Insights on Feed and Nutritional Requirements of *Tenebrio molitor*

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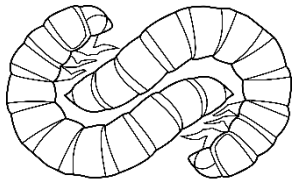
Background

During the inVALUABLE project feed and nutrition for *Tenebrio molitor* larvae have been investigated as part of WP2. The feed mixes were designed from **vegetable-based former foodstuffs** to implement a circular insect production.

The **objectives** in these studies was to explore and **optimize larval growth** focusing on feed composition; including the addition of commercial premixes and/or salts to the designed feed mixes.



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Outline of experimental work

- Monitoring of pilot-scale production
- Substrate particle size
- Premix and minerals
- Amino Acid requirements

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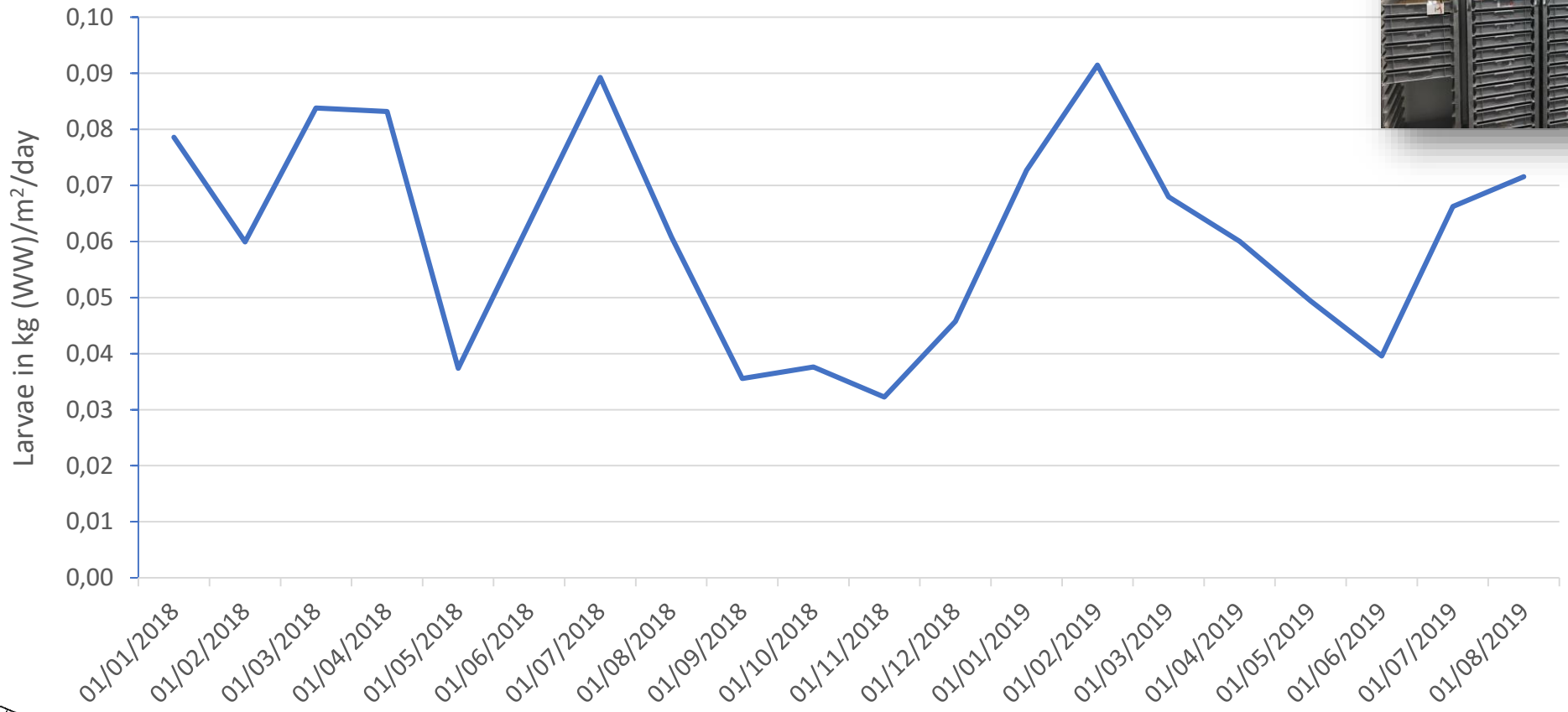
Monitoring of pilot-scale production



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Substrate particle size – set-up

Set-up of experiment

- 10 x 10 cm experimental trays
- 1500 eggs (avg. weight 0.97 g)
- 50 g feed and spent grains as water source
- 3 replicates of each feed mix (treatment)
- 5 feed mixes – comprised by oats, wheat, rye, rape seed and peas – named **0P, 1P, 1M and 2P and 2M**
  *Feed mixes 1 and 2 were blended with a piglet premix and were either ground into a fine powder (**P - particle size <0.5 mm**) or fed an untreated standard meal (**M - particle size between 0.5-1.8 mm**). Feed type 0P (reference) was like 2P, but without premix*

