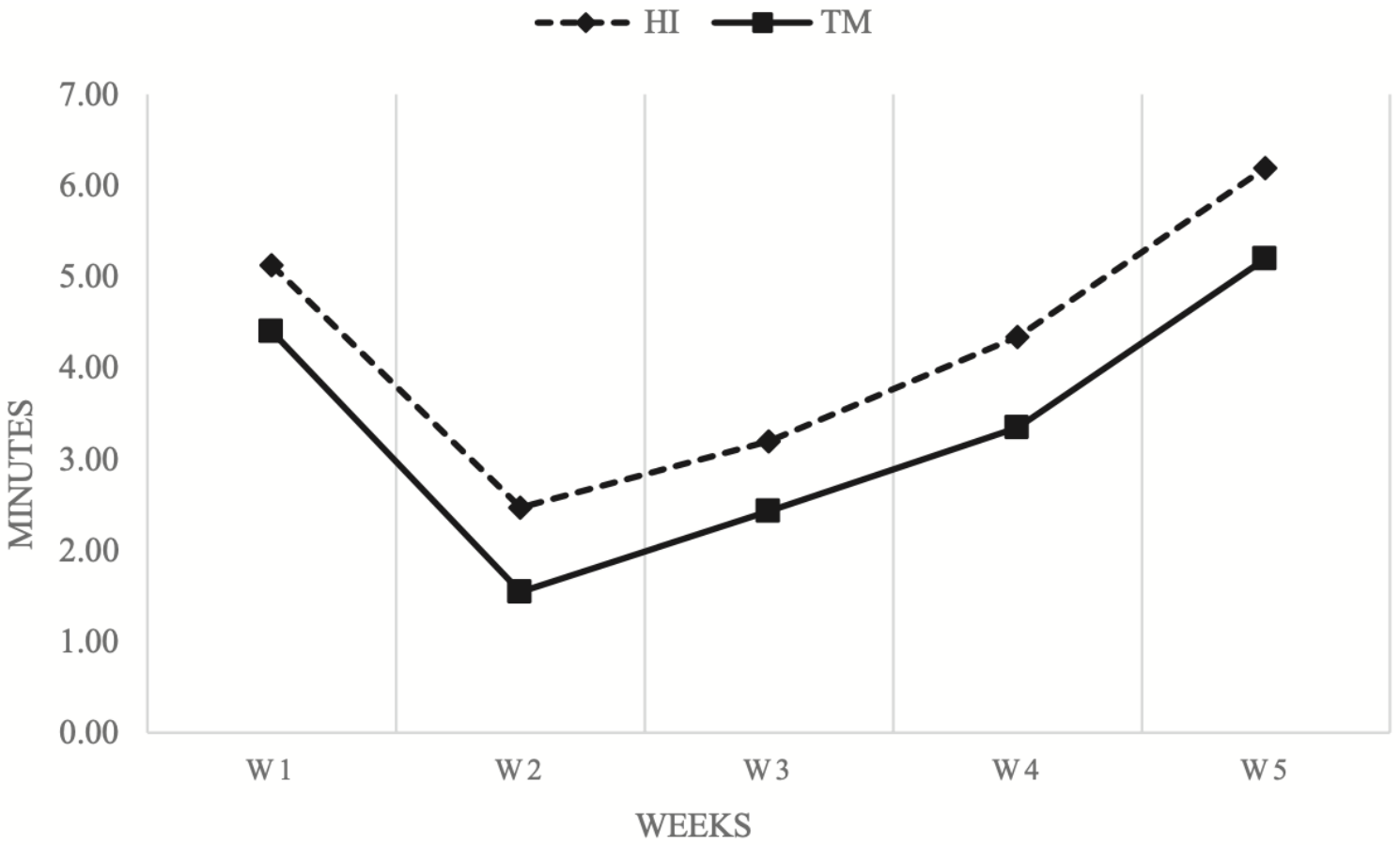
| Chemical composition[a] (as fed basis, %) | HI early instar larvae | HI late instar larvae | TM early instar larvae | TM late instar larvae |
|---|---|---|---|---|
| DM | 25.32 | 25.32 | 27.54 | 27.54 |
| CP | 12.01 | 8.07 | 16.78 | 10.82 |
| Ash | 3.05 | 2.00 | 1.69 | 0.90 |
| EE | 0.42 | 1.93 | 0.59 | 5.50 |
| GE (MJ/kg) | 5.03 | 6.76 | 5.90 | 7.65 |

Abbreviations: CP, crude protein; DM, dry matter; EE, ether extract; GE, gross energy.

[a]Values are reported as mean of duplicate analyses.

Bellezza Oddon et al., 2021

# time spent for eating 5% supplemented HI or TM live larvae




Bellezza Oddon et al., 2021

**TABLE 2** Effects of the dietary treatments on the growth performance of the broiler chickens ($n$ = 6)

| Items | Age (days) | Dietary treatments[a] C | HI | TM | SEM | $p$ Value[b] |
|---|---|---|---|---|---|---|
| LW, g | 4 | 87 | 87 | 88 | 0.38 | 0.796 |
| | 11 | 220 | 216 | 225 | 3.60 | 0.603 |
| | 38 | 2488 | 2527 | 2452 | 22.28 | 0.619 |
| ADG, g/d | 4–11 | 19 | 18 | 20 | 0.49 | 0.610 |
| | 12–38 | 76 | 72 | 80 | 1.98 | 0.348 |
| DFI, g/d | 4–11 | 24 | 22 | 22 | 0.67 | 0.679 |
| | 12–38 | 110 | 108 | 103 | 3.45 | 0.753 |
| FCR, g/G | 4–11 | 1.25 | 1.23 | 1.16 | 0.02 | 0.223 |
| | 12–38 | 1.36[ab] | 1.39[a] | 1.32[b] | 0.01 | ** |
| | 4–38 | 1.37[a] | 1.38[a] | 1.31[b] | 0.01 | ** |

| Dietary treatments[a] | | |
|---|---|---|
| C | HI | TM |

5% TM 1 time/day

5% BSF 1 time/day

Bellezza Oddon et al., 2021

# Welfare implications for broiler chickens reared in an insect larvae-enriched environment: Focus on bird behaviour, plumage status, leg health, and excreta corticosterone

Ilaria Biasato[1]*, Sara Bellezza Oddon[1], Giulia Chemello[2], Marta Gariglio[3], Edoardo Fiorilla[3], Sihem Dabbou[4], Miha Pipan[5], Dominik Dekleva[5], Elisabetta Macchi[3], Laura Gasco[1] and Achille Schiavone[3]

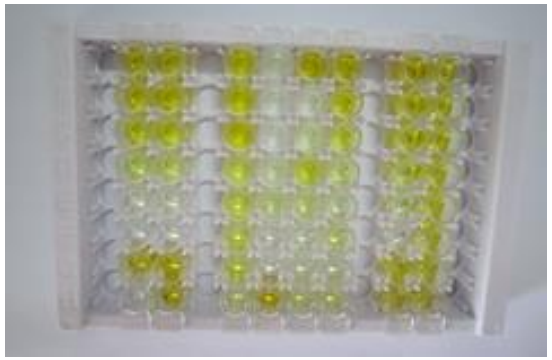
frontiers | Frontiers in Veterinary Science

