Resource conversion by black soldier fly larvae: towards standardisation of methods & reporting

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EAAP Annual Meeting 2019, Ghent, Belgium | 27 August 2019

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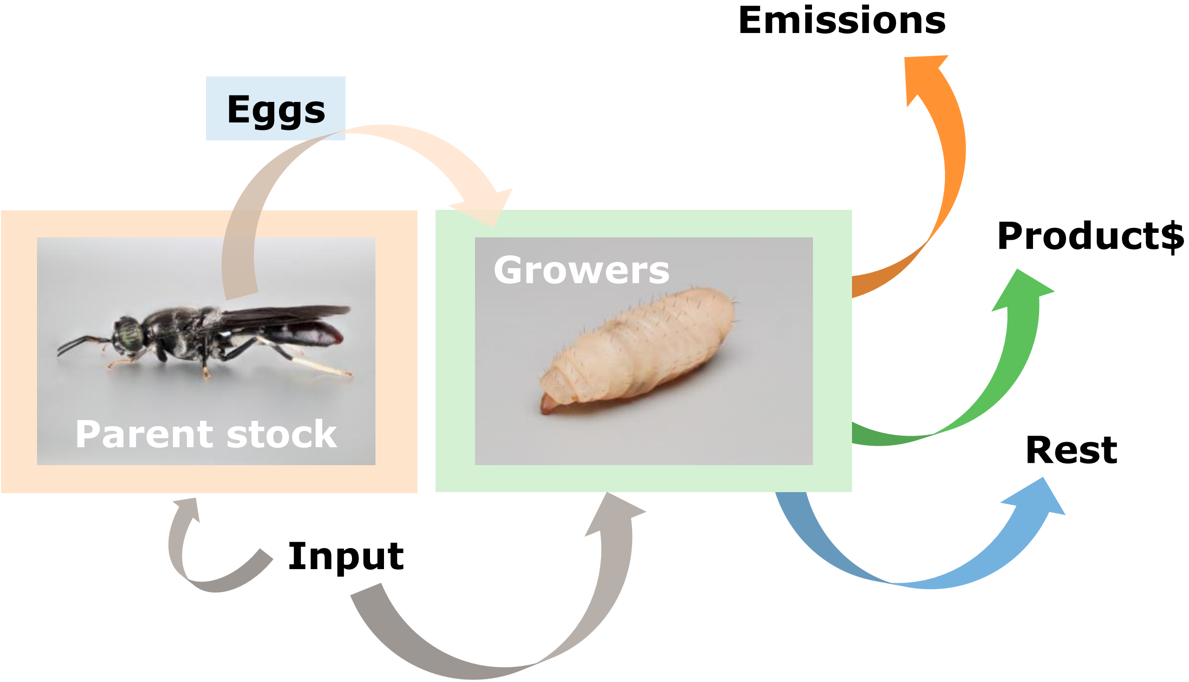
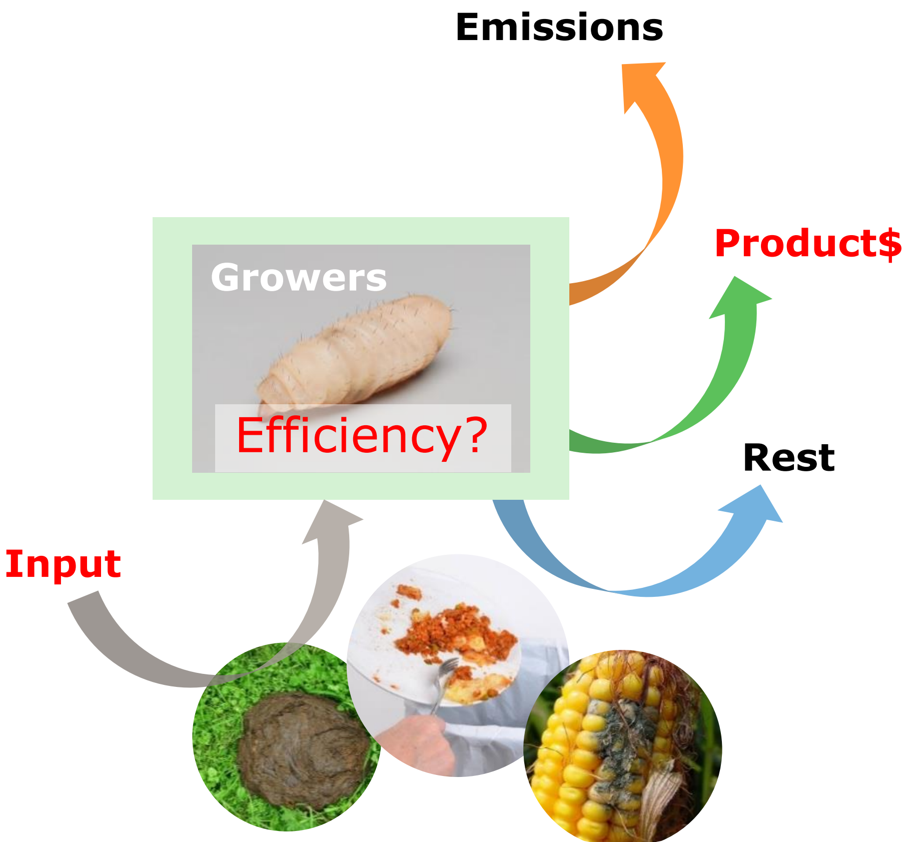
Mini-livestock production system