Nutritional information

	Feed type 1	Feed type 0+2
Dry matter (%)	85.8	86.6
Carbohydrates (%)	61.1	64.8
Protein (%)	14.7	15.5
Fat (%)	3.2	1.2
Ash	6.8	5.2

Data

- Subsampling were made every week from day 9-30, where larval size was based on area of larvae calculated from images
- From day 30 onwards, larvae were weighed

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Substrate particle size - Results

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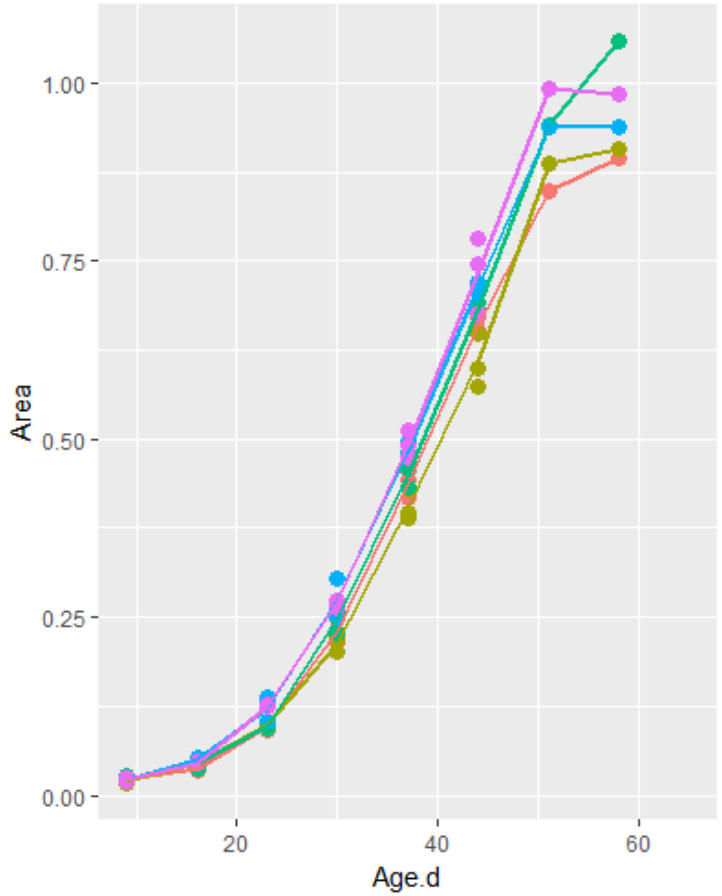
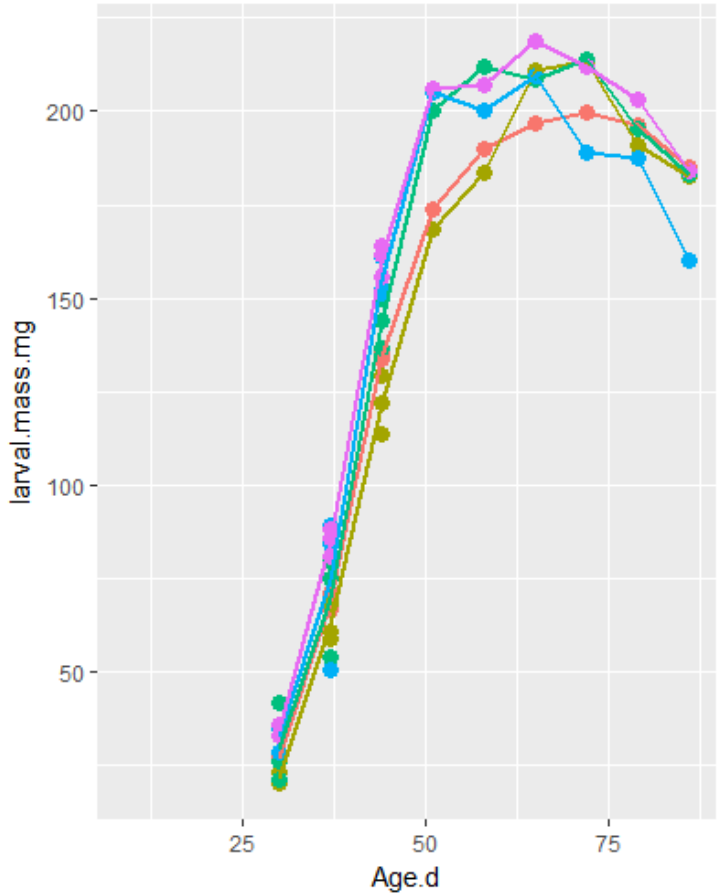
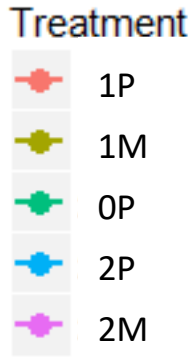
Larval size (based on area) from day 9



Larval mass (mg) from day 30





No significant difference between Powder (P) and Meal (M) feed mixes. Feed mix 2P and 2M performed better than the others until day 51.