PUBLISHED 25 August 2022
DOI 10.3389/fphys.2022.930158


**HI and TM live larvae as environmental enrichments**

**Behaviour analysis**

**Welfare of broiler chickens**



**Faecal corticosterone assessment**

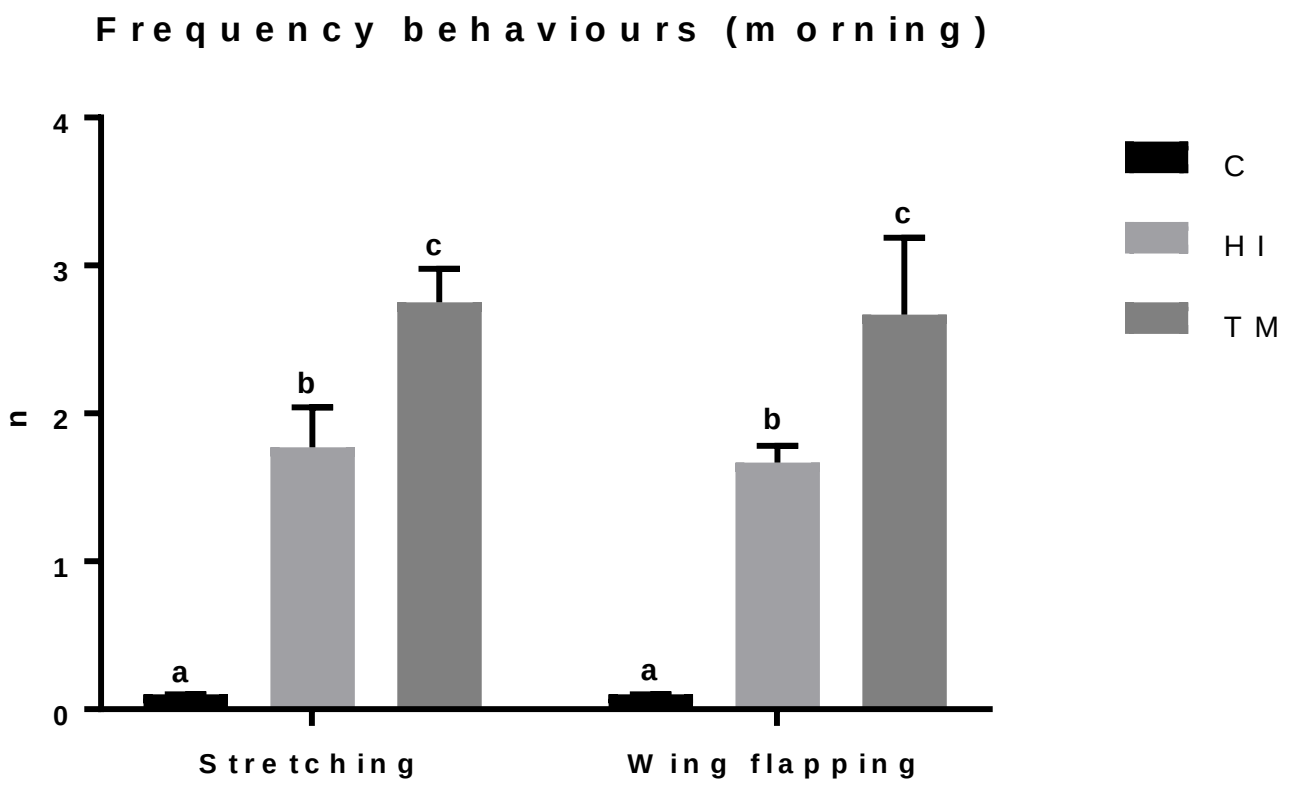
Biasato et al., 2022

**Dietary treatments[a]**

| C | HI | TM |
|---|---|---|

5% TM 1 time/day

5% BSF 1 time/day



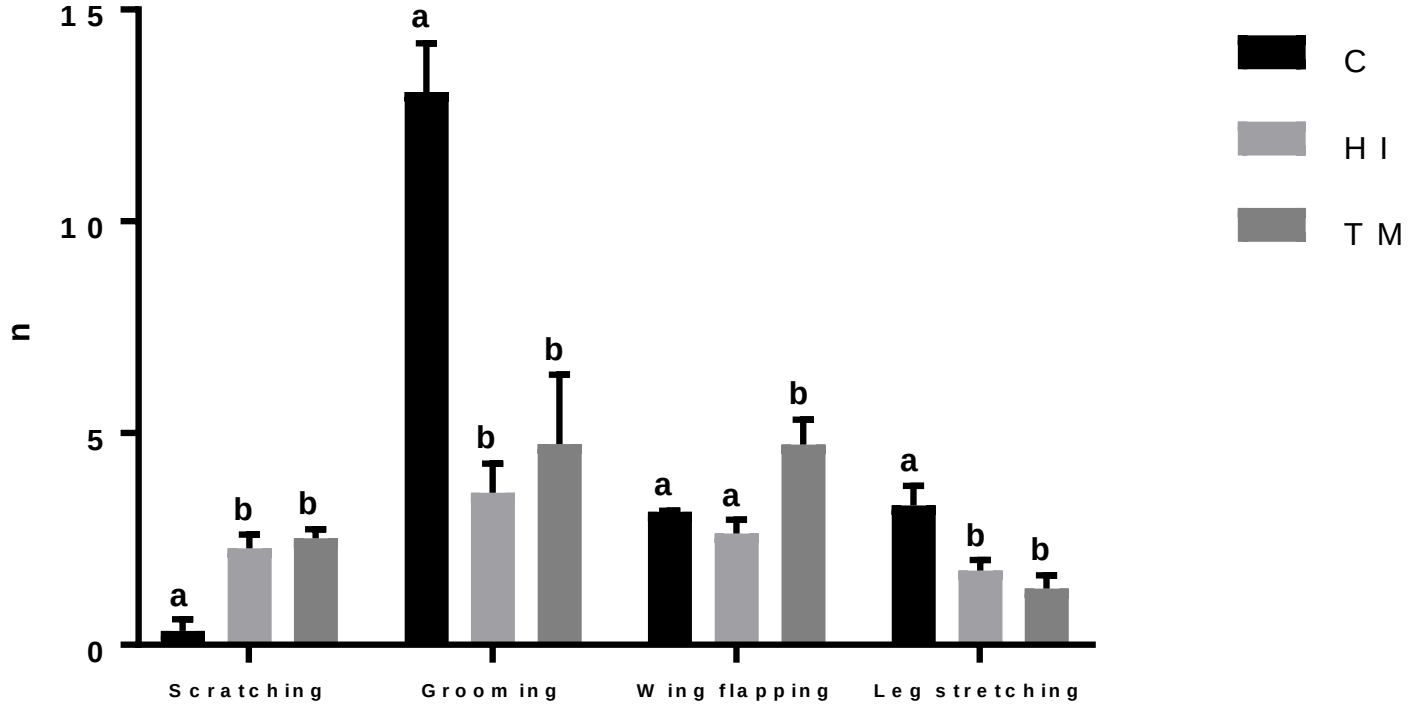

Biasato et al., 2022

**Dietary treatments[a]**

| C | HI | TM |
|---|---|---|

- TM → 5% TM 1 time/day
- HI → 5% BSF 1 time/day

**Frequency behaviours (larvae intake)**

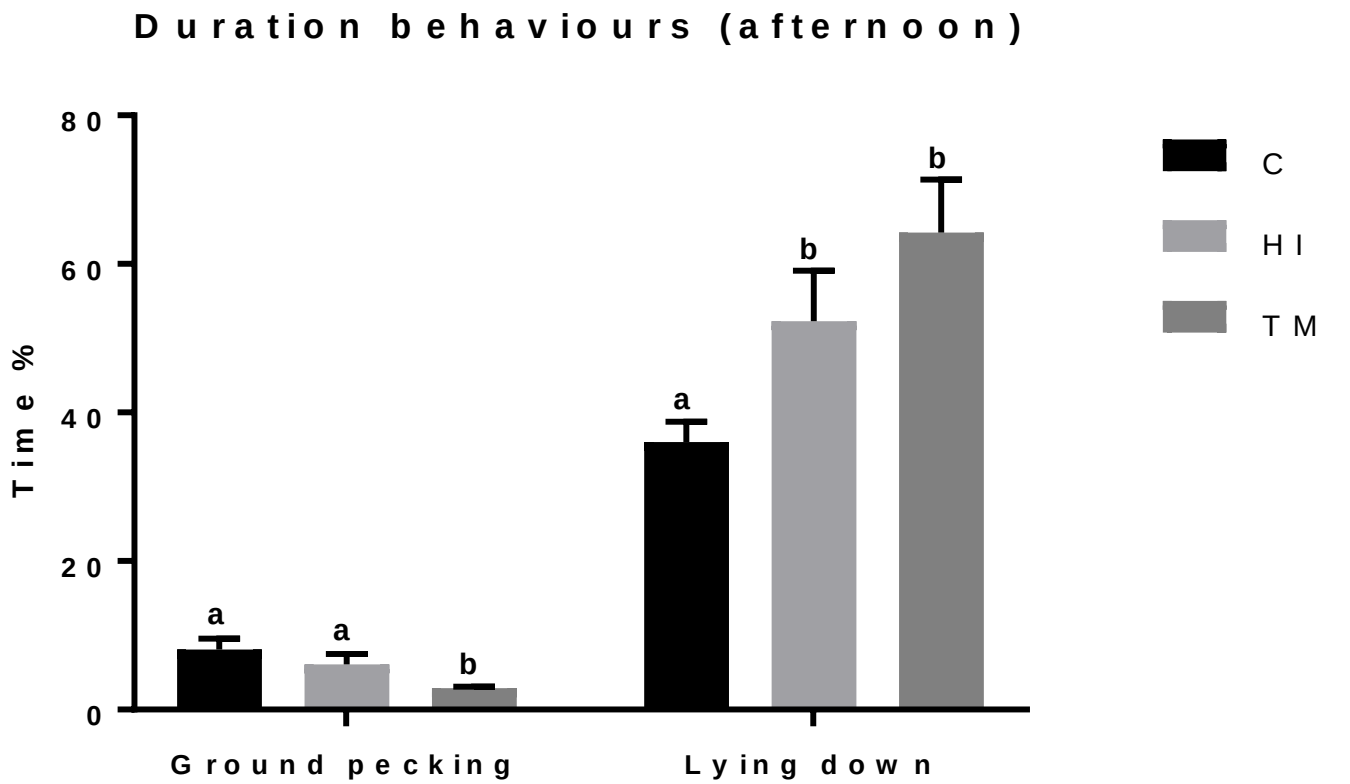



Biasato et al., 2022

| Dietary treatments[a] | | |
|---|---|---|
| C | HI | TM |

5% TM 1 time/day

5% BSF 1 time/day

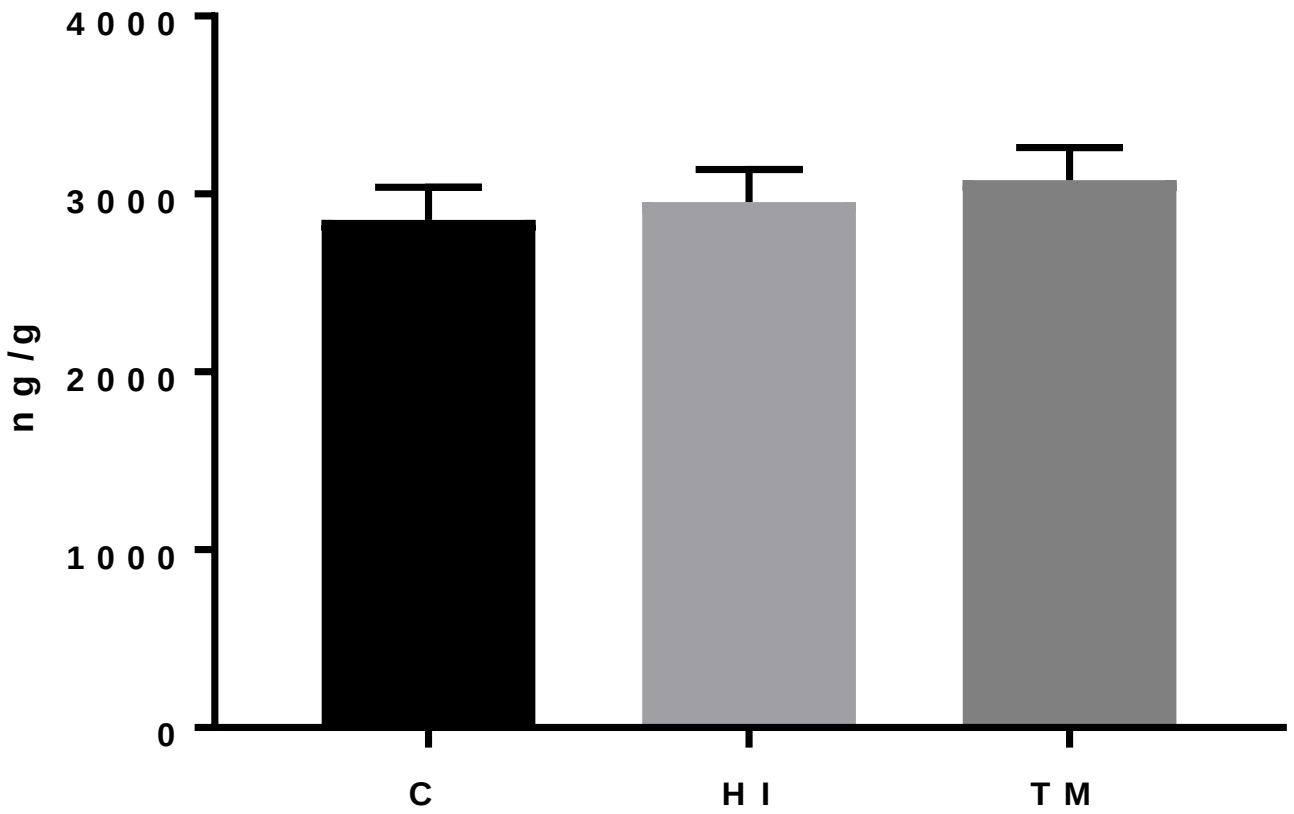



Biasato et al., 2022

| Dietary treatments[a] | | |
|---|---|---|
| C | HI | TM |

5% TM 1 time/day

5% BSF 1 time/day



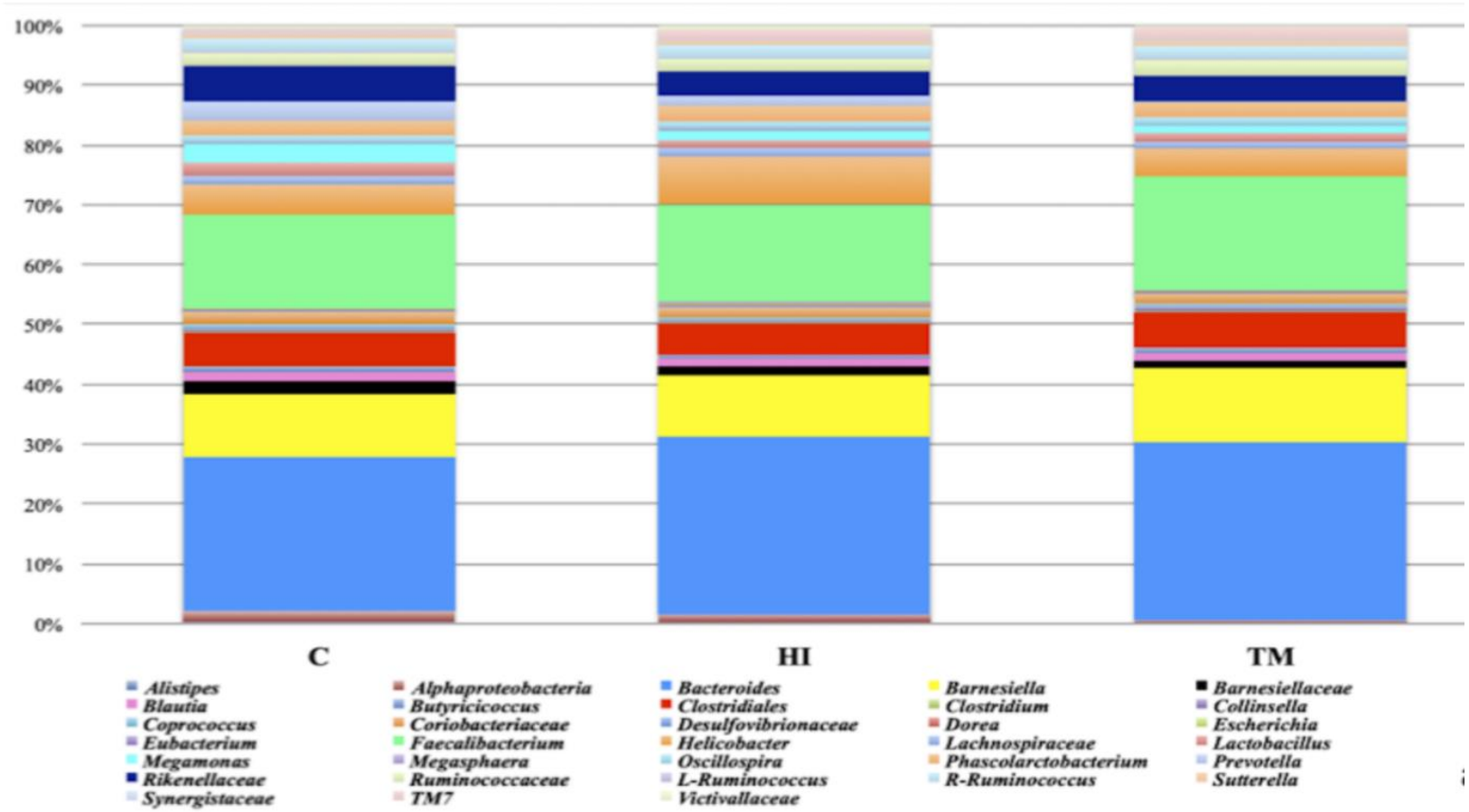

Biasato et al., 2022

# Composition of the caeca microbiota

| Dietary treatments[a] | | |
|---|---|---|
| C | HI | TM |

5% TM 1 time/day

5% BSF 1 time/day




Colombino et al., 2022

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

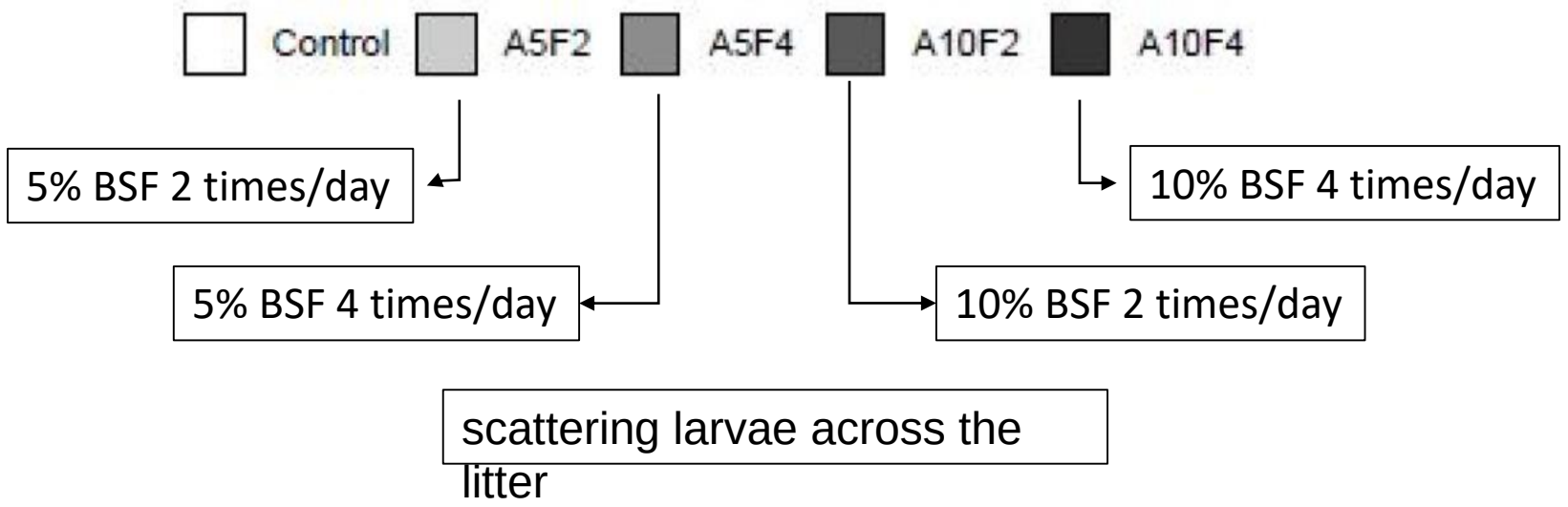
journal homepage: www.elsevier.com/locate/applanim

Applied Animal Behaviour Science 230 (2020) 105082

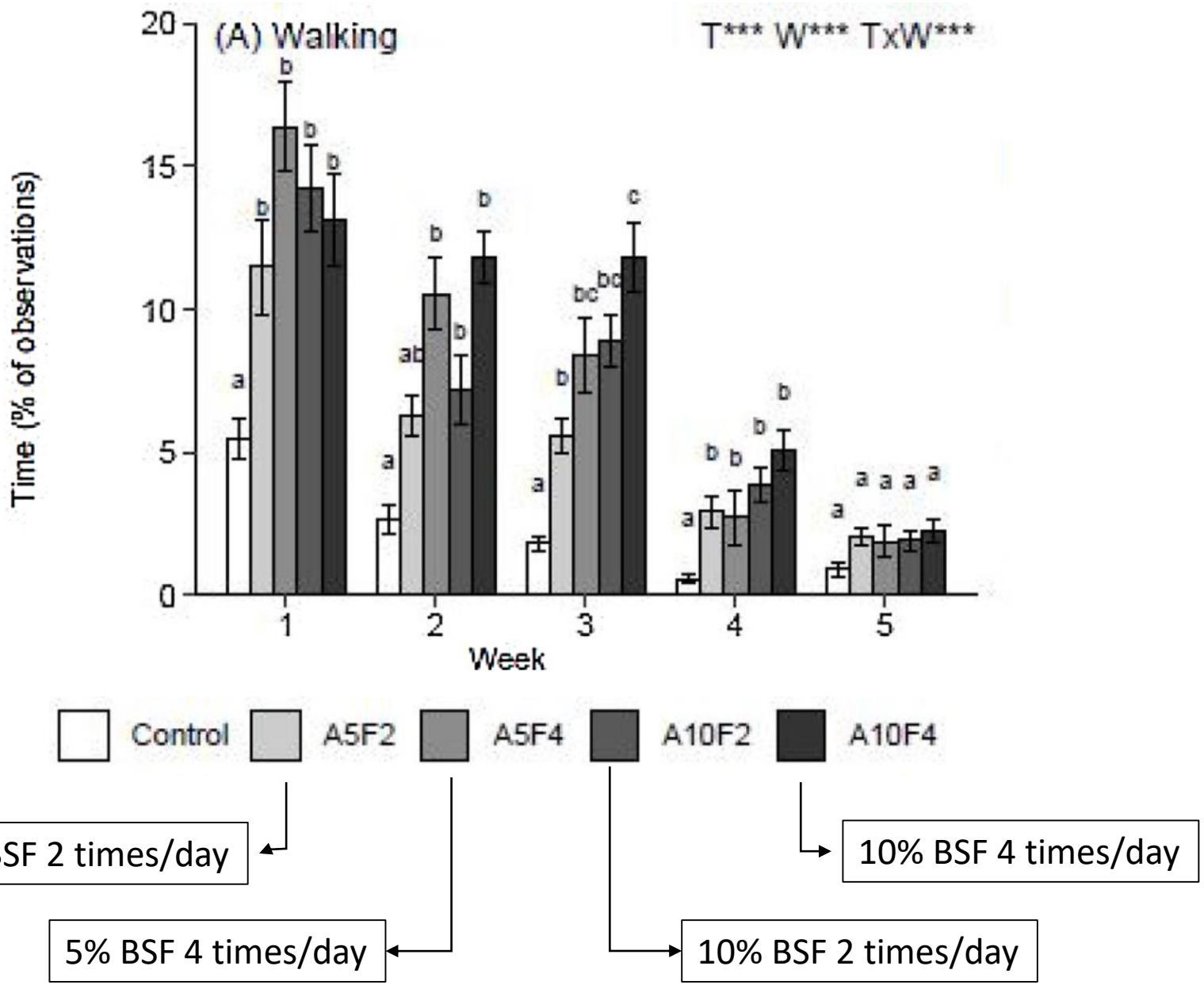
# Provisioning of live black soldier fly larvae *(Hermetia illucens)* benefits broiler activity and leg health in a frequency- and dose-dependent manner

Allyson F. Ipema[a,*], Walter J.J. Gerrits[b], Eddie A.M. Bokkers[c], Bas Kemp[a], J. Elizabeth Bolhuis[a]

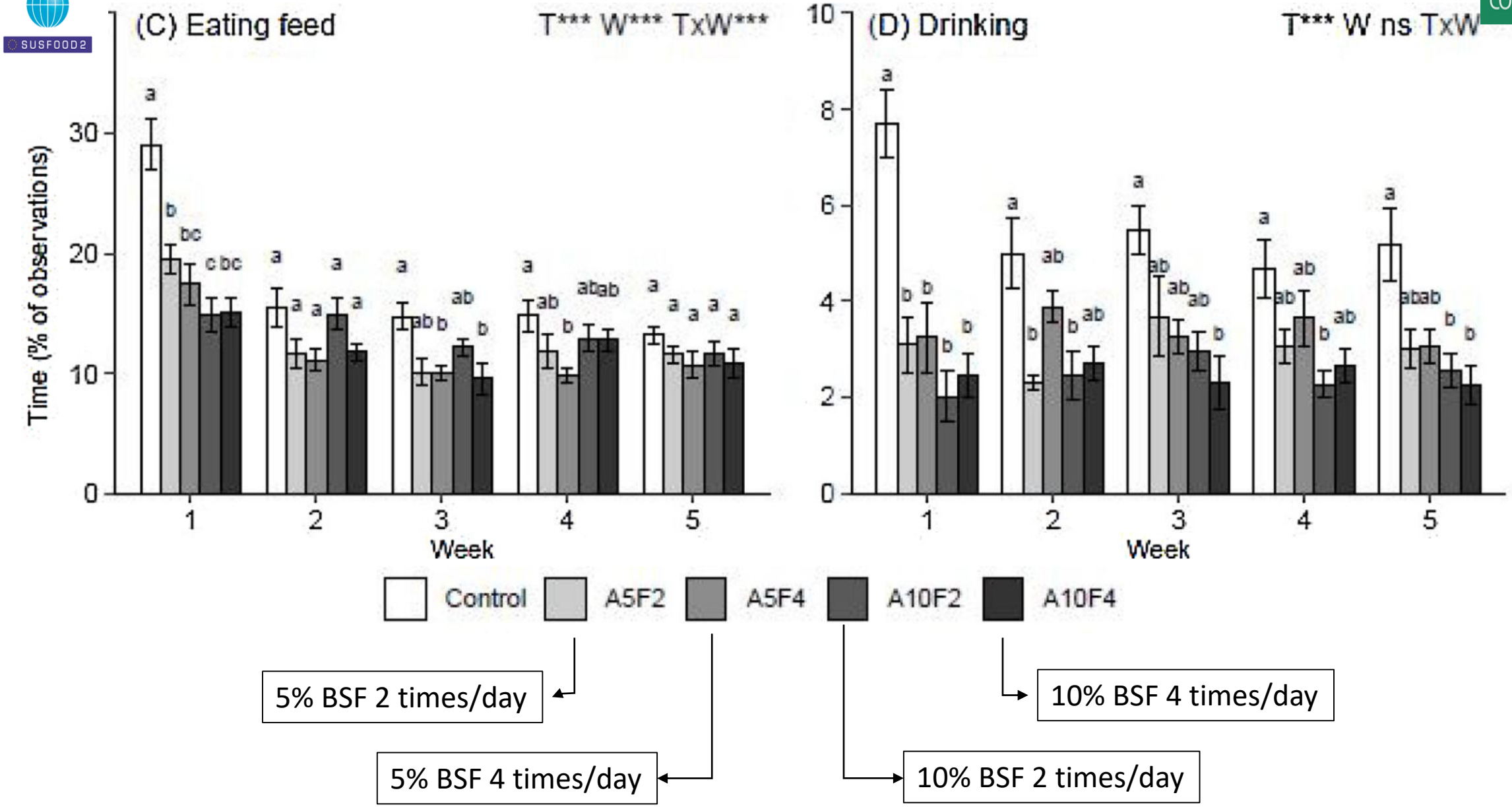
[a] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH, Wageningen, the Netherlands
[b] Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH, Wageningen, the Netherlands
[c] Animal Production Systems Group, Department of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH, Wageningen, the Netherlands