Mini-livestock production system



Many streams, few studies quantify conversion

Cat	Organic stream	Reference
A	Palm kernel expeller	Hem ea 2008
A	Rice straw	Zheng ea 2012a
A	Pig liver	Nguyen ea 2013; 2015
A	Vegetal (plantain, potato, cabbage) and fruit (banana, papaya) refuse	Parra Paz ea 2015
A	Wheat middlings, DDGS, beet pulp	Tschirner & Simon 2015
A	Rice straw	Manurung ea 2016
A	Fishmeal	Barroso ea 2017
A	Seaweed	Liland ea 2017
A	Brewer's waste	Nyakeri ea 2017
A	Soybean curd residue	Rehman ea 2017b
A	Sorghum, cowpeas	Tinder ea 2017
B1	Fruits and vegetables (grocery stores)	Nguyen ea 2013; 2015
B1	Spent grains, beer yeast, cookie remains, bread remains, potato steam peelings, beet molasses	Oonincx ea 2015a
B1	Fruit and vegetable waste (carrots, peas, salsify, celery), solid fraction from digestate of fermented fruit and vegetable waste (carrots, peas, salsify, celery)	Spranghers ea 2017
C	Fish rendering	Nguyen ea 2013; 2015
D	Municipal organic waste	Diener ea 2011
D	Restaurant waste	Zheng ea 2012a
D	Solid residual fraction of defatted raw waste from Chinese restaurants	Zheng ea 2012b
D	Restaurant waste (animal and plant matter)	Nguyen ea 2013; 2015
D	Dried digestate from biogas production, municipal biodegradable waste, catering waste, food scraps	Kalová & Borkovcová 2013
D	Canteen kitchen food waste (vegetable trimmings, spent coffee grounds, tea leaves), food leftovers from schools, hotels and hospitals	Cheng ea 2017
D	Banana peelings, restaurant food waste	Nyakeri ea 2017
D	Restaurant waste (potatoes, rice, pasta and vegetables)	Spranghers ea 2017
D	School cafeteria food waste	Surendra ea 2016

Cat	Organic stream	Reference
E	Layer hen manure	Sheppard 1983
E	Layer hen manure	Fatchurochim ea 1989
E	Layer hen manure	Sheppard ea 1994
E	Dairy cow manure, hog manure, hen manure	Erickson ea 2004
E	Cow manure with or without homogenised heads, viscera, bony structures from trout	St-Hilaire ea 2007
E	Dairy cow manure	Liu ea 2008
E	Dairy cow manure	Myers ea 2008
E	Cow manure, last month of growth with visceral organs and fat from rainbow trout	Sealey ea 2011
E	Dairy cow manure	Li ea 2011b
E	Cattle manure, pig manure, chicken manure	Li ea 2011a
E	Hen manure	Yu ea 2011
E	Dairy cow manure, pig manure, chicken manure	Zhou ea 2013
E	Pig manure	Nguyen ea 2013; 2015
E	Corncob plus pig manure biogas residue	Li ea 2015
E	Dairy cow manure, pig manure, chicken manure	Oonincx ea 2015b
E	Dairy cow manure, chicken manure	Rehman ea 2017a
E	Hen manure, cow manure	Kalová & Borkovcová 2013
E	Dairy manure	Rehman ea 2017b
F	Coffee berry pulp	Lardé 1990
F	Food scrap residue compost leachate	Popa & Green 2012
F	Waste plant tissues, garden waste, compost tea (garden waste and water)	Kalová & Borkovcová 2013
G	Sewage effluent	Popa & Green 2012
G	Human faeces	Lalander ea 2013
G	Mixture of human faeces, pig manure, dog food	Lalander ea 2014
G	Sludge from treatment of waste water, waste from rain drains	Kalová & Borkovcová 2013
G	Human faeces	Banks ea 2014
G	Human faecal sludge	Nyakeri ea 2017

Articles 40 total | 11 DM | 5 N

78 (mixtures) of streams | 21 DM | 13 N

Bosch *et al.*
Conversion of organic resources by black soldier fly larvae: Legislation, efficiency and environmental impact
Journal of Cleaner Production (2019) 22 | 355-363

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Conversion efficiency studies

Rearing					Harvest	Conv.		Reference
Diet g FM	Larvae #	Age d	Temp C	RH %	Life stage	DM %	N %	
NR	NR	0	31.8	NR	Prepup.	+	-	Diener ea 2011
1249	~1200	10	27	60-75	Prepup.	+	+	Li ea 2011b
1000	1000	8	26-29	65-75	50% Prepup.	+	-	Zheng ea 2012b
13-19	100	0	28	70	1st Prepup.	+	+	Oonincx ea 2015a
111-165	100	0	27	70	1st Prepup.	+	+	Oonincx ea 2015b
96-1194	59-333	NR	26-28	NR	50% Prepup.	+	-	Parra Paz ea 201[illegible]
19200-20000	~16000	8	NR	NR	5-6 inst. larvae	+	+	Tschirner & Simon 2015
3000-12000	~15000	8	30	65	16 d old larvae	+	-	Liland ea 2017
1000	1000	6	27	60-70	1st Prepup.	+	-	Rehman ea 2017a
1000	1000	6	27	60-70	1st Prepup.	+	-	Rehman ea 2017b
93-297	300	4	28±2	70	Prepup.	+	+	Tinder ea 2017

Articles 40 total | 11 DM | 5 N

Variations in

- Chemical analyses of diet
- Strains
- Scale
- Diet to larvae ratio
- Age/life stage at start & harvest
- Feeding regime
- ...

Need for standardisation!

Bosch *et al.*
Conversion of organic resources by black soldier fly larvae: Legislation, efficiency and environmental impact
Journal of Cleaner Production (2019) 22 | 355-363

Standardisation in other animal species

Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application

H. H. Stein, B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange[1]

Committee on Terminology to Report AA Bioavailability and Digestibility[2,3]

J. Anim. Sci. 2007. 85:172–180

[2]The committee on terminology to report AA availability and digestibility was appointed on the request of the late J. T. Yen, who for many years served as the chair of the international steering committee of the Symposia on Digestive Physiology in Pigs. Yen wanted to bring clarity to the manner in which experimental results were presented to use research results in animal production effectively. de Lange was appointed chair of the committee on terminology.

Determination of ileal digestibility of amino acids in raw materials for broiler chickens – Results of collaborative studies and assay recommendations

V. Ravindran[a,*], O. Adeola[b], M. Rodehutscord[c], H. Kluth[d], J.D. van der Klis[e,1], E. van Eerden[e], A. Helmbrecht[f]

fediaf
The European Pet Food Industry

6. Feeding Test Protocols

Table VI-1. Abbreviations

GE	Gross energy	CP	Crude protein
DE	Digestible energy	DP	Digestible protein
ME	Metabolisable en		
kJ	Kilojoule		
kcal	Kilocalorie		

6.1.2.1. Animals
6.1.2.2. Feeding procedures
6.1.2.3. Food
6.1.2.4. Food allowances
6.1.2.5. Times of feeding
6.1.2.6. Pre-trial termination
6.1.2.7. Collection
6.1.2.8. Sample preparation
6.1.2.9. Analytical determination
6.1.2.10. Calculation of digestible energy and digestible nutrients
6.1.2.11. Calculation of metabolisable energy

6.1. INDICATOR MET

6.1.1. Introduction

This feeding protocol has bee determine ME and nutrient digest in a way not harmful for cats an

6.1.2. Protocol

6.1.2.1. Animals

A minimum of six fully grown ani age shall complete the test. The a

6.1.2.2. Feeding procedures

Feeding procedures shall be standardized. The feeding shall consist of two phases. The first phase shall be the pre-collection period of at least three days for dogs and five days for cats *(Nott et al. 1994)* with the objective of acclimatising the test animals to the diet and adjusting food intake, as necessary, to maintain body weight.

The second phase shall be the total collection period; faeces and possibly urine will be collected during at least four days (96 hours) for dogs and five days (120 hours) for cats.

6.1.2.3. Food

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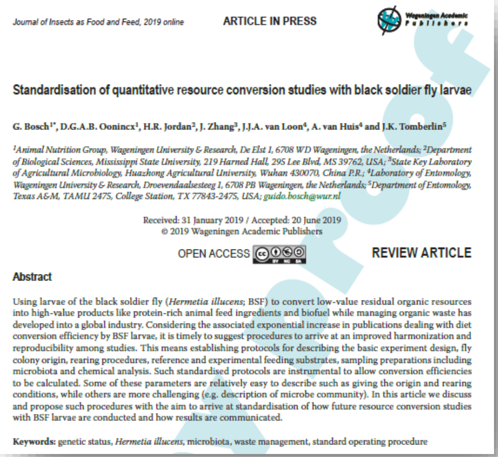
Towards standardisation of conversion studies

Aim | Harmonisation among studies and reproducibility of results to improve the overall scientific rigour in this developing field of research

- Present background information to increase awareness of factors impacting results
- Propose standardisation of methods
- Suggest avenues for future research


Journal of Insects as Food and Feed, 2019 online ARTICLE IN PRESS Wageningen Academic Publishers

Standardisation of quantitative resource conversion studies with black soldier fly larvae