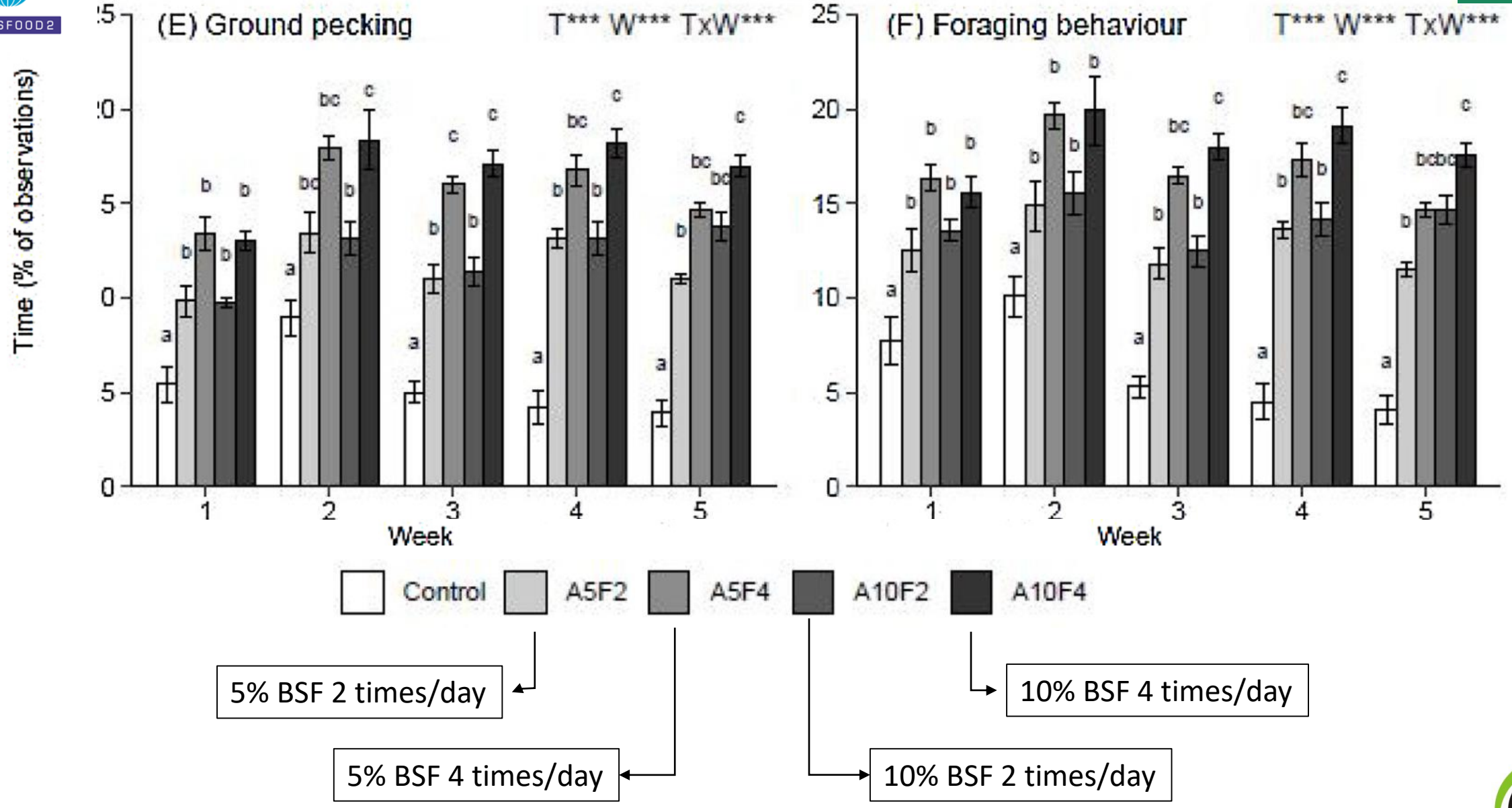

Ipema et al., 2020a



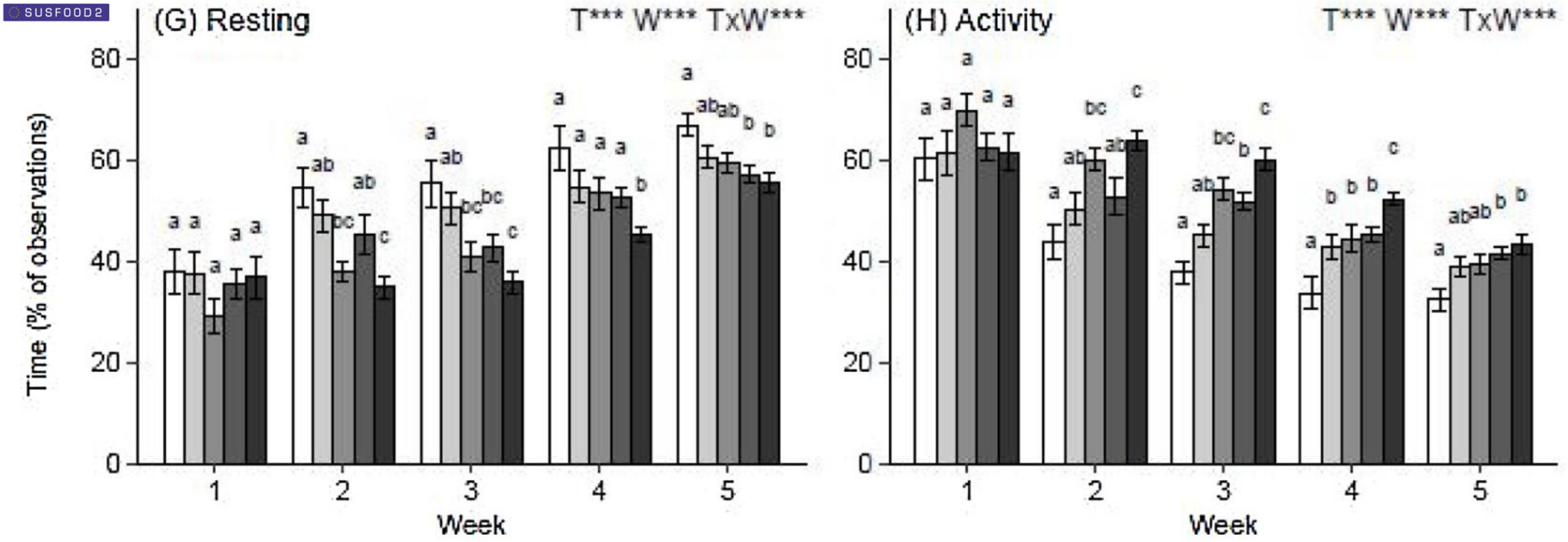

Ipema et al., 2020a




Ipema et al., 2020a




Ipema et al., 2020a



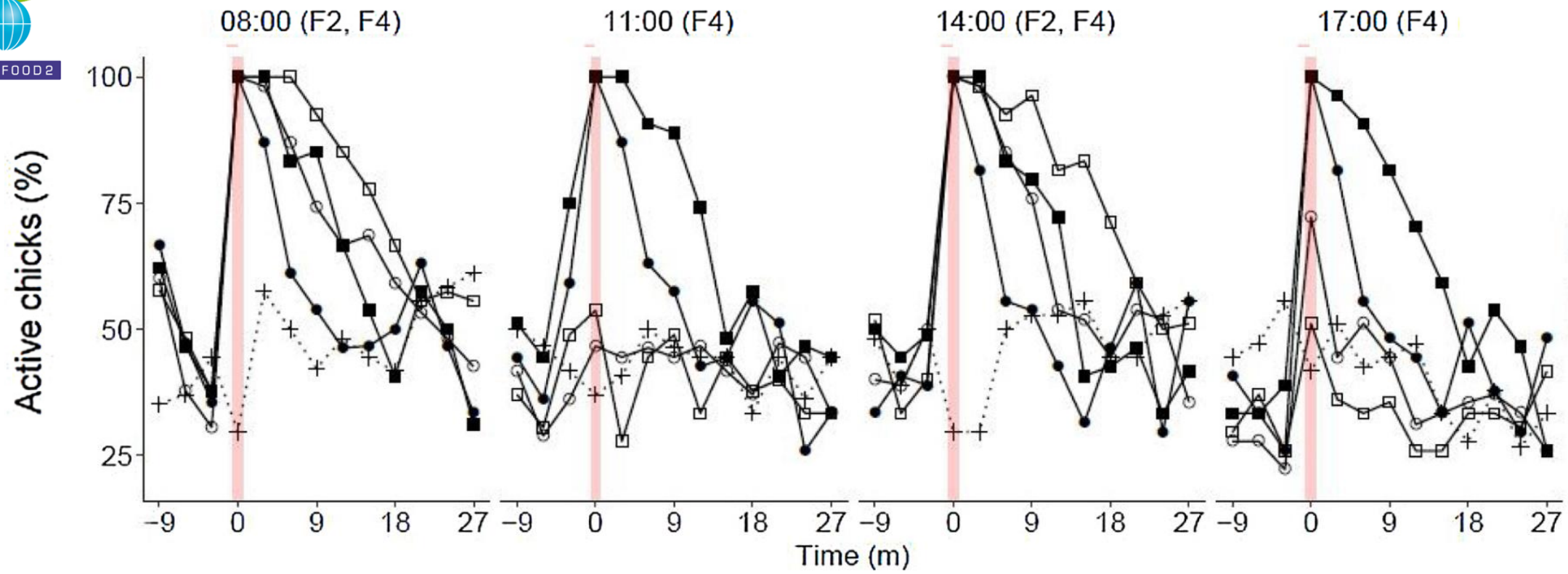

5% BSF 2 times/day

5% BSF 4 times/day

10% BSF 4 times/day

10% BSF 2 times/day

Ipema et al., 2020a



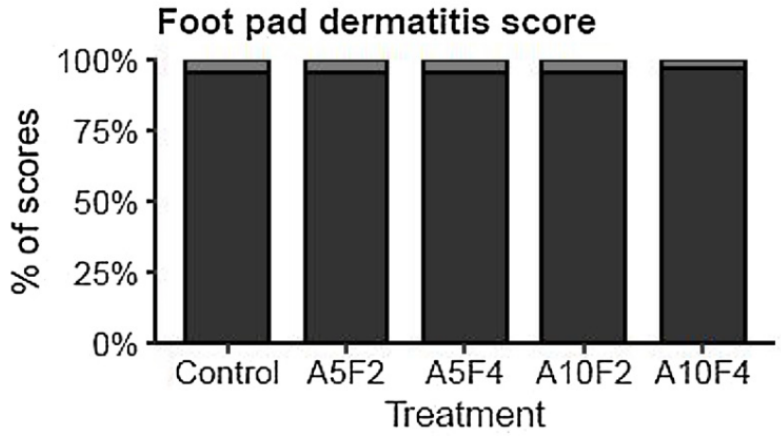

5% BSF 2 times/day

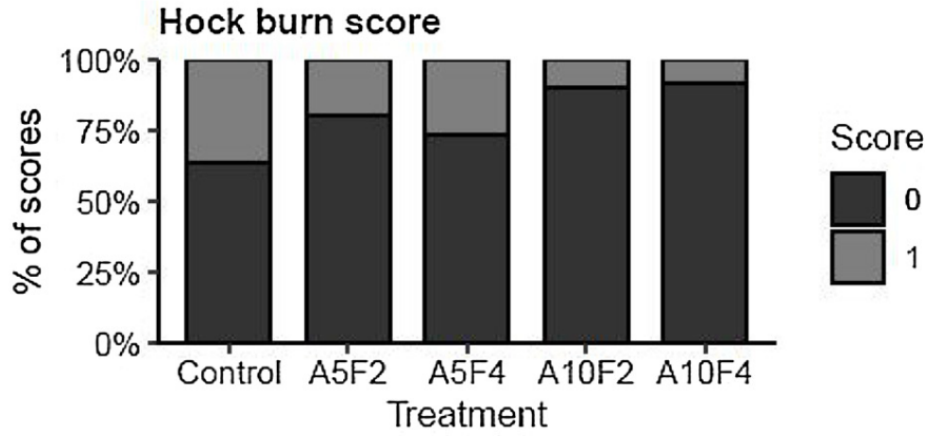
5% BSF 4 times/day

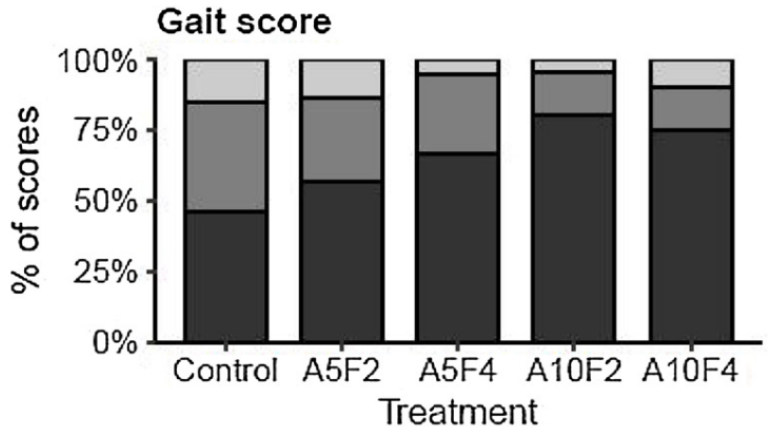
10% BSF 2 times/day

10% BSF 4 times/day

Ipema et al., 2020a




Control A5F2 A5F4 A10F2 A10F4

5% BSF 2 times/day

5% BSF 4 times/day

10% BSF 2 times/day

10% BSF 4 times/day

Ipema et al., 2020a

Contents lists available at ScienceDirect

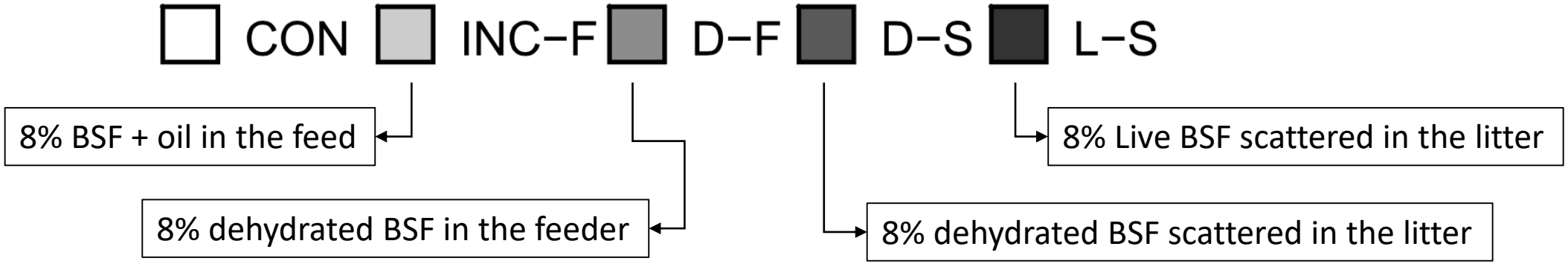
Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Physiology & Behavior 257 (2022) 113999

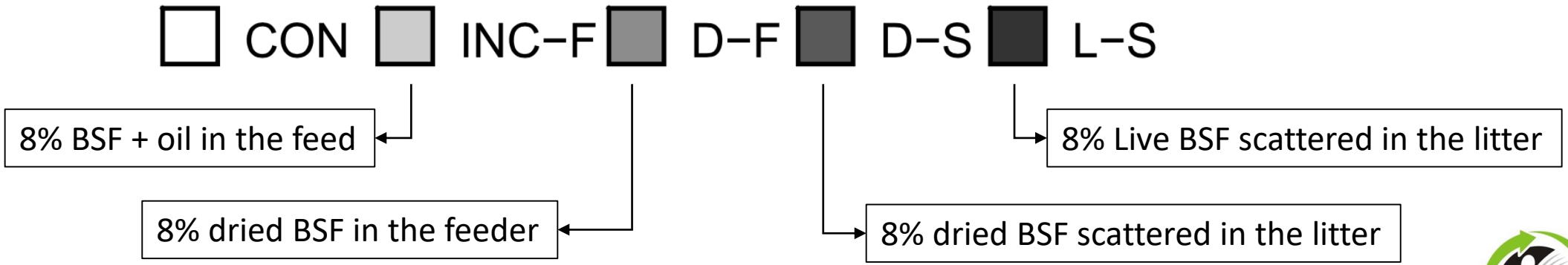
# Provision of black soldier fly larvae (Hermetia illucens) in different ways benefits broiler welfare and performance, with largest effects of scattering live larvae

Allyson F. Ipema [a,*], Eddie A.M. Bokkers [b], Walter J.J. Gerrits [c], Bas Kemp [a], J. Elizabeth Bolhuis [a]

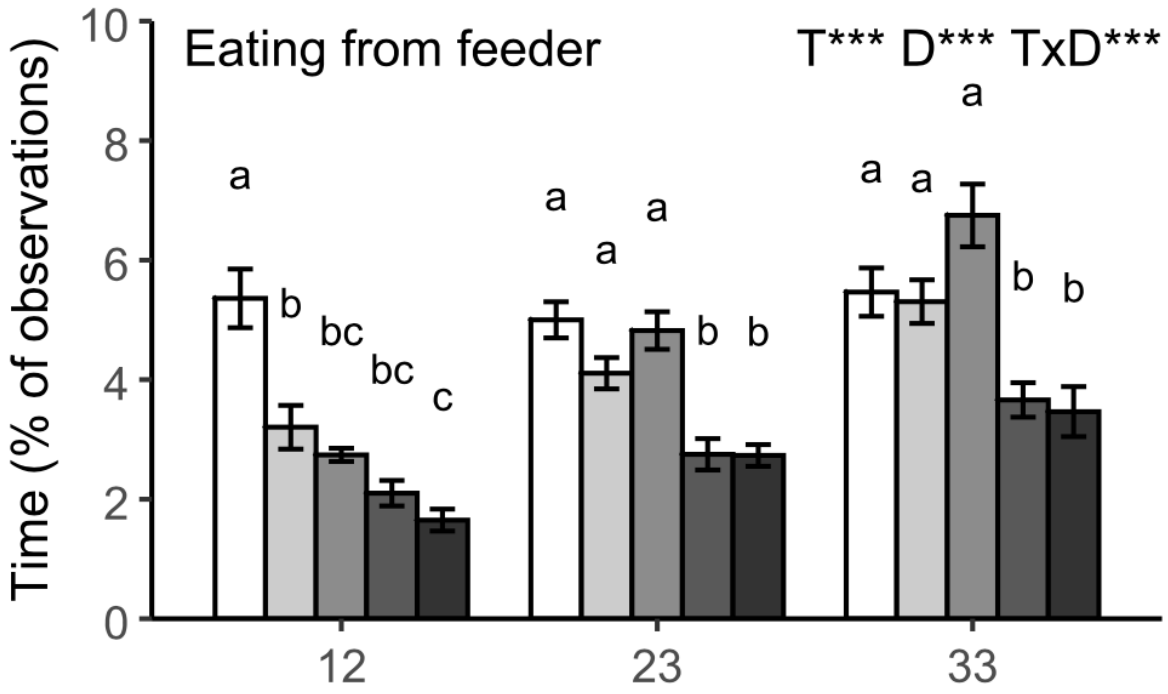


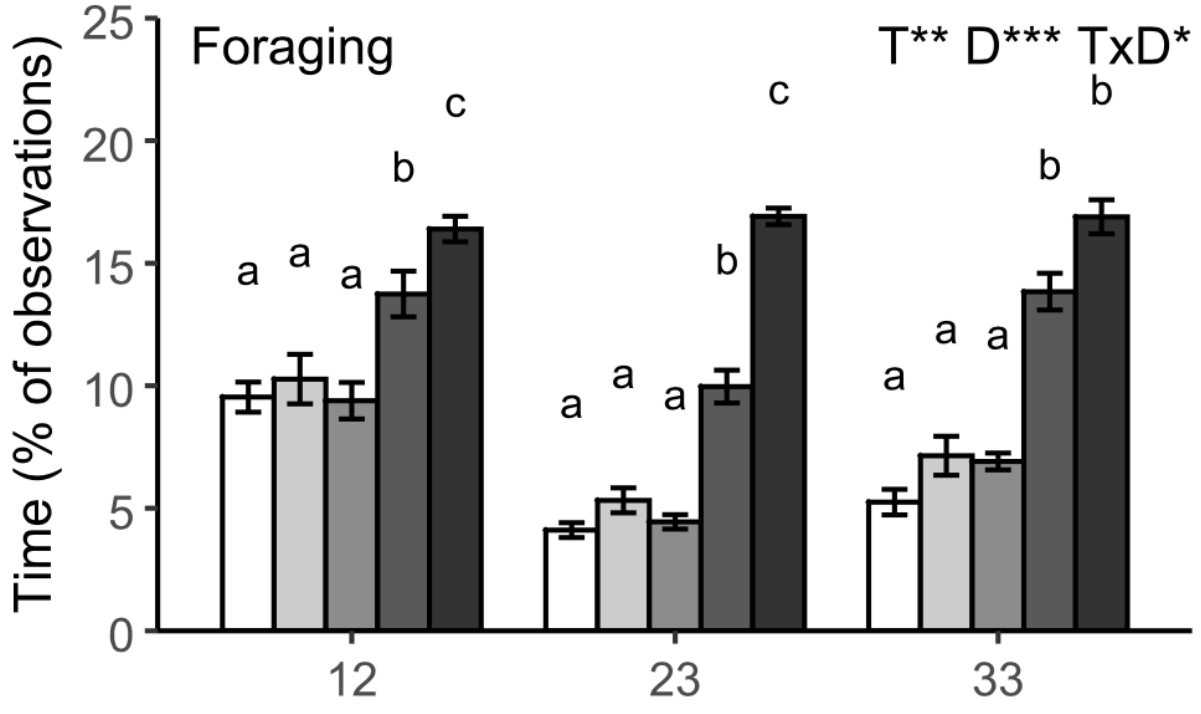

Ipema et al., 2022

| Measure | Period | CON | INC-F | D-F | D-S | L-S | Test-statistic and df | P-value |
|---|---|---|---|---|---|---|---|---|
| Average daily gain (g/d) | d1–9 | 22.6 ± 0.3$^{a}$ | 230±0.1$^{ab}$ | 23.7 ± 0.2$^{b}$ | 23.4 ± 0.3$^{ab}$ | 23.8 ± 0.3$^{b}$ | $F_{(4,44)} = 4.50$ | **0.004** |
| | d9–19 | 61.3 ± 0.6$^{a}$ | 61.6 ± 0.3$^{a}$ | 64.8 ± 0.4$^{b}$ | 64.2 ± 0.4$^{b}$ | 65.8 ± 0.7$^{b}$ | $F_{(4,44)} = 17.15$ | **<0.001** |
| | d19–27 | 102.4 ± 1.3$^{a}$ | 103.1 ± 0.9$^{ab}$ | 107.0 ± 1.3$^{b}$ | 107.6 ± 1.3$^{b}$ | 104.0 ± 1.4$^{ab}$ | $F_{(4,44)} = 4.16$ | **0.004** |
| | d27–35 | 122.4 ± 1.2 | 124.4 ± 1.2 | 123.6 ± 1.5 | 125.5 ± 1.5 | 124.6 ± 2.5 | $F_{(4,44)} = 0.57$ | 0.688 |
| Final weight (g) | d35 | 2660 ± 19.7$^{a}$ | 2694 ± 11.4$^{ab}$ | 2758 ± 9.8$^{bc}$ | 2772 ± 18.9$^{c}$ | 2747 ± 16.1$^{bc}$ | $F_{(4,43)} = 9.88$ | **<0.001** |
| Average daily dry matter intake of pellets (g/d) | d1–35 | 93.4 ± 0.7$^{a}$ | 92.4 ± 0.3$^{a}$ | 86.9 ± 0.6$^{b}$ | 85.8 ± 0.6$^{b}$ | 81.3 ± 0.6$^{c}$ | $F_{(4,44)} = 73.48$ | **<0.001** |
| Estimated average daily dry matter intake of pellets and larvae (g/d)* | d1–35 | 93.4 ± 0.7$^{ab}$ | 92.4 ± 0.3$^{b}$ | 95.0 ± 0.6$^{a}$ | 94.0 ± 0.6$^{ab}$ | 89.4 ± 0.5$^{c}$ | $F_{(4,44)} = 13.73$ | **<0.001** |
| Dry matter conversion ratio (g/g) | d1–35 | 1.25 ± 0.002$^{a}$ | 1.23 ± 0.003$^{b}$ | 1.24 ± 0.008$^{ab}$ | 1.22 ± 0.004$^{b}$ | 1.16 ± 0.004$^{c}$ | $F_{(4,44)} = 49.63$ | **<0.001** |