G. Bosch[1*], D.G.A.B. Oonincx[1], H.R. Jordan[2], J. Zhang[3], J.J.A. van Loon[4], A. van Huis[4] and J.K. Tomberlin[5]

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REVIEW ARTICLE

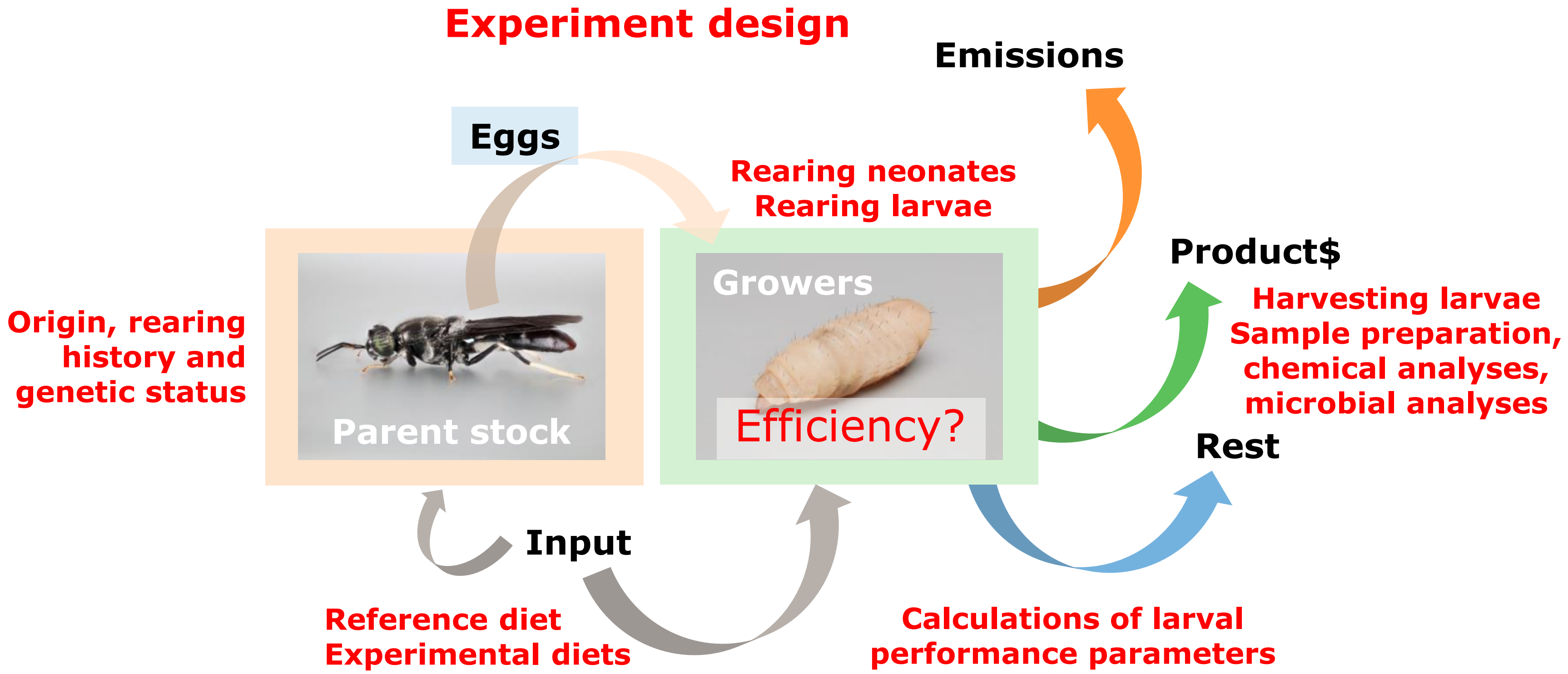
Abstract

Using larvae of the black soldier fly (*Hermetia illucens*; BSF) to convert low-value residual organic resources into high-value products like protein-rich animal feed ingredients and biofuel while managing organic waste has developed into a global industry. Considering the associated exponential increase in publications dealing with diet conversion efficiency by BSF larvae, it is timely to suggest procedures to arrive at an improved harmonization and reproducibility among studies. This means establishing protocols for describing the basic experiment design, fly colony origin, rearing procedures, reference and experimental feeding substrates, sampling preparations including microbiota and chemical analysis. Such standardised protocols are instrumental to allow conversion efficiencies to be calculated. Some of these parameters are relatively easy to describe such as giving the origin and rearing conditions, while others are more challenging (e.g. description of microbe community). In this article we discuss and propose such procedures with the aim to arrive at standardisation of how future resource conversion studies with BSF larvae are conducted and how results are communicated.

Keywords: genetic status, *Hermetia illucens*, microbiota, waste management, standard operating procedure

Bosch *et al.*
Standardisation of quantitative resource conversion studies with black soldier fly larvae
Journal of Insects as Food and Feed | in press

Towards standardisation of studies



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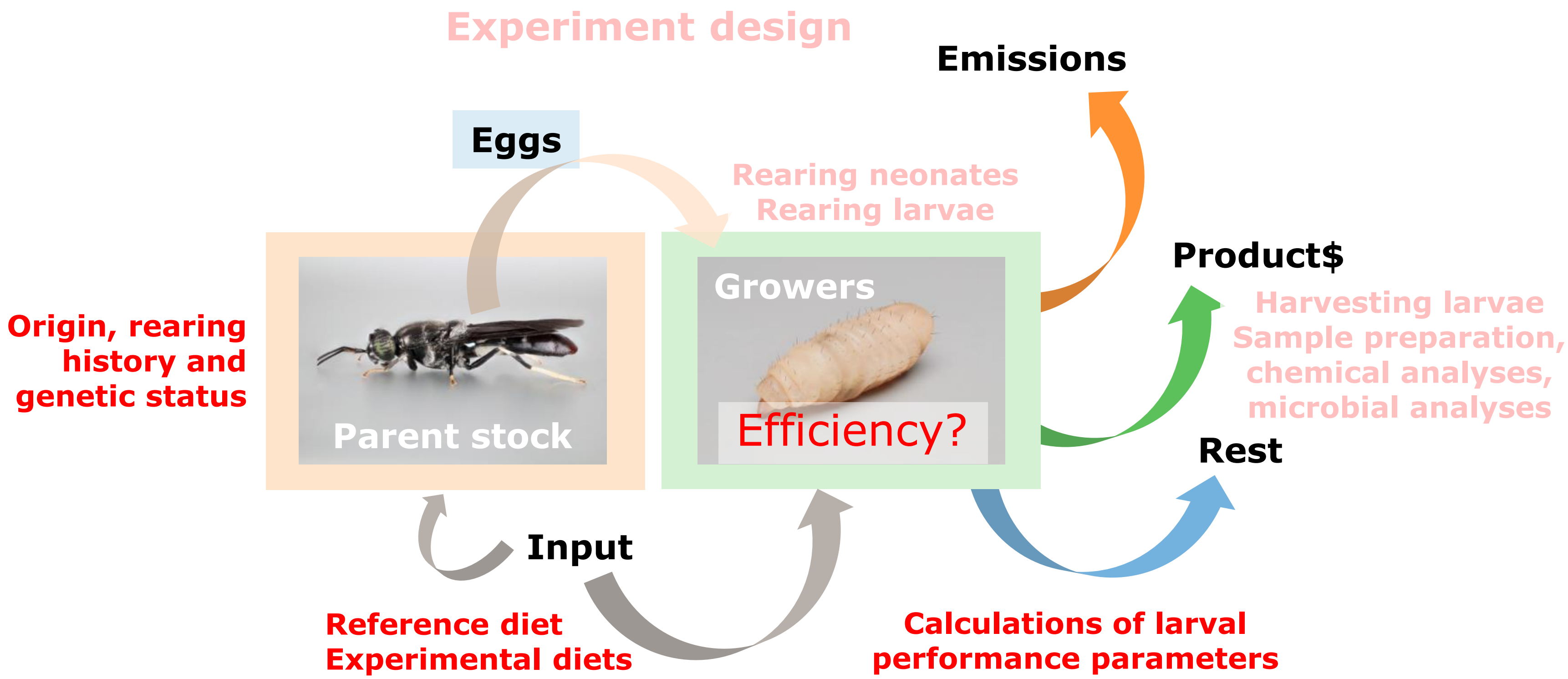

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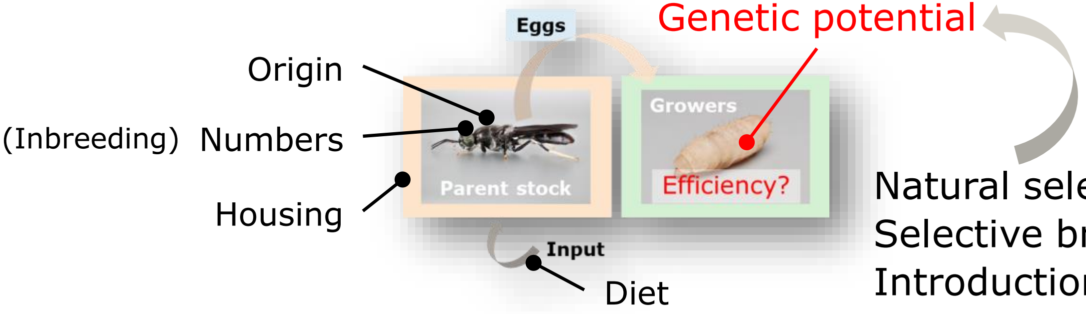
Journal of Insects as Food and Feed | in press