Ipema et al., 2022




CON INC−F D−F D−S L−S

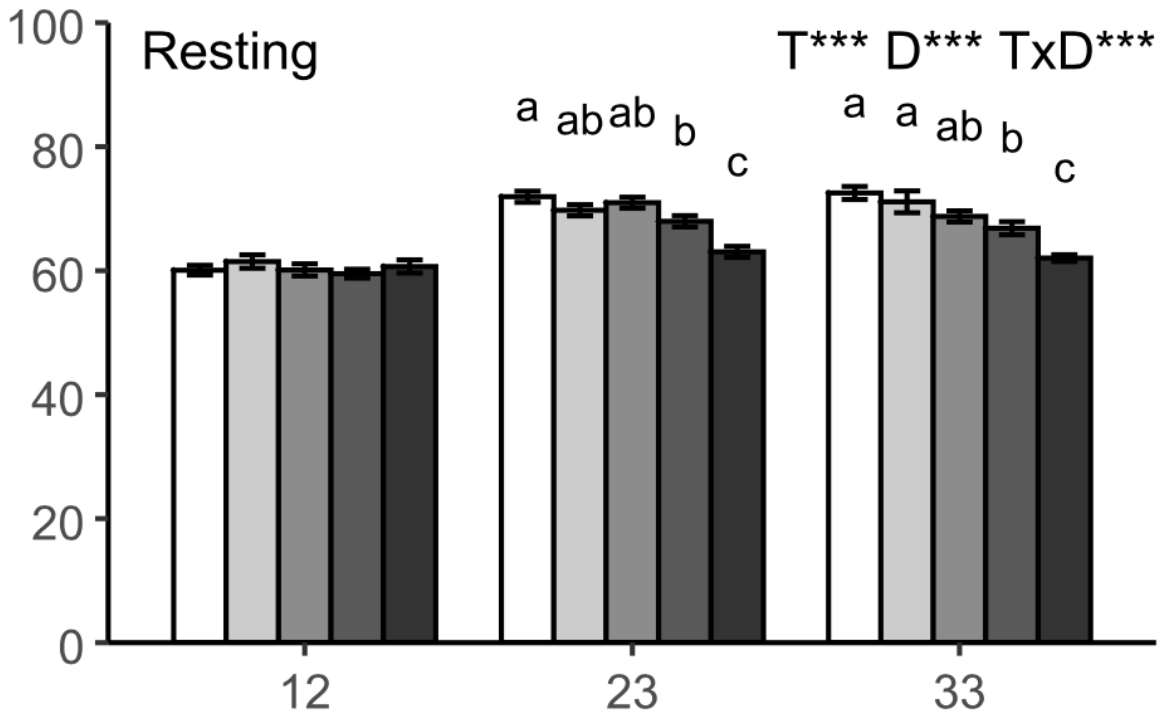
8% BSF + oil in the feed

8% dried BSF in the feeder

8% dried BSF scattered in the litter

8% Live BSF scattered in the litter

Ipema et al., 2022




CON INC–F D–F D–S L–S

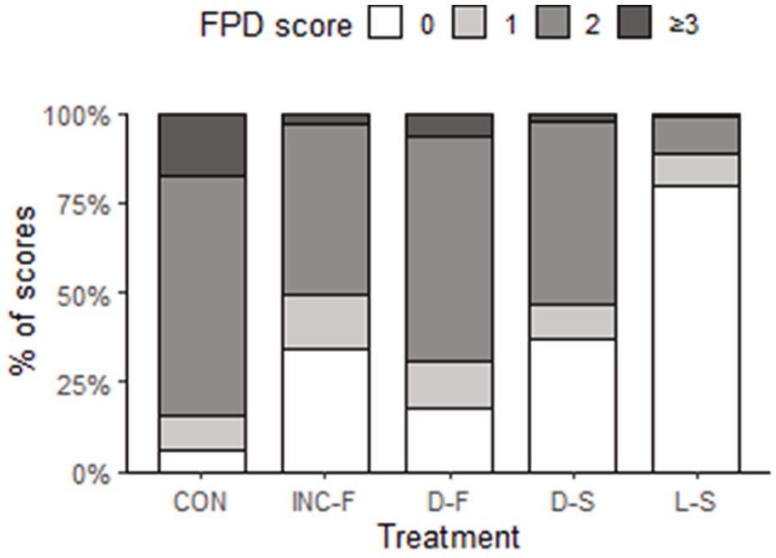
8% BSF + oil in the feed

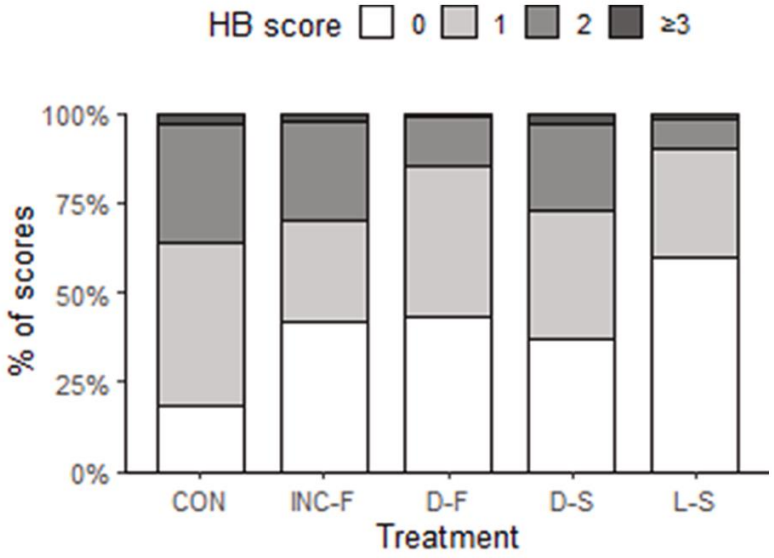
8% dried BSF in the feeder

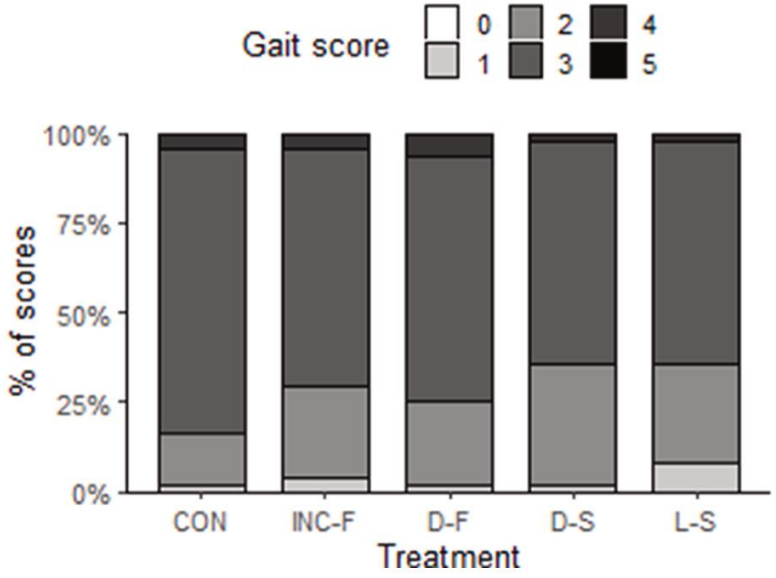
8% dried BSF scattered in the litter

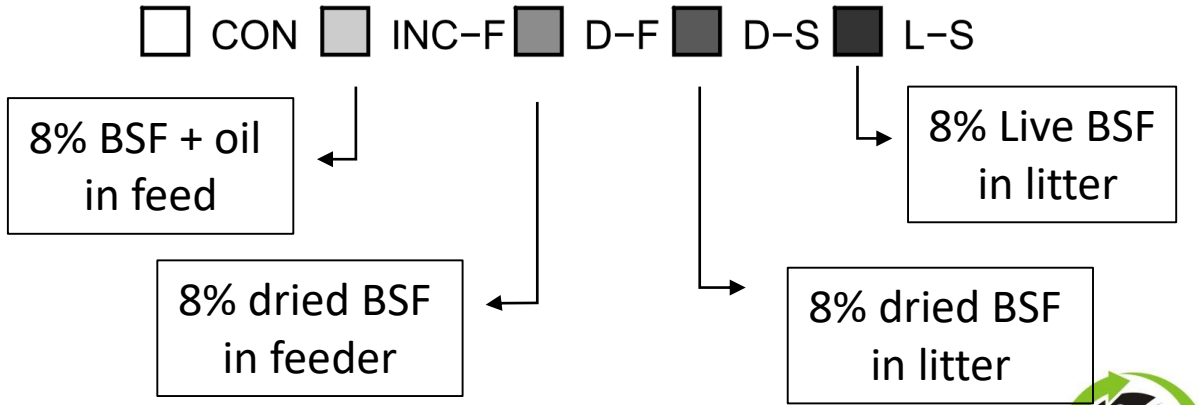
8% Live BSF scattered in the litter

Ipema et al., 2022



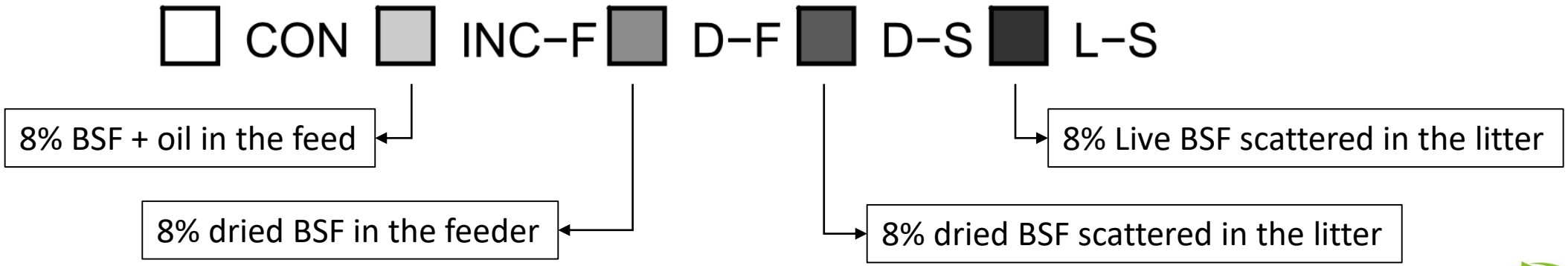




Ipema et al., 2022

| Measure | CON | INC-F | D-F | D-S | L-S | Test-statistic and df | P-value |
|---|---|---|---|---|---|---|---|
| Feather CORT (pg/mm) | 0.44 ± 0.13 | 0.24 ± 0.06 | 0.30 ± 0.11 | 0.41 ± 0.14 | 0.38 ± 0.12 | $F_{(4,55)} = 2.76$ | **0.037** |

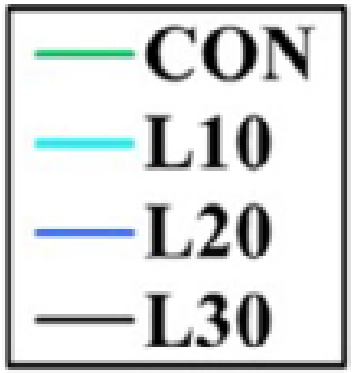



Ipema et al., 2022

# Effects of increasing levels of whole Black Soldier Fly (*Hermetia illucens*) larvae in broiler rations on acceptance, nutrient and energy intakes and utilization, and growth performance of broilers

M. M. Seyedalmoosavi,* M. Mielenz ,* S. Görs,* P. Wolf,† G. Daş ,*,[1] and C. C. Metges *

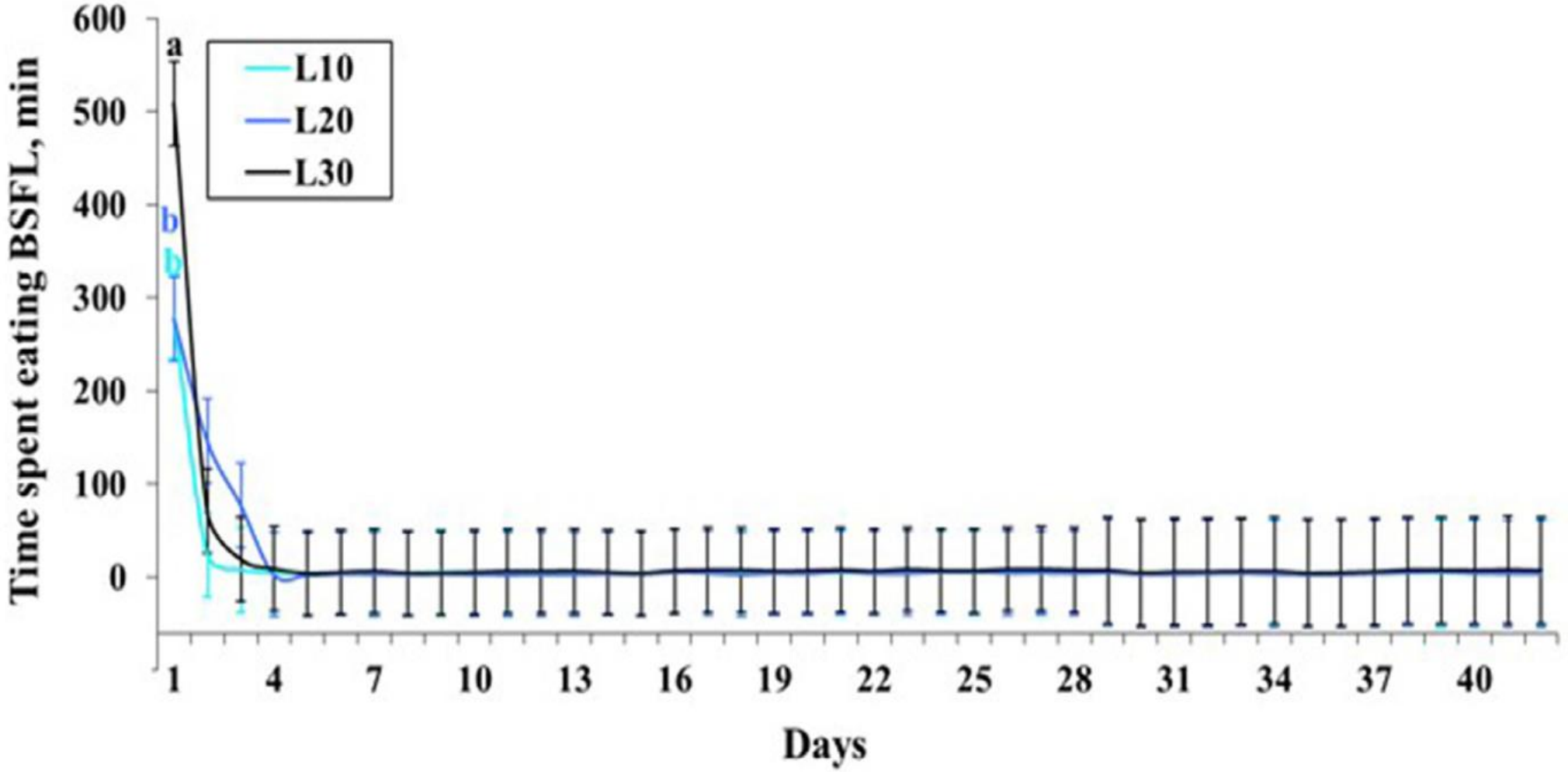
2022 Poultry Science 101:102202



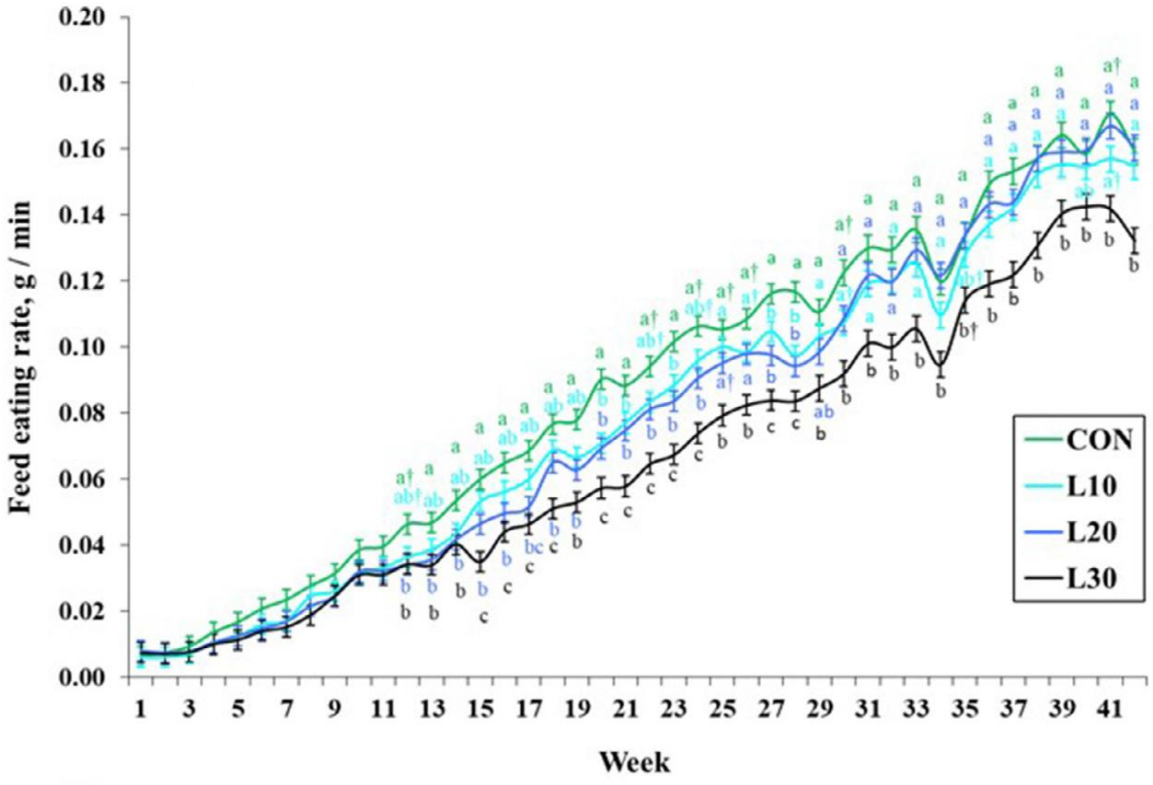

→ 10% DEFROZEN BSLF, 1 time/day

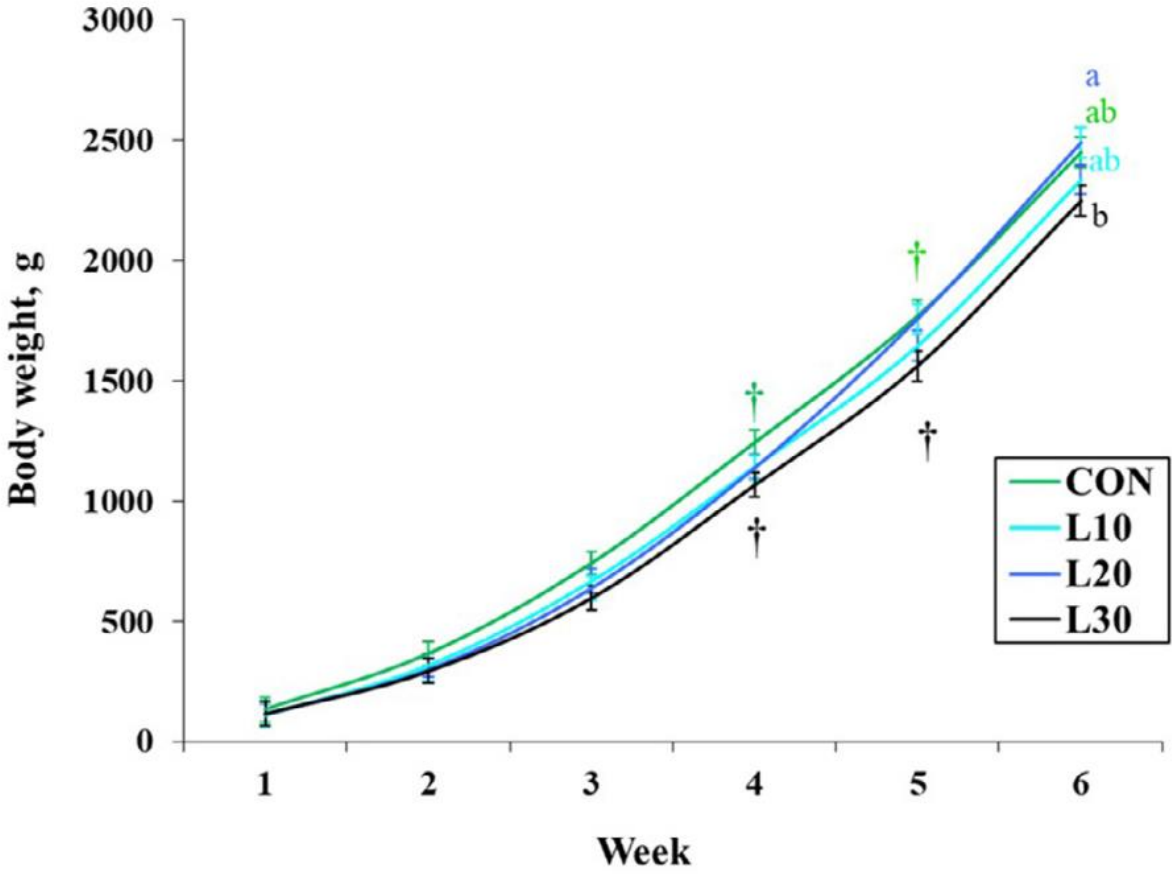
→ 20% DEFROZEN BSLF, 1 time/day

→ 30% DEFROZEN BSLF, 1 time/day




Seyedalmoosavi et al., 2022




Seyedalmoosavi et al., 2022

# 4. WHOLE INSECT LARVAE in SLOW GROWING CHICKENS

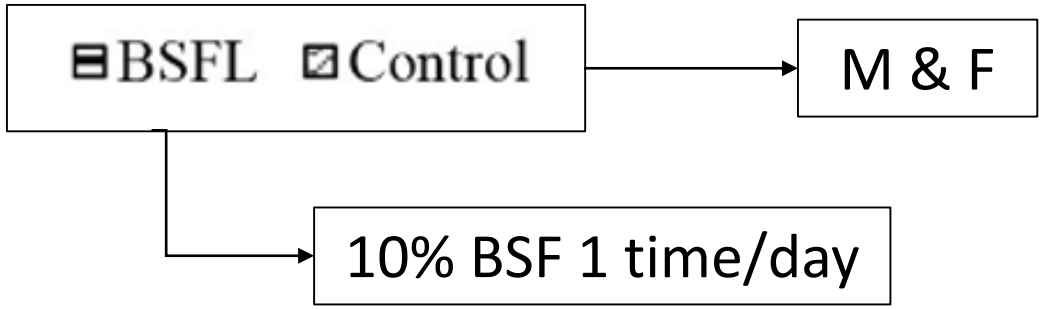
# Black soldier fly larvae used for environmental enrichment purposes: Can they affect the growth, slaughter performance, and blood chemistry of medium-growing chickens?