Origin, rearing history and genetic status

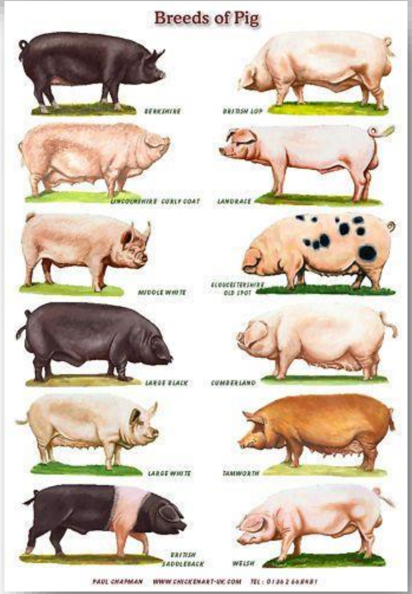


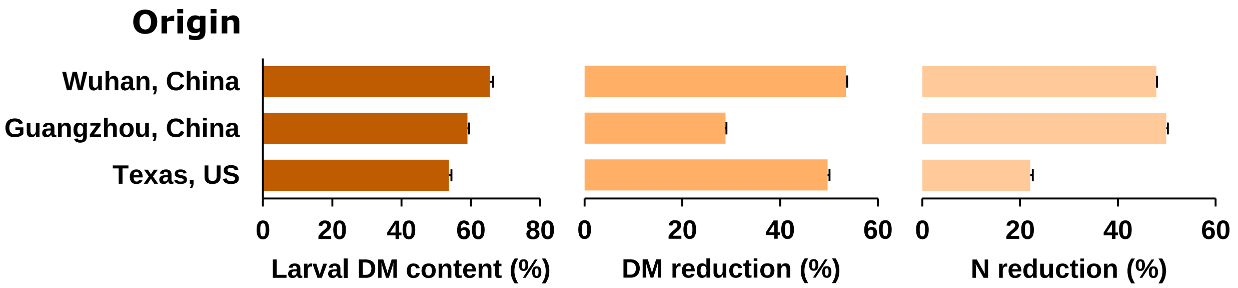
Natural selection
Selective breeding
Introduction individuals


ON FEBRUARY 16, 2018

Protix, Hendrix Genetics to develop insect breeding program

Program aims to improve potential of insects as an efficient protein converter





n=300 6-d old, 100 g pig manure; n=3
28 C, 75% RH, 16:8 h LD
harvest at 50% prepupae

Zhou *et al.*
Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures | *Journal of Medical Entomology* (2013) 50 | 1224-1230

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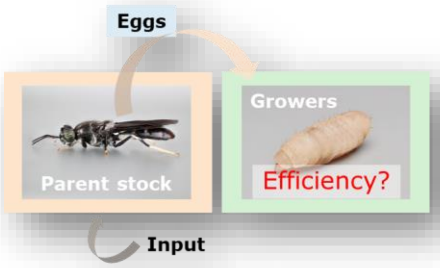
Origin, rearing history and genetic status



- ✓ **Origin of the insects used to set up the colony**
- ✓ **Founder population size**
- ✓ **Minimum effective population size**
- ✓ **Number of generations in the lab**
- ✓ **Introduction of individuals from outside the colony**
- ✓ **Current rearing conditions (diet, abiotic conditions)**
- ✓ **Duration of rearing cycle**
- ✓ **Substrate for pupation and eclosion**
- ✓ **Conditions for reproduction**

Laboratory Populations as a Resource for Understanding the Relationship Between Genotypes and Phenotypes: A Global Case Study in Locusts

Karine Berthier,[*,1] Marie-Pierre Chapuis,[*,†,1] Stephen J. Simpson,[*] Hans-Jörg Ferenz,[‡] Chérif M. Habib Kane,[§] Le Kang,[¶] Angela Lange,[||] Swidbert R. Ott,[**] Mohammed A. Babah Ebbe,[§] Kees W. Rodenburg,[††] Stephen M. Rogers,[**] Baldwin Torto,[‡‡] Jozef Vanden Broeck,[§§] Joop J. A. van Loon,[¶¶] Gregory A. Sword[*]

Shift in Phenotypic Variation Coupled With Rapid Loss of Genetic Diversity in Captive Populations of *Eristalis tenax* (Diptera: Syrphidae): Consequences for Rearing and Potential Commercial Use

LJUBINKA FRANCUSKI,[1] MARKO DJURAKIC,[1] JASMINA LUDOŠKI,[1] PILAR HURTADO,[2] CELESTE PÉREZ-BAÑÓN,[2] GUNILLA STÅHLS,[3] SANTOS ROJO,[2] AND VESNA MILANKOV[1,4]