Valentina Bongiorno[1], Marta Gariglio[1]*, Valeria Zambotto[2], Eleonora Erika Cappone[1], Ilaria Biasato[3], Manuela Renna[1], Claudio Forte[1], Carl Coudron[4], Stefania Bergagna[5], Francesco Gai[2] and Achille Schiavone[1]

frontiers | Frontiers in Veterinary Science
PUBLISHED 14 December 2022
DOI 10.3389/fvets.2022.1064017

Bongiorno et al., 2022

| Proximate composition, g/100 g on an as fed basis | Values[a] |
|---|---|
| DM | 33.63 |
| CP | 14.39 |
| EE | 9.56 |
| Ash | 4.34 |
| Chitin | 2.00 |
| GE, MJ/kg | 8.69 |




Bongiorno et al., 2022

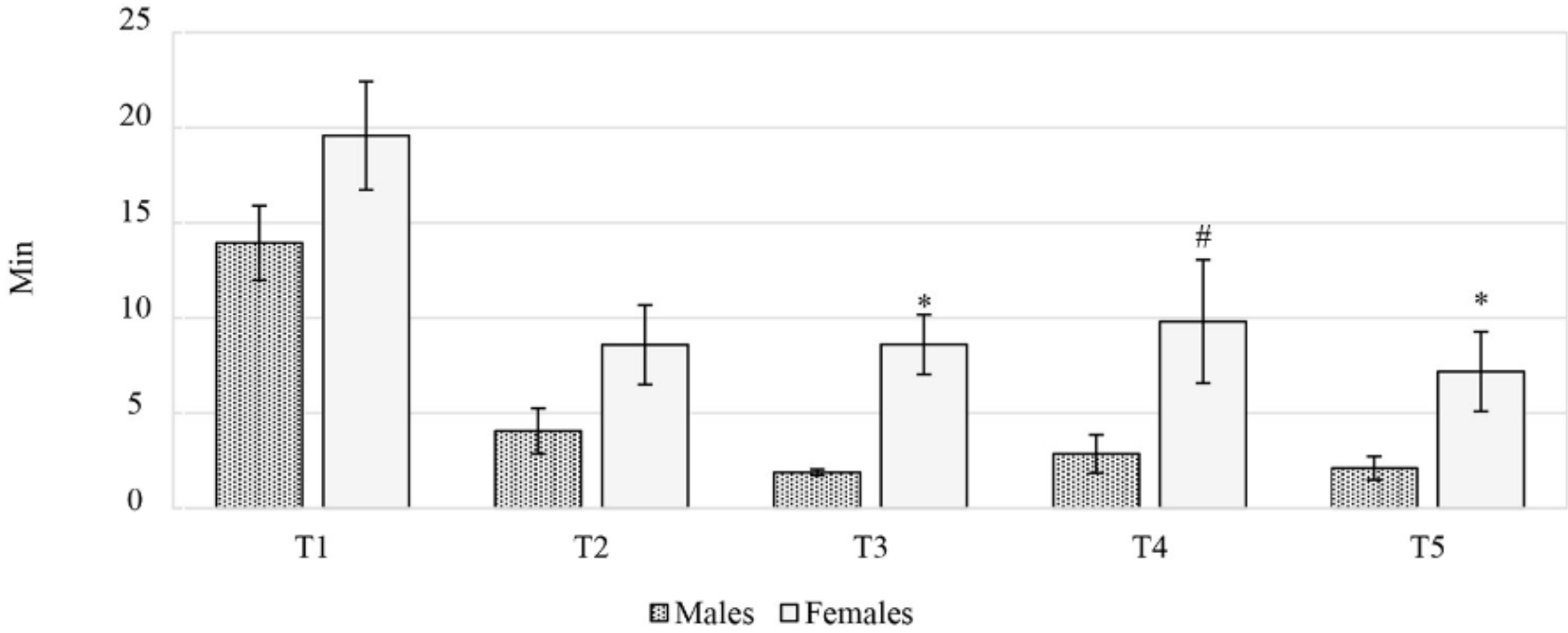
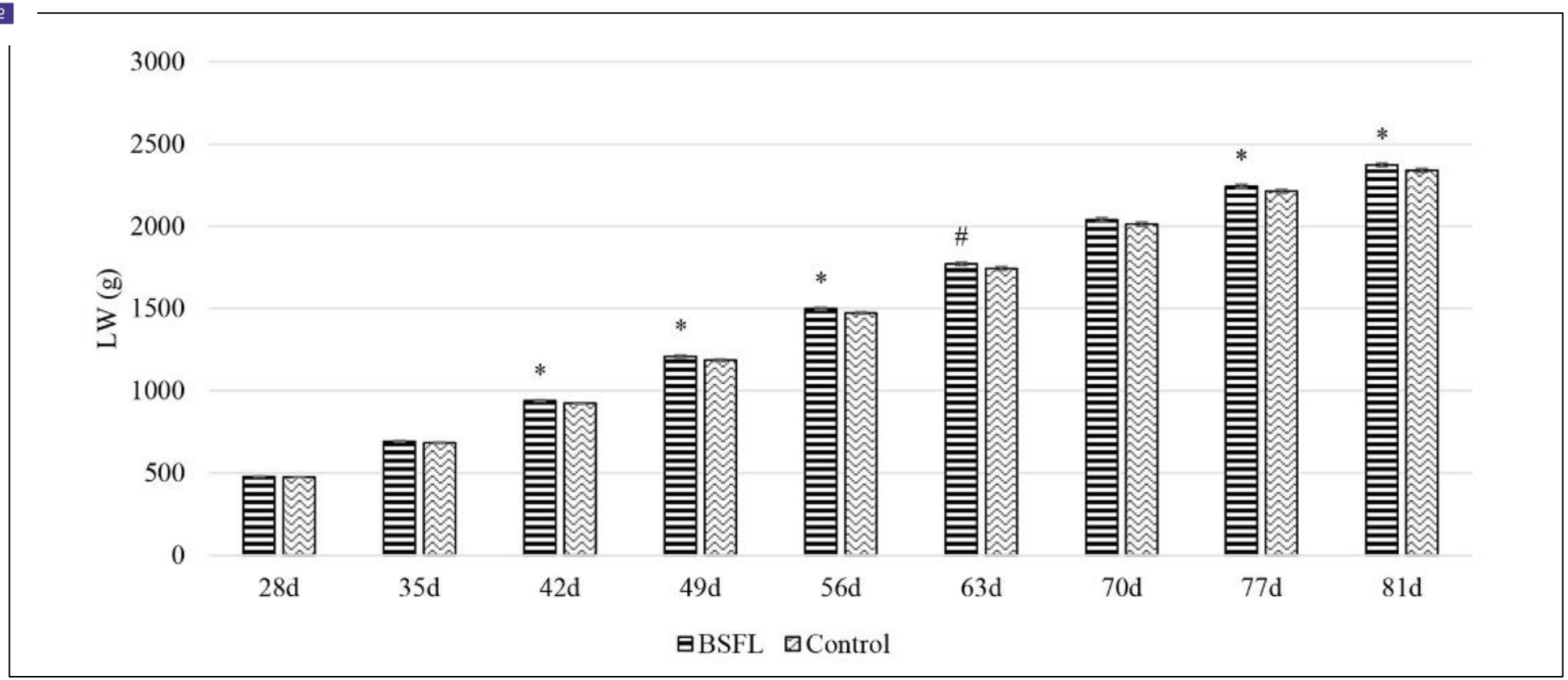



FIGURE 3
The time spent by the Label Rouge Naked Neck birds on eating live black soldier fly larvae ($n = 10$). *$P \leq 0.05$; #$P \leq 0.10$. T1, 28–39 days of age; T2, 40–50 days of age; T3, 51–62 days of age; T4, 63–74 days of age; T5, 75–81 days of age.

BSFL Control

10% BSF 1 time/day

Bongiorno et al., 2022




BSFL Control

10% BSF 1 time/day

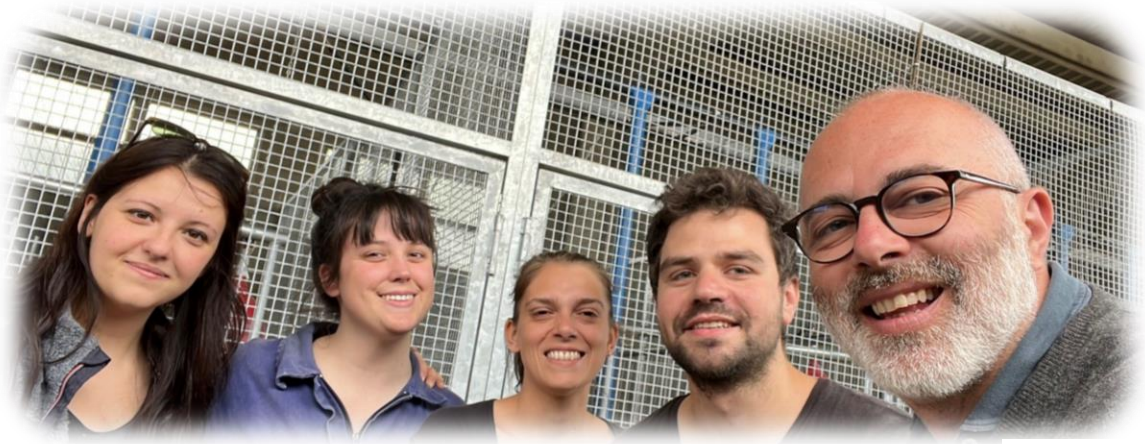
Bongiorno et al., 2022

## PS2 - Nutritional intervention for climate changes

*PS2-011* - **Growth and slaughtering performance of a local chicken breed fed dried and live Black soldier fly larvae as environmental enrichment**

E. Fiorilla, M. Gariglio, V. Bongiorno, E.E. Cappone, V. Zambotto, F. Gai, J. Cortes, C. Coudron, I. Biasato, A. Schiavone


- C
- P → Alternative protein sources
- PE → P+ 4% DM drehydrated BSFL, 1 time/day
- PL → P+ 4% DM live BSFL, 1 time/day



23rd European Symposium on Poultry Nutrition

ESPN 2023

Fiorilla et al., 2023

Piedmont


# Whole larvae in autochthonous chicken breeds

Biondа Piemontese

Millefiori Piemontese

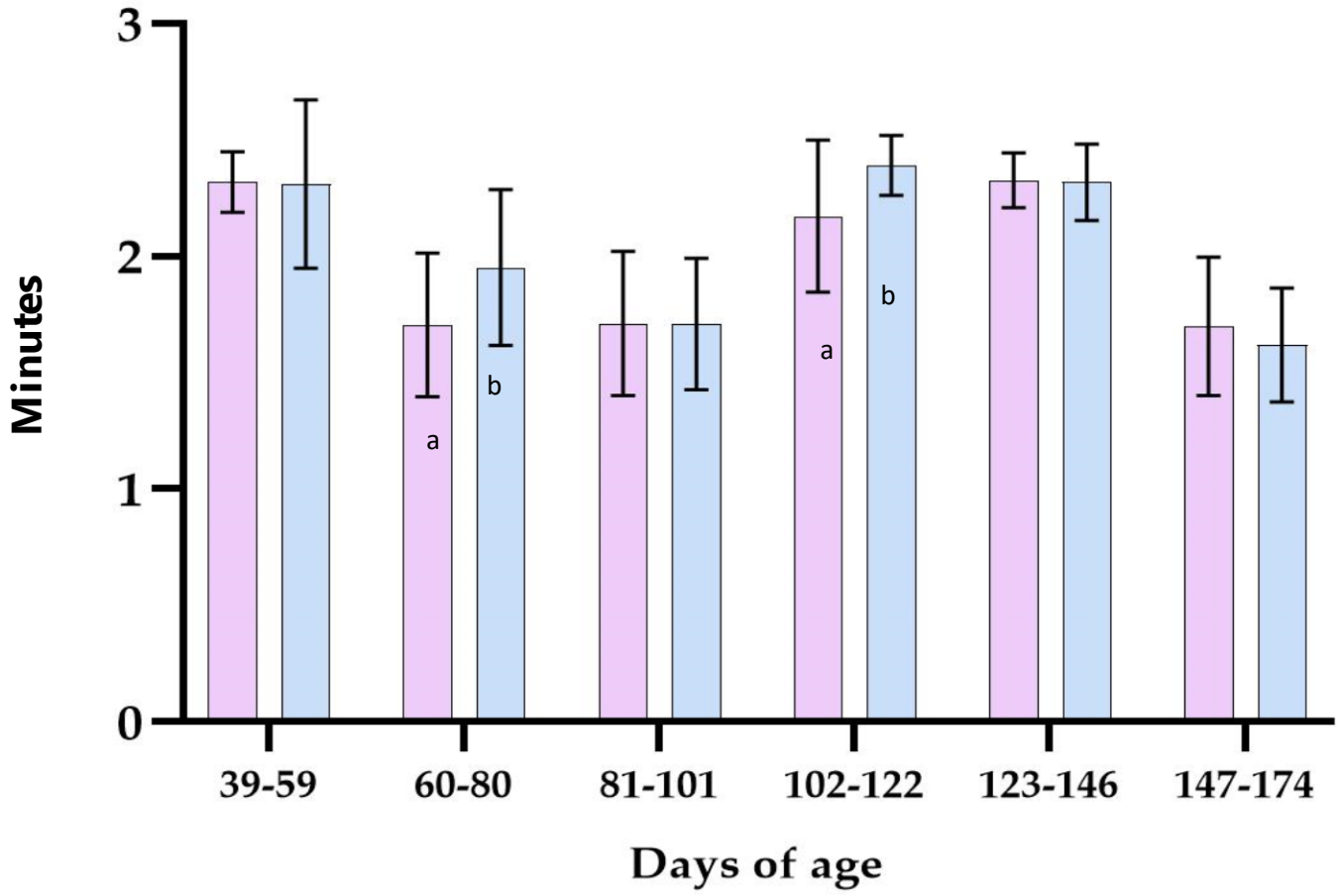
Bianca di Saluzzo

Slow Food®

Fiorilla et al., 2023

POULTRYNSECT

# Larvae **consumption** time




PE — P + 4% DM dehydrated BSFL

PL — P+ 4% DM live BSFL

Fiorilla et al., 2023

# 5. WHOLE INSECT LARVAE in OTHER AVIAN SPECIES

| | | BIRD'S AGE (Days) | INSECT INCLUSION | INSECT DISTRIBUTION | EFFECTS ON BIRD |
|---|---|---|---|---|---|
|  | BSF | 0 to 35 | 10 % DFI | Once | Increased daily feed intake and body weight gain; lower feed conversion ratio; reduced aggressive pecking; a tendency of lowered incidence of feather and skin damage (Veldkamp & van Niekerk 2019) |
|  | Dried maggot | 308 to 357 | 50 g | Three times | Preference for cereal grains rather than dried maggots (Traore et al. 2020) |
|  | Live BSF<br>Live YMW | 3 to 62 | 5% DFI | Once | Reduced H/ L ratio; reduced fecal corticosterone<br>(Gariglio et al. *2023 in press*) |

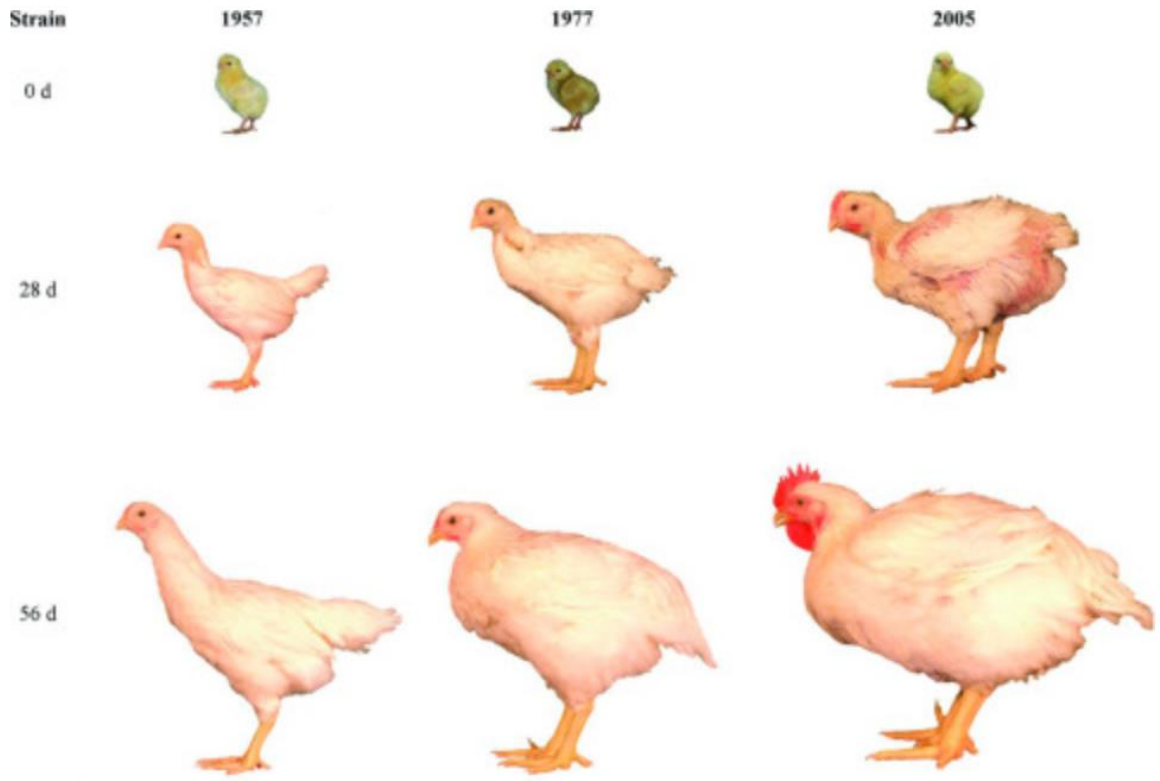
# 6. CONCLUSION AND RECOMENTADTION

# FUTURE PERSPECTIVE

- ✓ POULTRY GUT HEALT
- ✓ DRIED LARVAE THE BEST? (no water transport, easy storage and handling, biosecurity, etc.)
- ✓ TOOLS FOR LARVAE ADMINISTRATION
- ✓ DIGESTIBILITY TEST IN POULTRY
- ✓ POULTRY PRODUCT QUALITY
- ✓ WELFARE RELATED TO AVIAN GENOTYPE and ADMINISTRATION SYSTEM
- ✓ GAMEBIRDS?

# CONCLUSIONS

- ✓ IN CHICKENS DEHYDRATED/ FROZEN LARVAE ACCEPTABILITY SIMILAR TO LIVE LARVAE
- ✓ WHOLE LARVAE STIMULATES BROILER CHICKEN ACTIVITY
- ✓ WHOLE LARVE IMPROVE RELATIONSHIP BETWEEN HUMANS AND CHICKENS
- ✓ WHOLE LARVAE PROMISING TO IMPROVE AVIAN BEAHVIOUR and WELFARE




# THE BROILER CHICKEN IS STILL A BIRD!

# POULTRYNSECT

## Animal welfare: the live insect larvae tool

*Valentina Bongiorno, PhD student (UNITO, Italy)*


Consiglio Nazionale delle Ricerche
**(Coordinator)**

## Final Symposium
## Rome, 27th of October 2023

POULTRYNSECT FINAL MEETING
ROME 2023

# WELFARE ANALYSES

## First trial

# The «why?»

From the knowledge acquired…

- Better plumage condition of laying hens
- Reduced broiler chickens' fear and increased foraging behavior

(Star et al., 2020; Ipema et al., 2020ab; Biasato et al., 2022)

# The «why?»

…to the research question:

can the live black soldier fly larvae improve the welfare of medium-growing chickens?

(van de Weerd et al., 2009; Riber et al., 2018)

# The «why?»

…to the research question:

can the live black soldier fly larvae improve the welfare of medium-growing chickens?

Consumers' empowerment

in sustainable production

Organic production

≠

ensured welfare

(van de Weerd et al., 2009; Riber et al., 2018)

# Materials and methods: chicken reared

Hubbard
JA57 hybrid

Medium

growing

hybrid

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

+10% BSFL provision based on DFI*

DFI: daily feed intake
BSFL: black soldier fly larvae

CF: control female

LF: larva female

+10% BSFL provision based on DFI*

# Materials and methods:

## ethological tests and animal-based welfare measurements

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

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

*Parameters evaluated*

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

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





→ 29d = start live BSFL provision

Days of age

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

(Dabbou et al., 2022; Welfare Quality®, 2009; Costa et al., 2016; Palme et al., 2013; Campbell, 1995; Salamano et al., 2010)

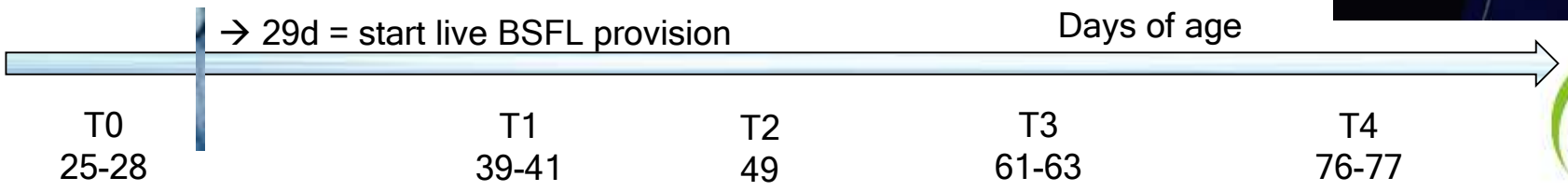
# Materials and methods: behavioral observations

- 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)

BORIS

(Friard and Gamba, 2016)

→




(Veldkamp, T., van Niekerk, T. G. C. M., 2019)

# Materials and methods:

## ethogram of specific behavior repertoire and activity of chickens

| Clas | Denomination | Description | References |
|---|---|---|---|
| Foraging related behaviors | 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) |
| Comfort behaviors | Preening | Self-feather grooming by beak | (McCowan et al., 2006) |
| Activity Behaviors | 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 | - |
| Social behaviors | Sparring | Play fighting | (Veldkamp and van Niekerk, 2019) |
| | Chasing | Running after a conspecific | (Biasato et al., 2022) |
| | Pecking conspecifics | Pecking movements directed at a pen mate | (McCowan et al., 2006) |
| | Allopreening | Social preening | (Kenny et al., 2017) |

# Materials and methods: statistical analyses

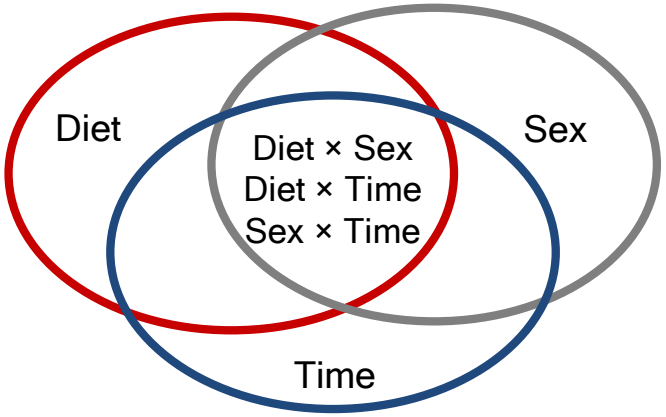
SPSS software

General Linear Mixed Model (GLMM)

↓

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

↓




Spearman correlation

↓

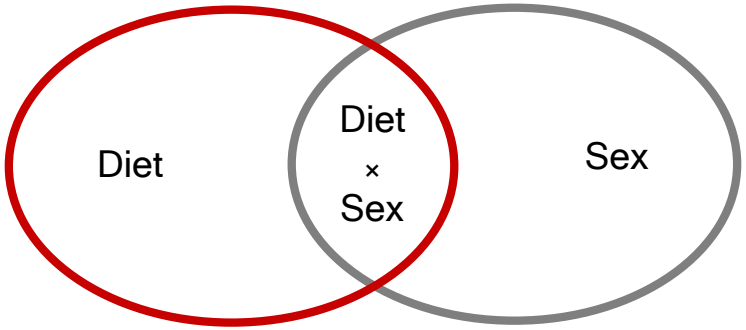
- Excreta corticosterone metabolites
- Tonic immobility duration.