>Estimate degree of inbreeding in a colony

Reference diet and Experimental diets

Input

Reference diets

3 out of 11 studies

Different

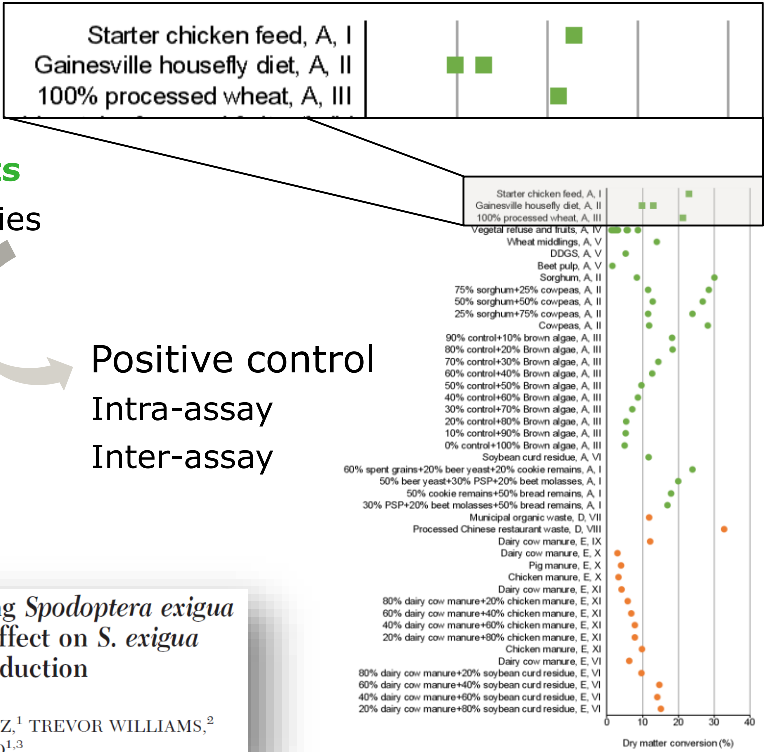
Comparison studies

Cross laboratory

Positive control

Intra-assay

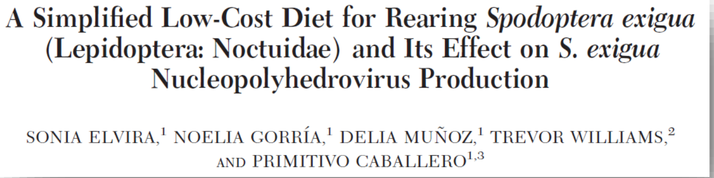
Inter-assay



Reference diet

- ✓ Well-defined ingredients
- ✓ Available around the globe
- ✓ Low cost

>Develop and evaluate



A Simplified Low-Cost Diet for Rearing *Spodoptera exigua* (Lepidoptera: Noctuidae) and Its Effect on *S. exigua* Nucleopolyhedrovirus Production

SONIA ELVIRA,[1] NOELIA GORRÍA,[1] DELIA MUÑOZ,[1] TREVOR WILLIAMS,[2] AND PRIMITIVO CABALLERO[1,3]

Bosch *et al.*

Conversion of organic resources by black soldier fly larvae: Legislation, efficiency and environmental impact

Journal of Cleaner Production (2019) 22 | 355-363

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Reference diet and Experimental diets

- ✓ **Diet ingredients: supplier and product name**
- ✓ **Storage method and duration if applicable**
- ✓ **Preparation methods**
- ✓ **Analysis of contents: dry matter, ash, N and total fat**

Input

Experimental diet

Origin ingredients in too general terms

Limited chemical characterization

Understanding nutrition

Cutrignelli *et al.*
Res Vet Sci (2018) 117 | 209-215

Table 2
Ingredient composition and proximate analysis of the test diets.

	HIML	SBM
Ingredient composition (g/kg)		
Maize grain	653.0	583.0
Soybean meal	–	235.0
Insect meal	170.0	–
$CaCO_3$ grains	80.0	80.0
Dehulled sunflower meal	50.0	50.0
Vegetable oil	10.0	15.0
Mineral and Vitamin supplement[a]	30.0	30.0
Monocalcium phosphate	5.00	5.00
Salt	2.00	2.00
Proximate analysis (% as feed) and energy content (Kcal/kg)		
Dry matter[b]	90.5	90.1
Crude protein[b]	17.9	18.1
Crude fiber[b]	4.1	4.0
Ether extract[b]	4.3	4.3
ADF[b]	3.8	3.5
ADF linked protein[b]	2.88	1.52
Ash[b]	14.2	14.2
NDF[b]	15.2	14.0
Metabolizable Energy[c]	2745	2780
Mineral and EAA content (% as feed)		
Ca[c]	4.96	4.26
Total P[c]	0.67	0.69

Bosch *et al.*
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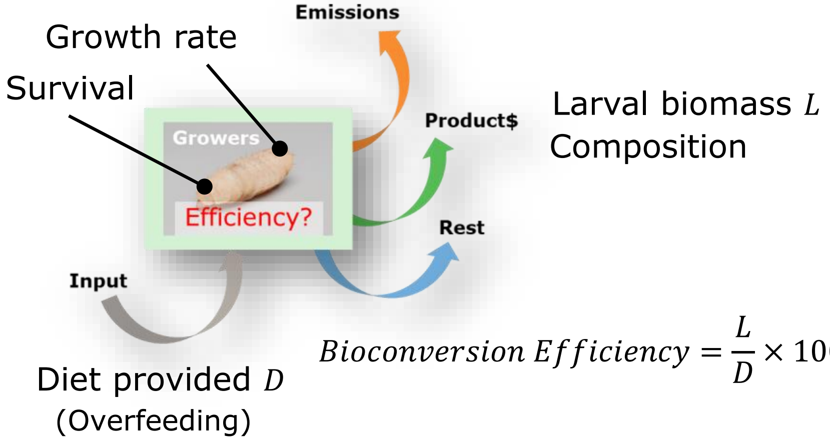
Calculations of larval performance parameters



$$Bioconversion\ Efficiency = \frac{L}{D} \times 100$$

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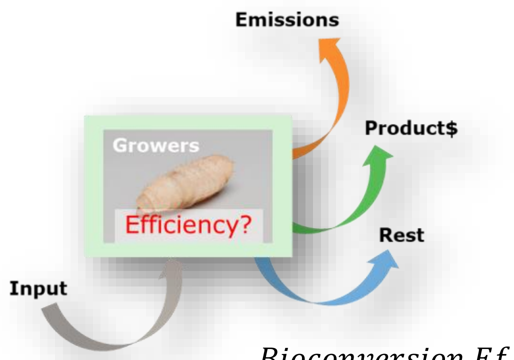
Calculations of larval performance parameters

Fresh basis

	Stream A	Stream B
Diet provided, g fresh	1000	1000
Biomass yield, g fresh	200	200
Conversion efficiency	20%	20%



$$Bioconversion\ Efficiency = \frac{L}{D} \times 100$$

Profound impact on conclusions...