General Linear Model (GLM)

↓

-Heterophile lymphocite 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:

## 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 ≠ commercial housing conditions

- Better leg health (Hall, 2001)
- Diminished lameness (Dawkins et al., 2004)
- Enhanced feather condition (van Hierden, 2003)

**Higher number of birds**

↓

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

of the live larvae provision both

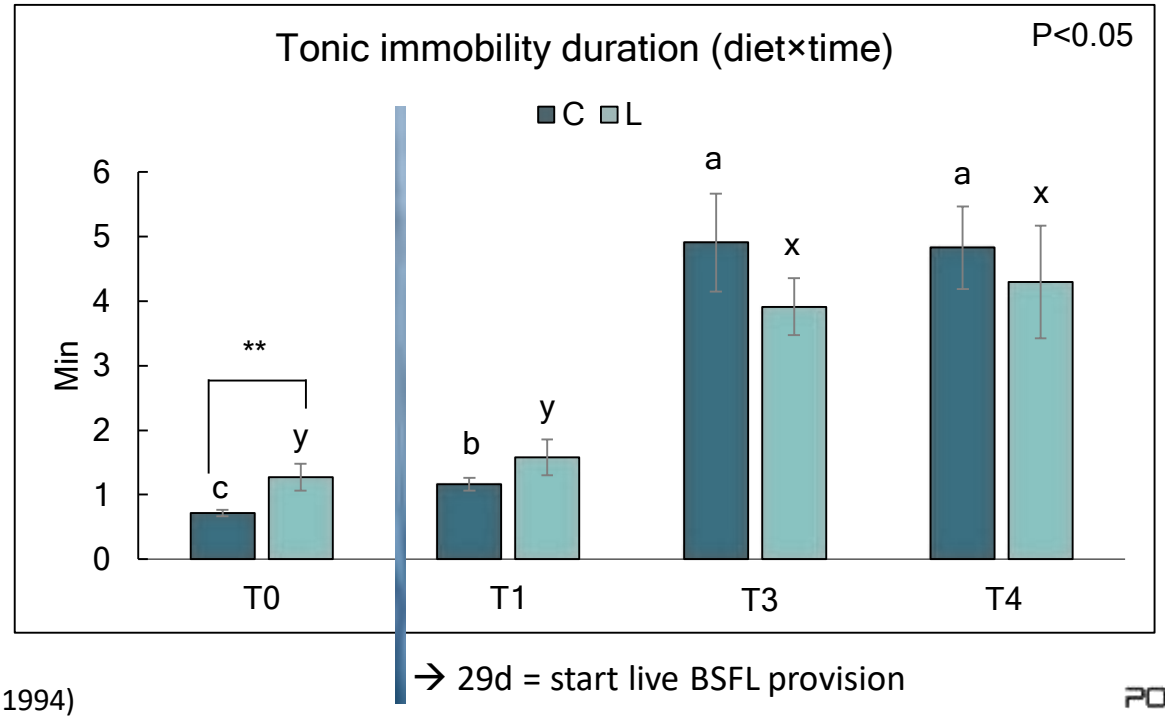
on the tonic immobility and excreta

corticosterone metabolites

- No significant correlation

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

Higher weight and the reduced activity level of adult birds

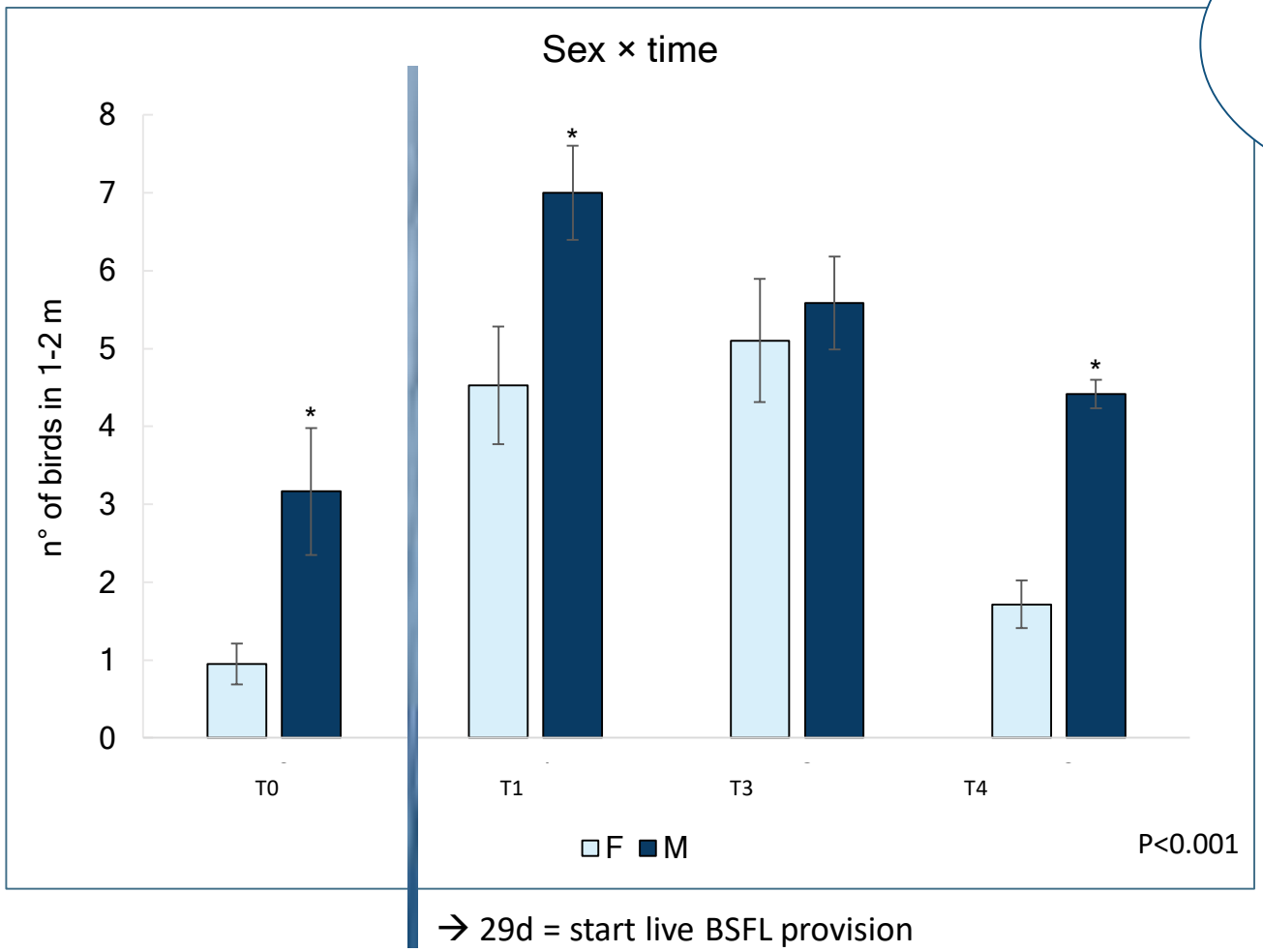



(Campo and Carnicer, 1993; Brake et al., 1994)

# Results and discussion:

## ethological tests and animal-based welfare measurements

*Avoidance distance test*




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

- major boldness of males than females
- Hens' responsibility for brooding and offspring protection

↓

major prudence than males

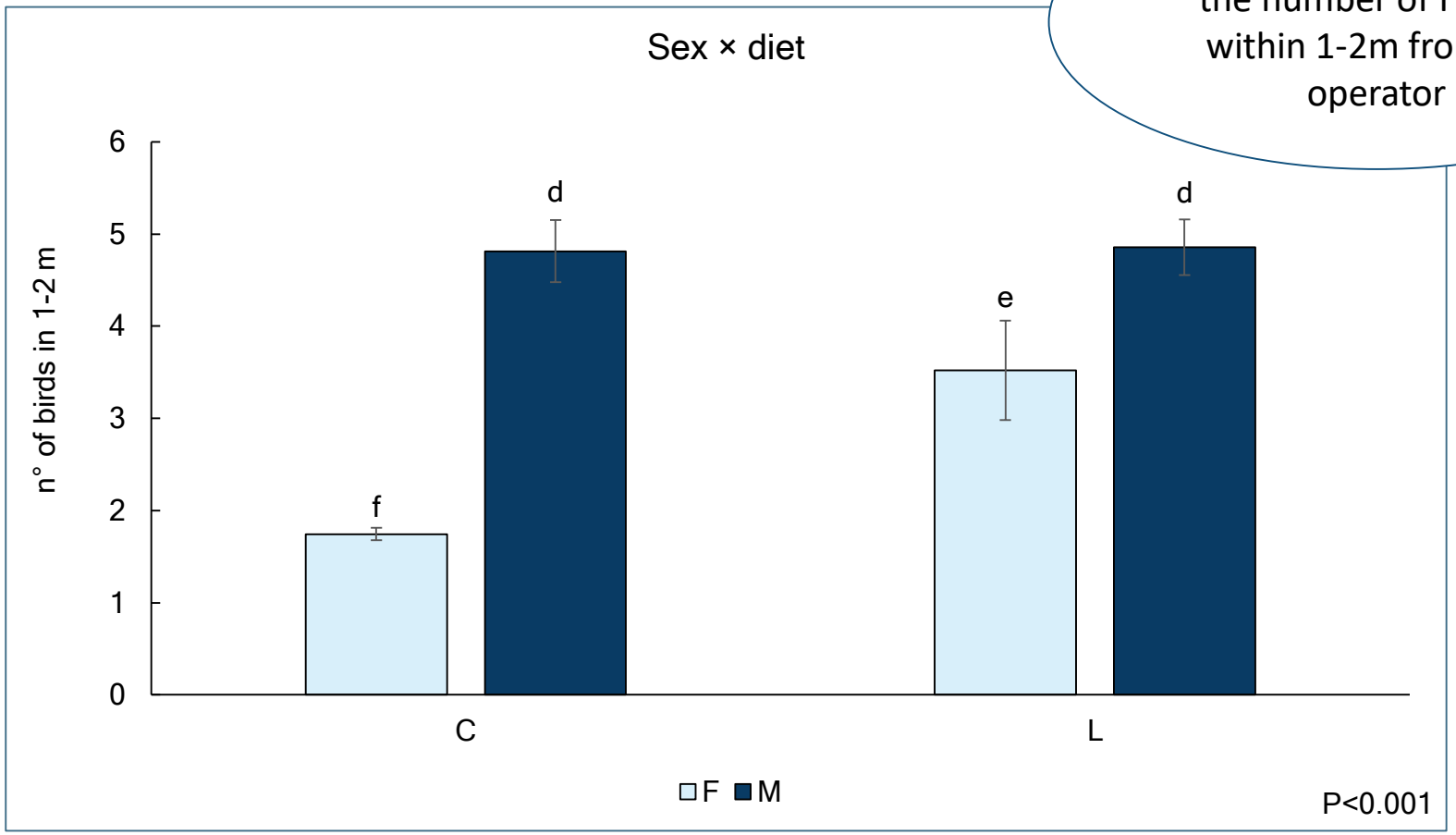
(Collias and Collias, 1967)

# 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

exploration ⟷ fear

Reduction of F fear related to the larvae provision

↓

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

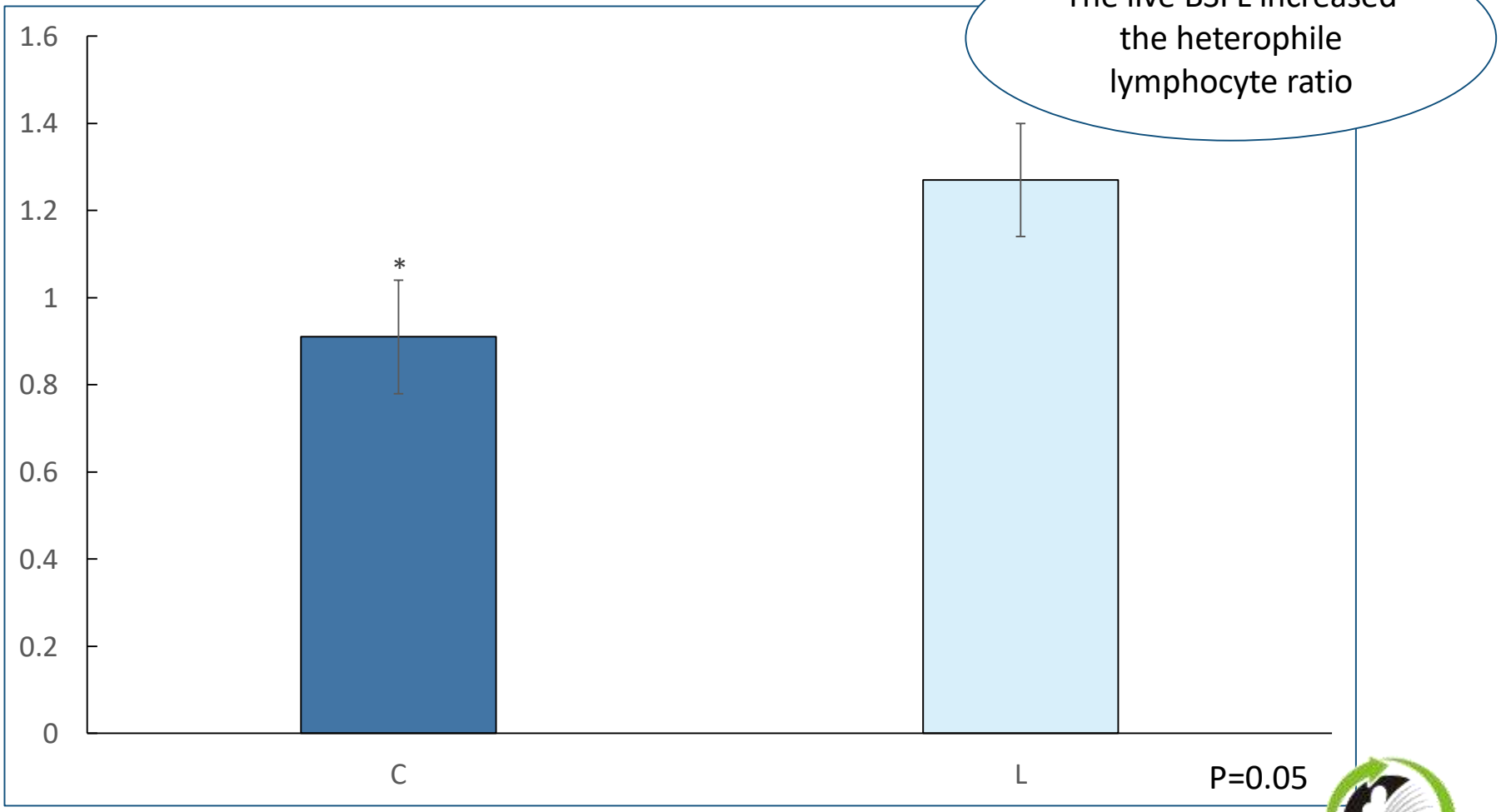
| Generalized fear towards humans? | Capacity of the birds to associate humans to the larvae provision? |
|---|---|

Adaptation of the test based on my research question

# Results and discussion:

## ethological tests and animal-based welfare measurements

*Heterophile lymphocyte ratio*




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

# Results and discussion:

## ethological tests and animal-based welfare measurements

Heterophile lymphocyte ratio

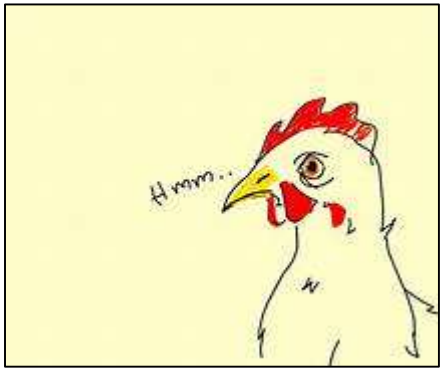
Absence of chickens' exposure to intense and prolonged stress conditions

What about the competion for the larvae access?

Heterophile lymphocyte ratio variation among strains

Results obtained might not be directly related to a negative bird experience

What about the anticipatory behavior??

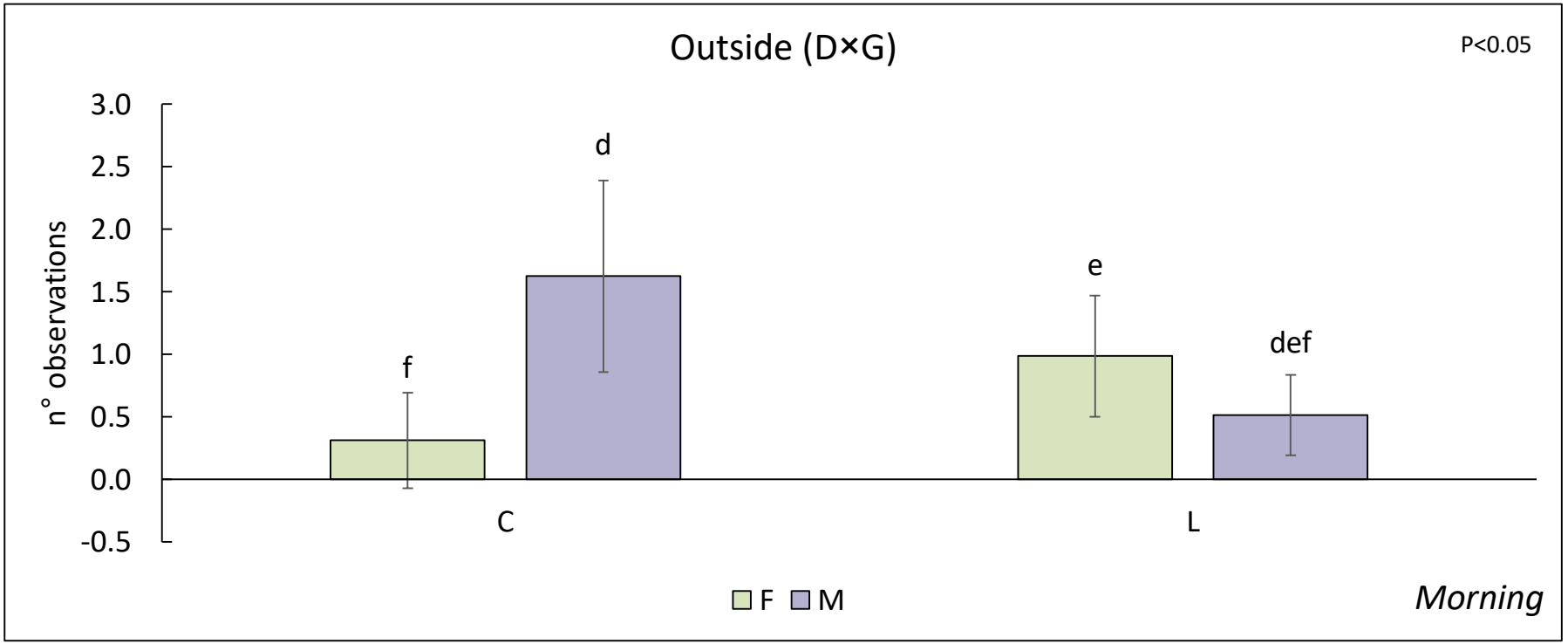



(Bongiorno et al., 2023 under revision)

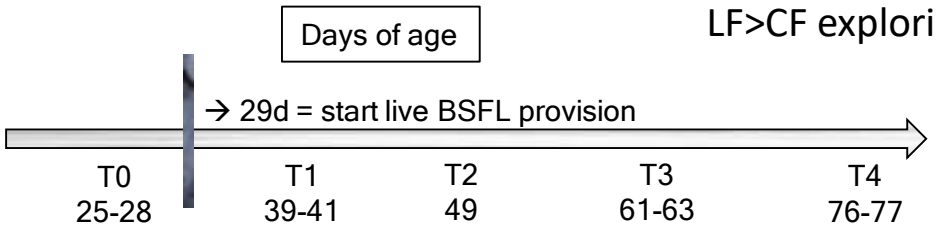
# Results and discussion:

## ethological tests and animal-based welfare measurements

*Video recordings*




LF>CF exploring the outside

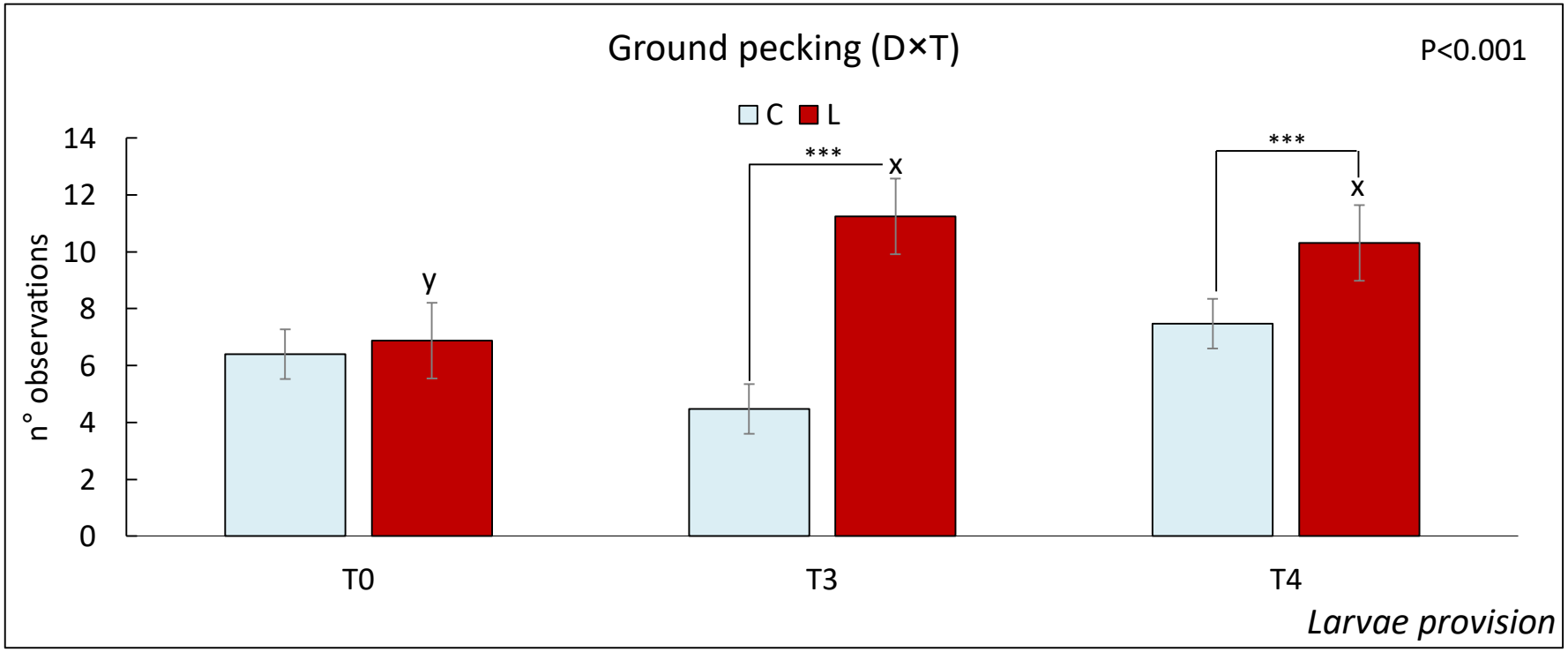



(Bongiorno et al., 2023 under revision)

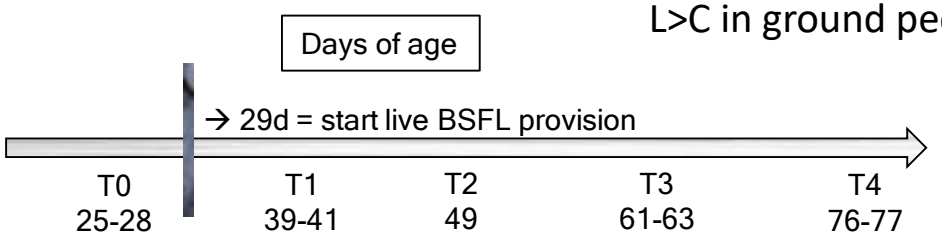
# Results and discussion:

## ethological tests and animal-based welfare measurements

*Video recordings*




L>C in ground pecking at T3 and T4

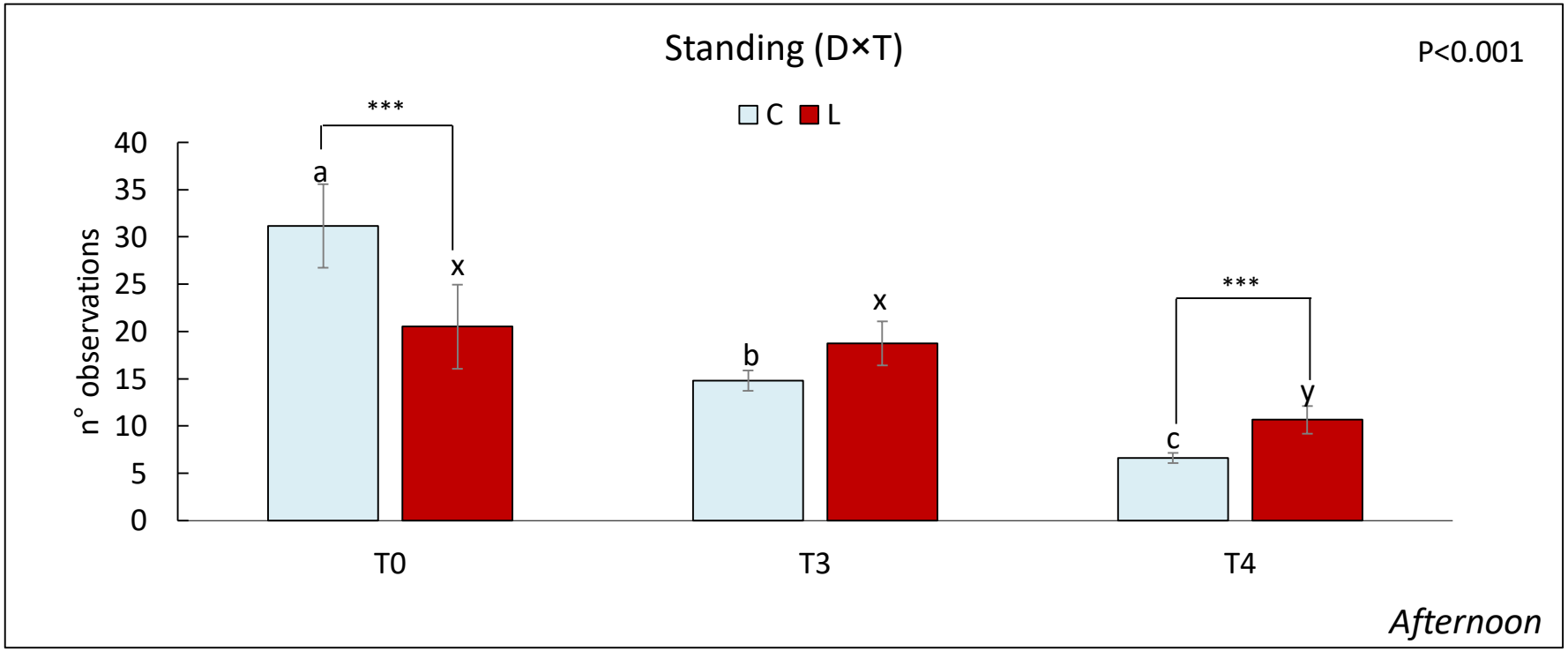



(Bongiorno et al., 2023 under revision)

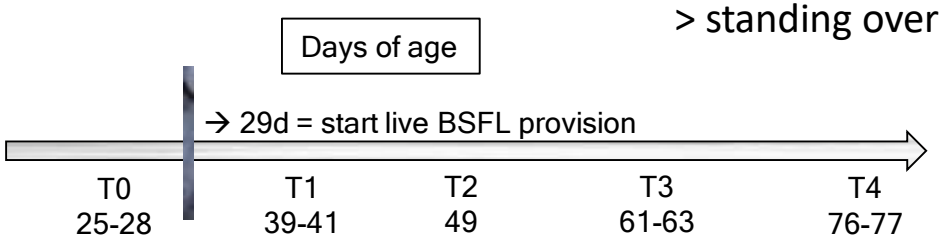
# Results and discussion:

## ethological tests and animal-based welfare measurements

*Video recordings*




> standing over time in L groups




(Bongiorno et al., 2023 under revision)

# Results and discussion:

## ethological tests and animal-based welfare measurements

Behavioral analysis

- Increased exploration behavior in females (avoidance distance test + video recordings)

=

benefits in animal management

- Increased foraging behavior

=

over time stimulation of natural behavioral repertoire

- Increased standing behavior

=

maintainance of birds' activity over aging

(Bongiorno et al., 2023 under revision)

# Take home messages

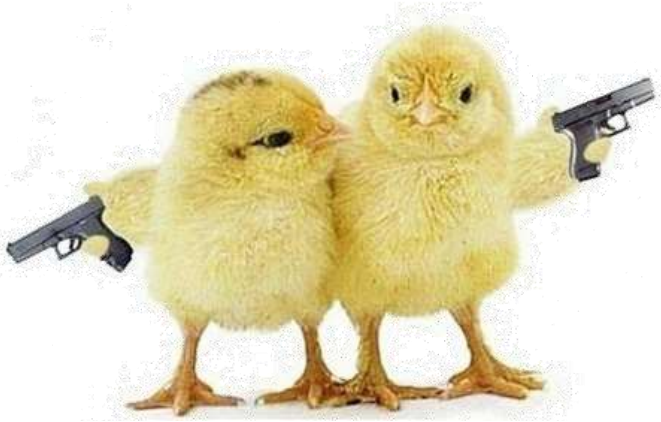
- No negative implications related to birds' feather, leg and foot condition, and skin damage
- No significant effect on the tonic immobility and corticosterone level
- Advantages in fear reduction, denoted especially in F birds
- Higher heterophile lymphocyte ratio in L birds than C ones

→ competition based stress or anticipatory behavior

? Open question

# WELFARE ANALYSES

## Second trial



# Future analyses

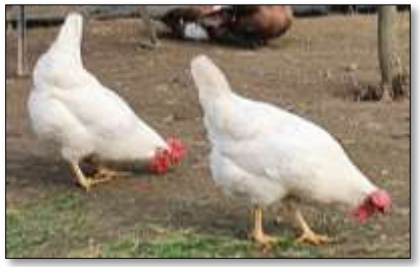
## What about the effect of live larvae on welfare of local chicken breeds?

Bianca di Saluzzo

Slow-growing breed

Growth Cycle 150 days

198 males



Experimental chicken

Eggs incubation

Breeders

Internal breeding

# Future analyses

3 experimental groups; 11 birds/pen;
(6 Replicates; 66 birds/treatment)

C

[C] - Control diet

S

[S] - Sustainable diet

D

[D] - Sustainable diet +
15% live larvae provision
based on the DFI*

| Diet Composition (g/kg) | Control (g/kg) | Sustainable diet (g/kg) |
|---|---|---|
| Corn meal | 617 | 461 |
| Soybean meal | 320 | |
| Field bean meal | | 110 |
| Pea meal | | 108 |
| Barley meal | | 47 |
| Sunflower meal | | 95 |
| Corn gluten meal | | 116 |
| Soybean oil | 20 | 16 |
| Dicalcium phosphate | 13.5 | 13.5 |
| Calcium carbonate | 19 | 20 |
| Sodium chloride | 1.5 | 1.5 |
| Sodium bicarbonate | 1.4 | 1.4 |
| DL-methionine | 1.7 | 0.7 |
| L-lysine | | 4 |
| Vitamin and mineral starter/grower premixA | 5.9 | 5.9 |
| TOTAL | 1000 | 1000 |

*DFI: daily feed intake

# Ongoing analyses

Heterophile lymphocyte ratio

plumage, skin lesions, hock-burn, footpad dermatitis scores

Excreta corticosterone

Video recordings

Tonic immobility test

Avoidance distance test

# POULTRYNSECT

## Poultry gut microbiota: the influence of live insect larvae administration

*Ilaria Biasato, DVM, PhD (UNITO, Italy)*


Consiglio Nazionale delle Ricerche
**(Coordinator)**

## Final Symposium
## Rome, 27th of October 2023

POULTRYNSECT FINAL MEETING
ROME 2023

# MICROBIOTA

”The assemblage of living microorganisms present in a defined environment”

# METAGENOME

”The collection of genomes and genes from the members of a microbiota”

# MICROBIOME

”The genes and genomes of the gut microbiota, as well as their products and the host environment”

Who is there?

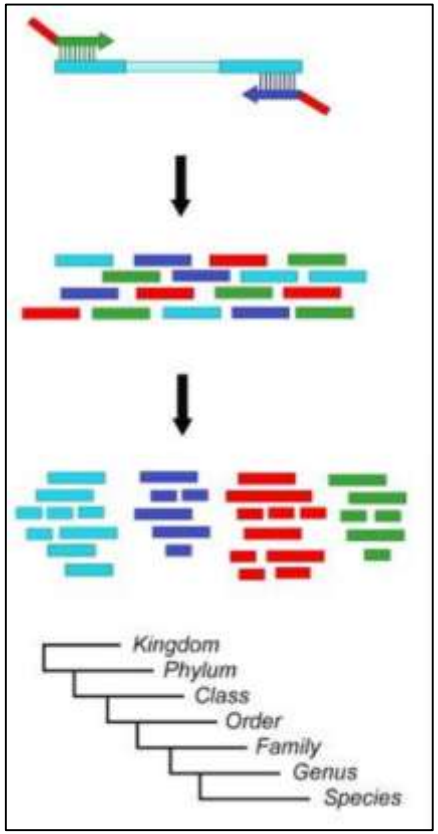
What can they do?

What can they produce?

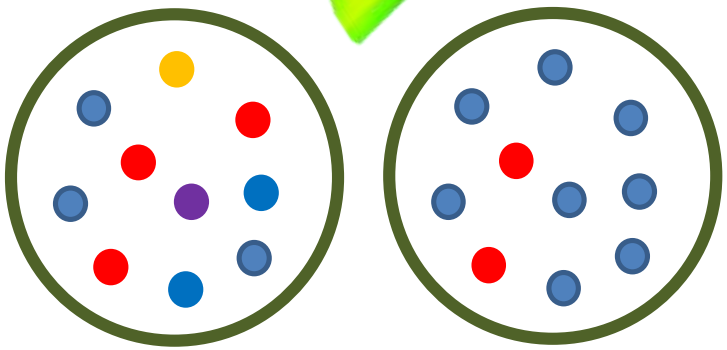
# METABOLOME

”The complete set of small molecule metabolites present within an organism or cell”

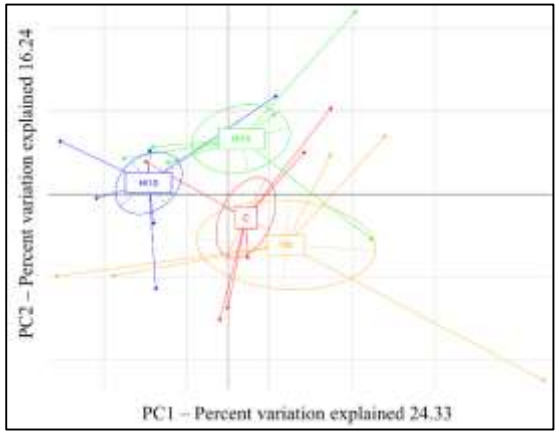
# MICROBIOTA




16S rRNA gene sequencing

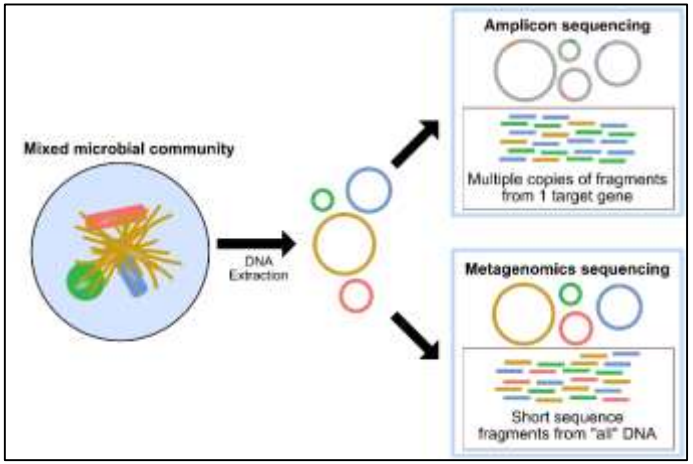


Alpha diversity (Chao1, Simpson and Shannon indices)

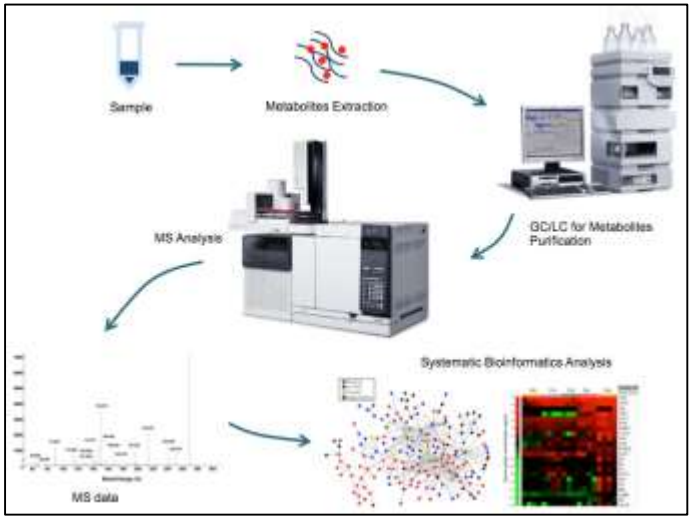



Beta diversity (Bray-Curtis, Jaccard or Unifrac distances visualized by Principal Component Analysis [PCA] or Principal Coordinate Analysis [PCoA])

# METAGENOME




**Shotgun metagenomic sequencing**




# METABOLOME

**Nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (HPLC-MS)**

HOST-RELATED FACTORS

ENVIRONMENT-RELATED FACTORS


Why?