- When considering protein economy: efficiency on N basis

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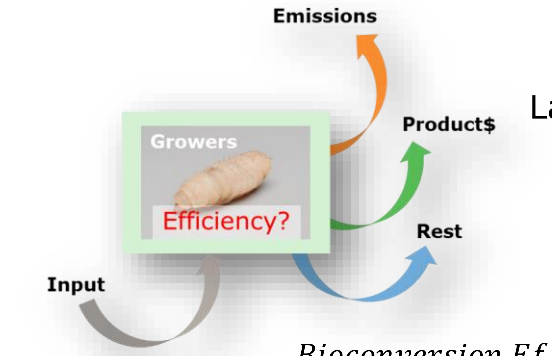
Calculations of larval performance parameters

$$BE = \frac{L_{end} - L_{start}}{D} \times 100$$

$$ECI = \frac{L_{end} - L_{start}}{D_{start} - \cancel{D_{end}}} \times 100$$

$$ECD = \frac{L_{end} - L_{start}}{D_{start} - \cancel{D_{end}} - \cancel{F_{end}}} \times 100$$



Larval biomass L

Diet provided D

$$Bioconversion\ Efficiency = \frac{L}{D} \times 100$$

BE, Bioconversion Efficiency

ECI, Efficiency of Conversion of Ingested Food (Waldbauer, 1968)

EDI, Efficiency of Conversion of Digested Food (Waldbauer, 1968)

>Develop digestibility method

Bosch *et al.*
Standardisation of quantitative resource conversion studies with black soldier fly larvae
Journal of Insects as Food and Feed | in press
Waldbauer
The consumption and utilization of food by insects
Advances in Insect Physiology (1968) 5 | 229-288

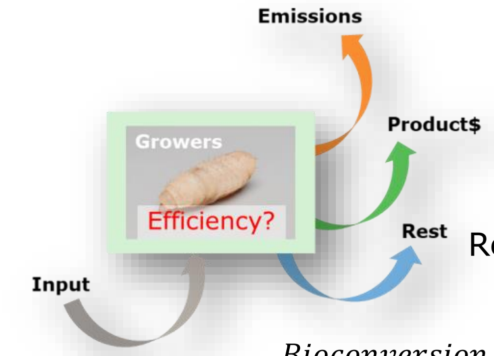
Calculations of larval performance parameters

$$BE = \frac{L_{end} - L_{start}}{D} \times 100$$

$$BER = \frac{L_{end} - L_{start}}{D_{start} - R_{end}} \times 100$$

BE, Bioconversion Efficiency

BER, BE corrected for Residue



Larval biomass L

Residue R

Diet provided D

$$Bioconversion\ Efficiency = \frac{L}{D} \times 100$$

$$Reduction\ Rate = \frac{D_{start} - R_{end}}{D_{start}} \times 100$$

$$Waste\ Reduction\ Index = \frac{RR}{d} \times 100$$

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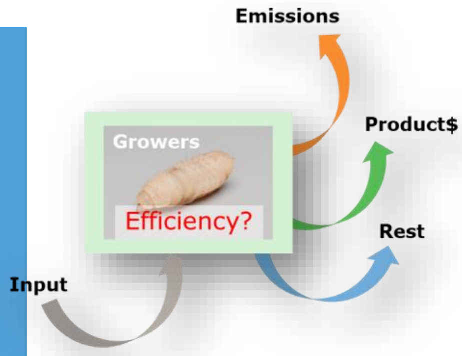
Calculations of larval performance parameters

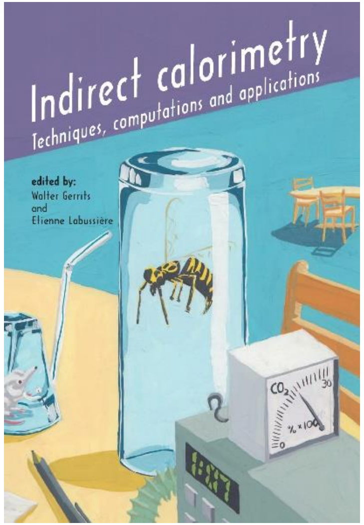
Larval performance

- ✓ **Survival (%)**
- ✓ **Growth rate, preferably reported as growth curves**
- ✓ **Fresh weight of larvae and of prepupae at harvest**
- ✓ **Total DM, OM, and N in insect biomass at harvest**

Conversion efficiency

- ✓ **Bioconversion Efficiency and/or Bioconversion Efficiency corrected for Residue**
- ✓ **If applicable, overall Degradation, Reduction Rate and/or Waste Reduction Index**





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Key messages

- First effort to provide directions and checklists for researchers
- Currently impossible to establish unambiguous standardized procedures; lack of basic knowledge and reference values

Stimulate investments in studies that improve and extend our methods

- Further enhance fundamental understanding of biological principles of resource utilisation by BSF larvae and microbiota

Thank you for your attention

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