DIET-RELATED FACTORS

GUT HEALTH ☺

Increased microbial alpha diversity

Selection of short chain fatty acids (SCFAs)-producing bacteria (chitin degradation)

INSECT-BASED FEEDS

Reduction of pathogenic bacteria (lauric acid, AMPs or chitin)

Reduction of alpha diversity and potentially beneficial bacteria + selection of pathogenic bacteria (high inclusion levels)

..but very limited information about live larvae ☺



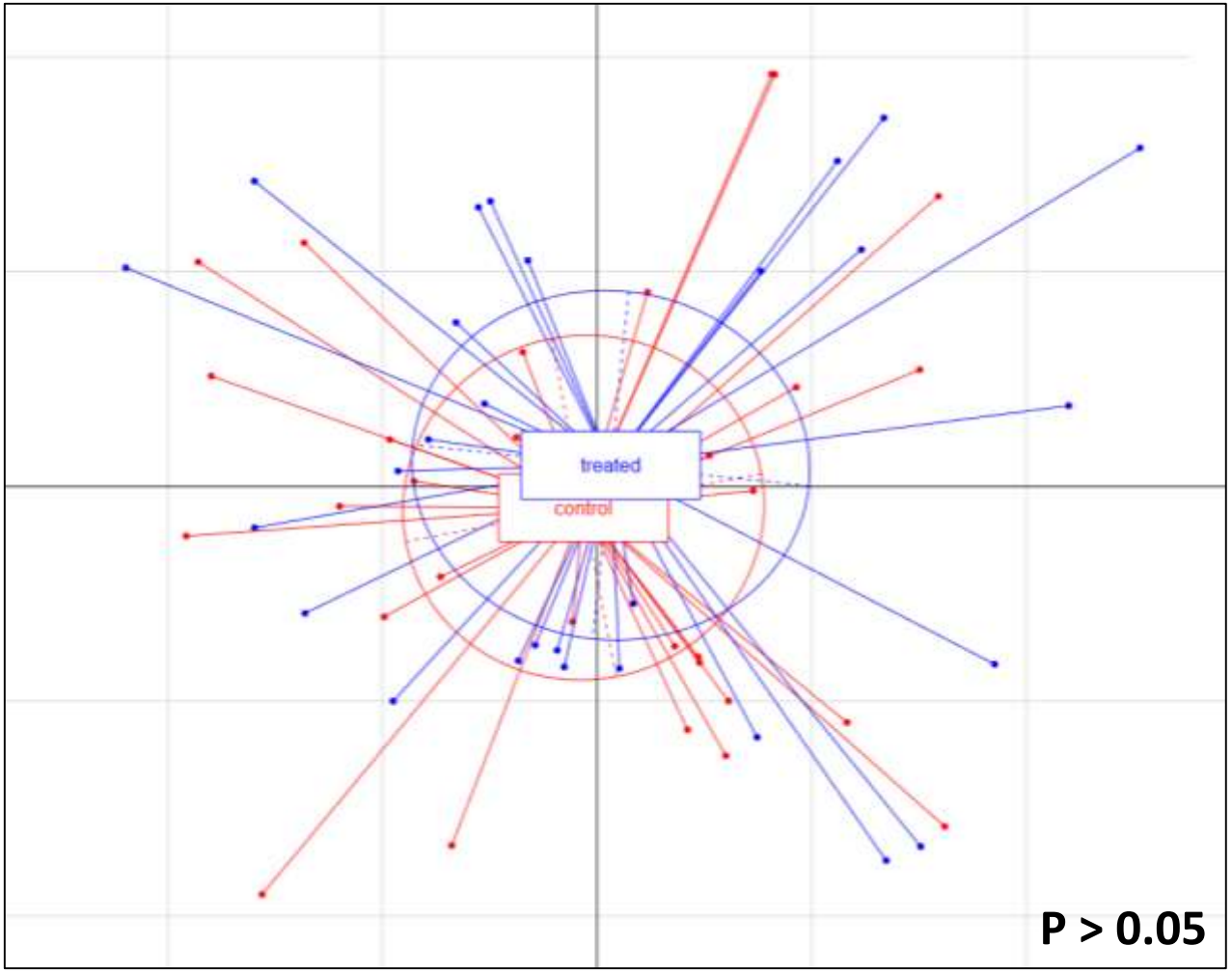
**Influence of live BSF larvae on gut microbiota of medium-growing chickens and autochthonous, dual-purpose Bianca di Saluzzo**

**10% of expected DFI vs control diet (male and female Label Rouge Naked Neck chickens)**

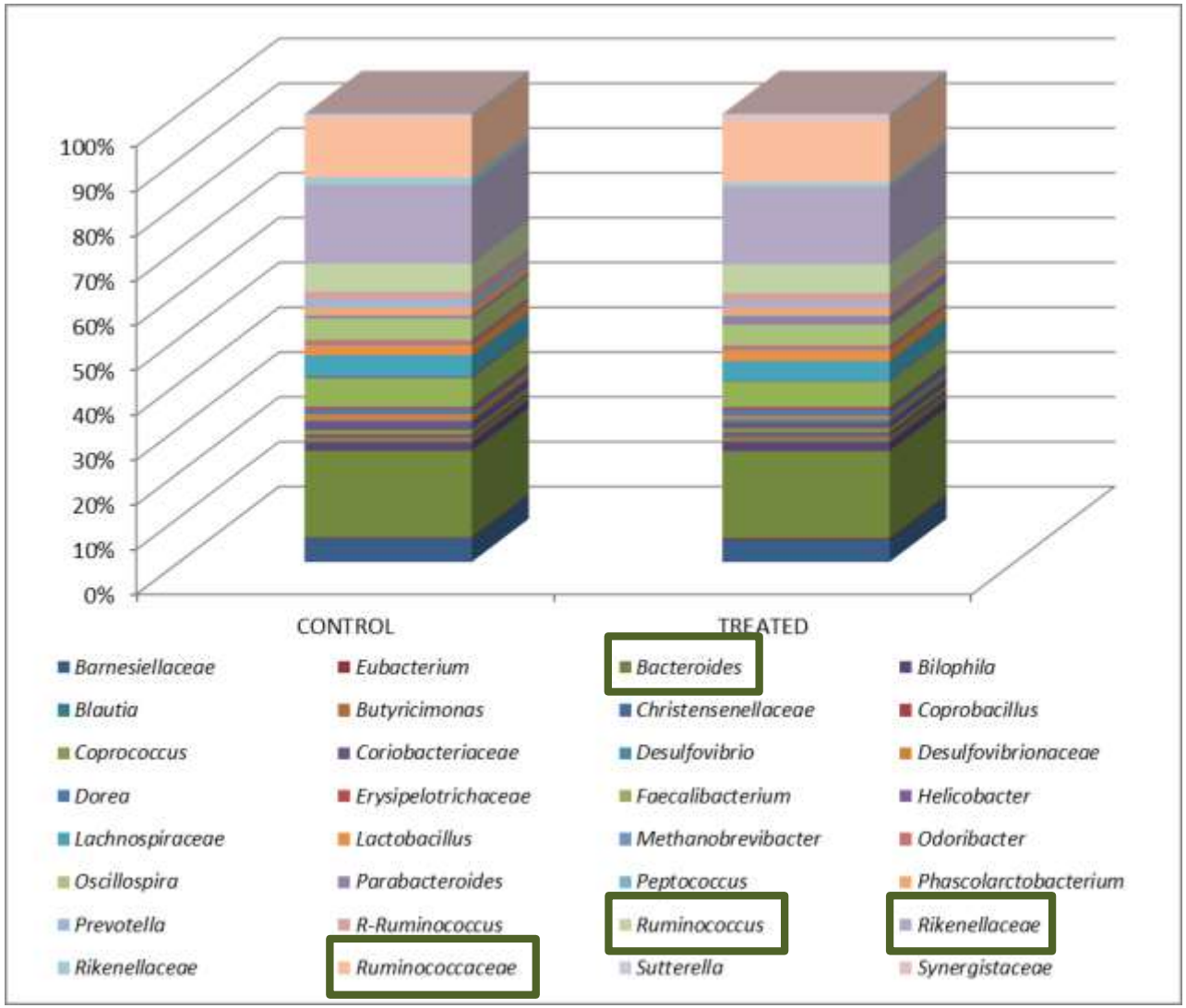
1

**15% of expected DFI vs two control diets (commercial and sustainable) and 5% of expected DFI of dry larvae (male Bianca di Saluzzo)**

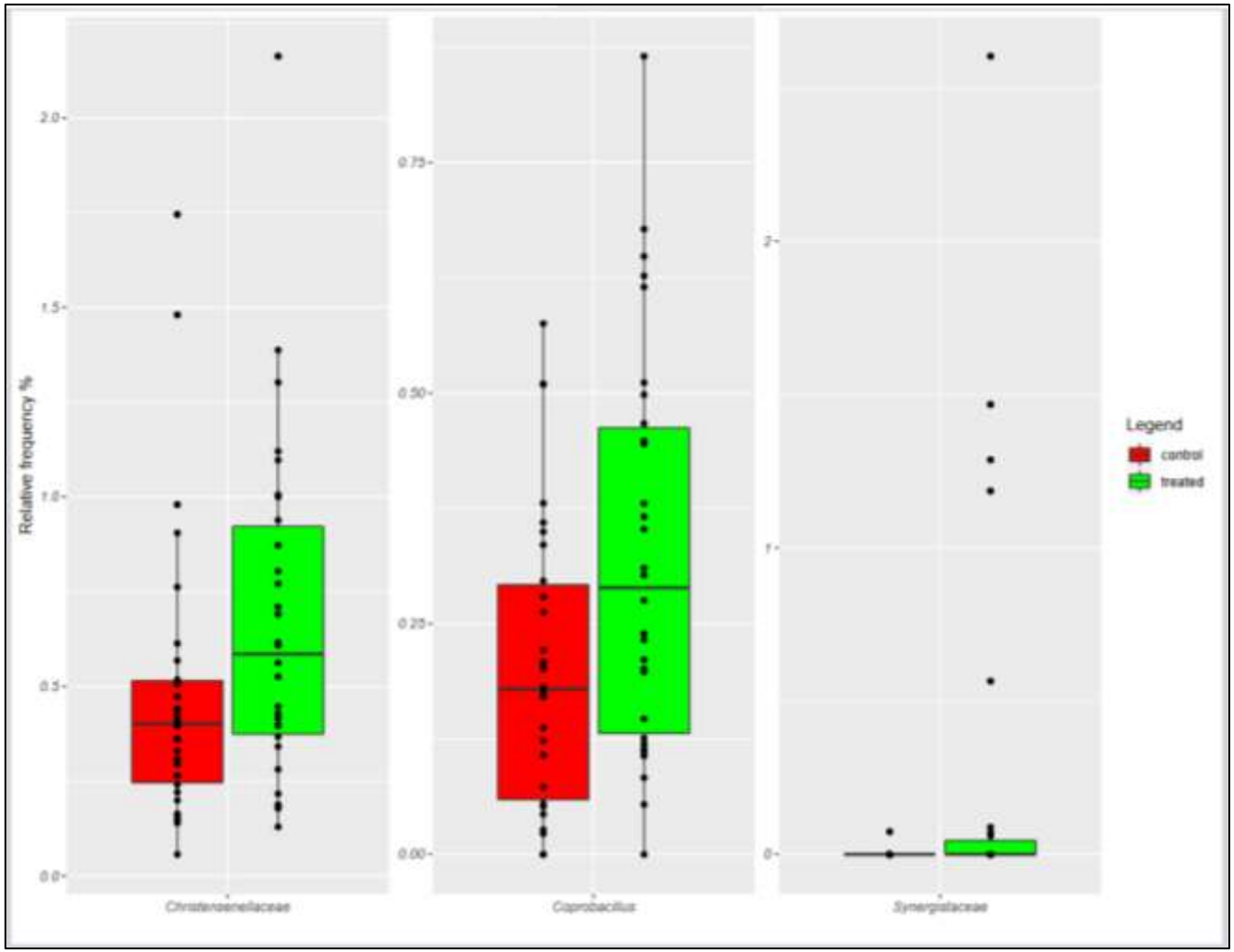
2



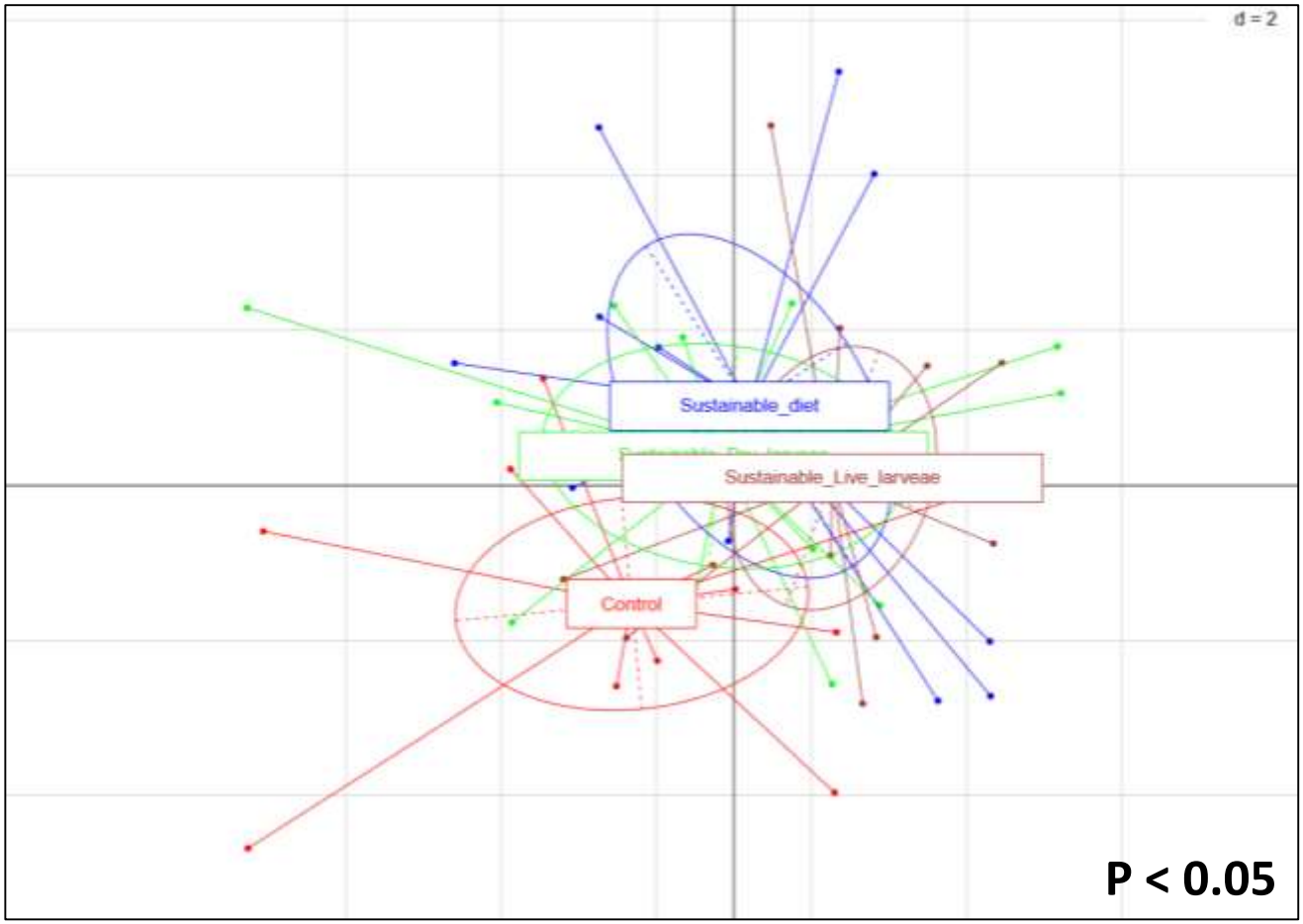

**Live BSF larvae have high water content (70-75%) ☺**



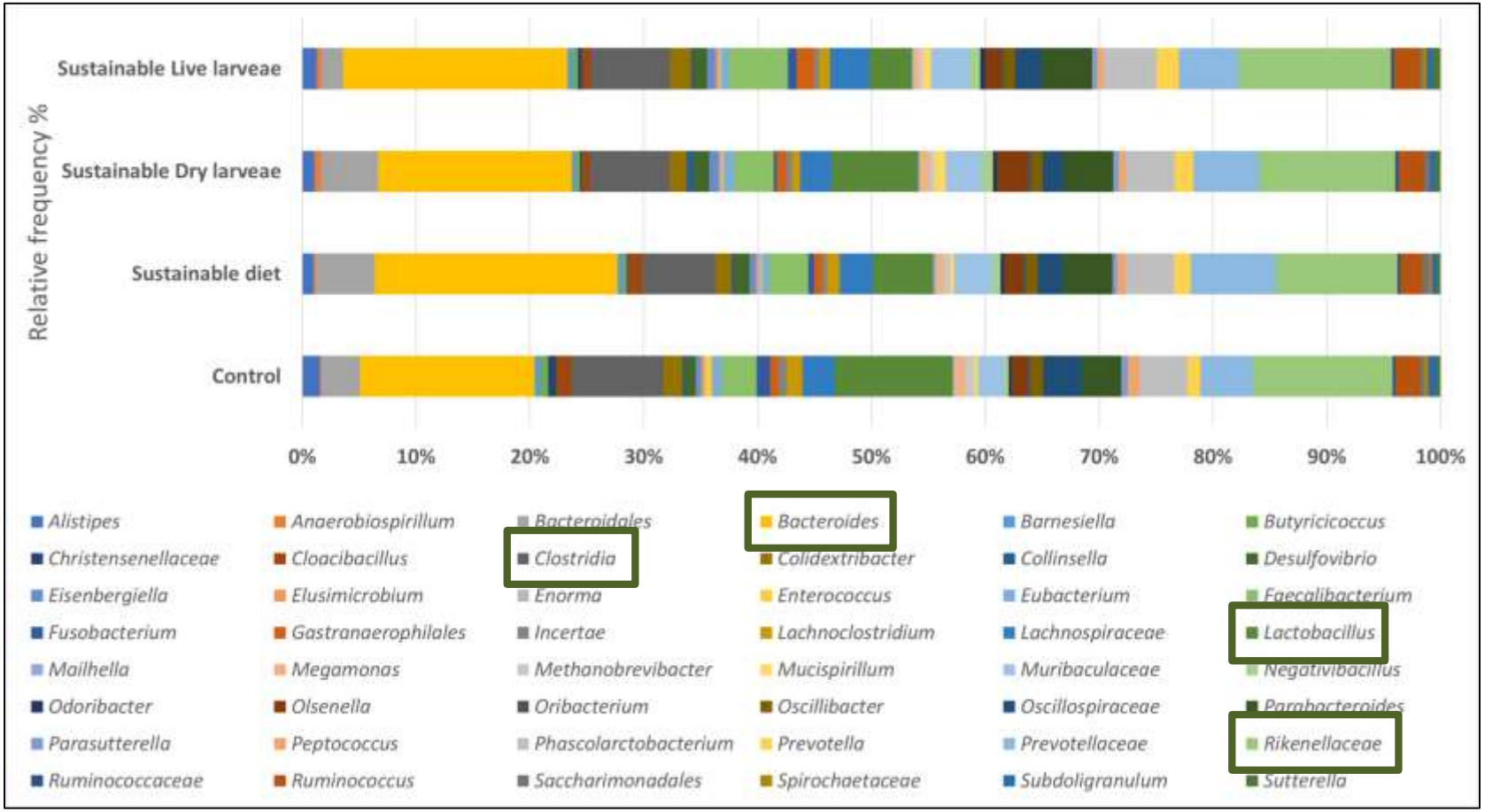

**Preservation of physiological caecal microbiota ☺**



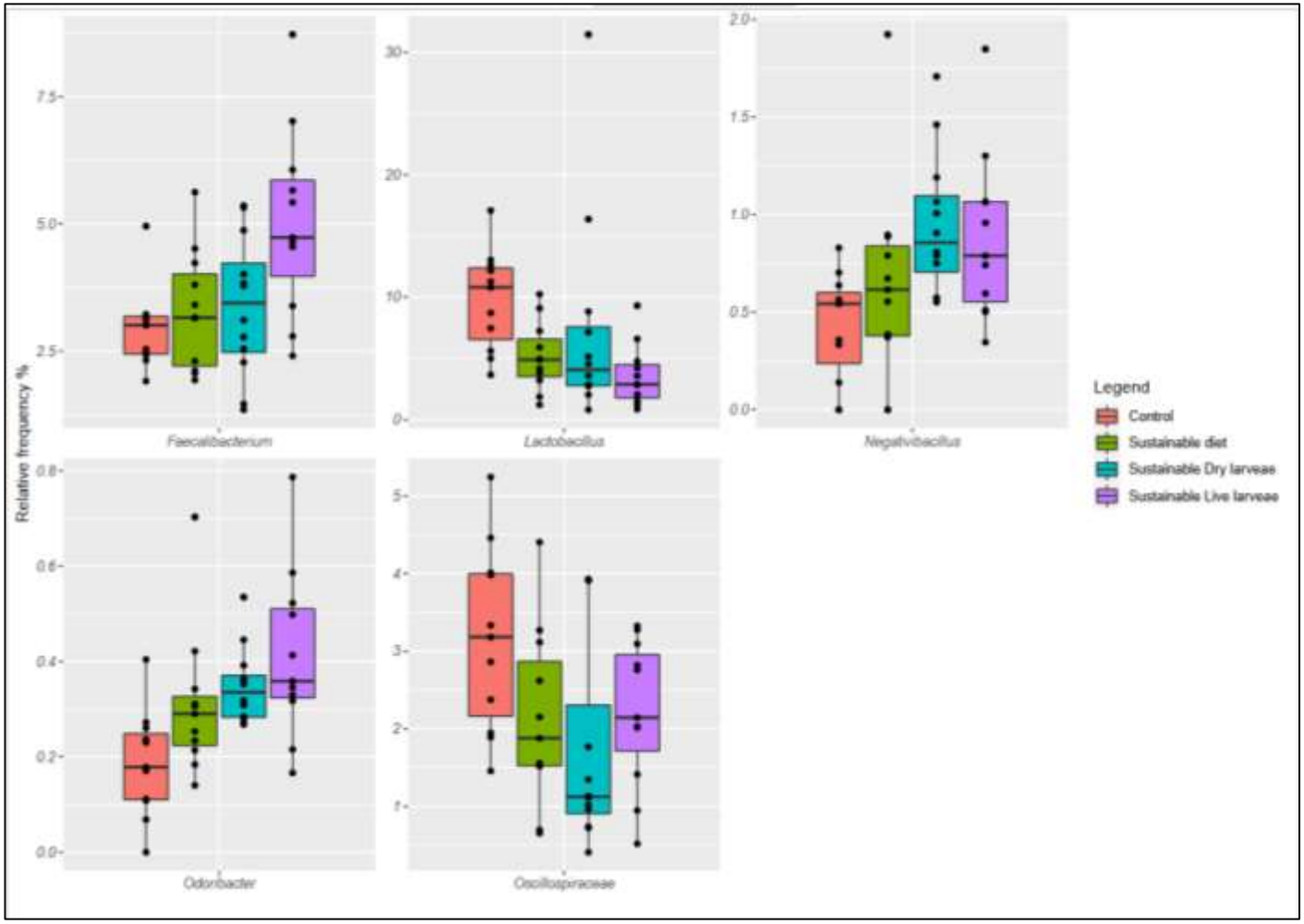

**Positive modulation of the minor ASVs fraction (<1%) ☺**




**..higher supplementation level (10 vs 15%) or different breed response? ☺**




**Preservation of physiological caecal microbiota ☺**




**Selection of specific SCFAs-producing bacteria at the expense of others** 🤔

2

**Live BSF larvae supplementation may positively modulate the poultry gut microbiota**

1

**Live BSF larvae supplementation does not alter the physiological caecal microbiota of medium-growing and dual-purpose chickens**

3

**Use of live BSF larvae as feed ingredient will potentially generate more pronounced effects on poultry gut microbiota ☺**


ilaria.biasato@unito.it
+390116708570 / +393489164702

**FOR MORE INFORMATION ABOUT POULTRYNSECT PROJECT FOLLOW US ON:**

# INFO & CONTACTS

https://poultrynsect.eu/

https://susfood-db-era.net/main/Poultrynsect

**Facebook:**
https://www.facebook.com/Poultrynsect-111484687644519/

**Twitter:**
@poultrynsect