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Premix and minerals

- ‘Scratching the surface’ – numerous dose-response studies are needed to assess the micro-nutritional requirements
- Top-down approach – testing a range of commercial premix blends for piglets and pigs at different inclusion levels (1-5%) in a few experimental feed mixes
- Supplementary studies to evaluate the inclusion of certain minerals, e.g. sodium

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Premix and minerals (sodium) – set-up

Hypothesis

Larvae will grow more effective on feed with added NaCl compared to feed without, as e.g. sodium is essential for amino acid accumulation and protein synthesis (e.g. Kuchler, 1967)

Set-up

- 2 feed mixes
  - Feed A: 442 ('Standard inVALUABLE feed mix' with 1% premix piglets)
  - Feed B: 442+0.1% NaCl
- 60 x 40 cm boxes
- 4 replicates
- 1800 g feed + spent grains
- 15 g eggs

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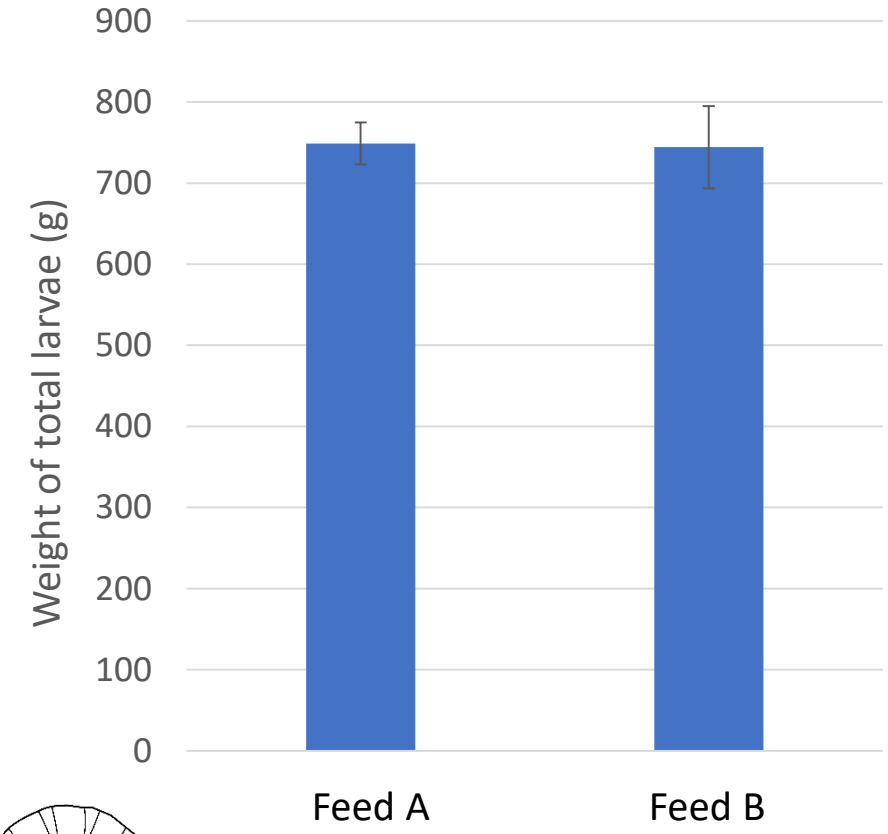
Premix and minerals (sodium) - Results



- No significant effect on total weight of larvae on NaCl diet (P=0.926)
- No significant effect of individual larvae size on NaCl diet (P=0.901)
- Sodium levels of Feed A (0.09%) appear to be sufficient

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Amino Acid requirements for *T. molitor*

What do we need to know?

- Feed composition
- Nutrient digestibility and availability
- Nutrient requirements

What do we know?

- Old studies (1970's)
- Only basic knowledge on digestion and anatomy
- Limited knowledge on amino acid requirements from old studies

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Amino Acid requirements for *T. molitor*

Hypotheses

a) It is possible to apply methods used on common livestock species on mealworms

b) The crude protein content of the feed affects the growth of mealworm larvae

c) Different AA's are required in different amounts.

d) It is possible to find the first limiting AA in mealworms.

Three experiments

1) Effect of crude protein concentration on growth

2) Screening of the effect of different amino acids on growth

3) Effect of graded methionine concentrations on growth

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Amino Acid requirements for *T. molitor*

Method

- All experiments were carried out in aluminum containers measuring approx. 60 cm^2
- Larvae aged 28 days ± 1.5 days from oviposition
- Larvae picked individually according to age and size
- *Ad libitum* feeding (including water every second day)

Collection of data

- All larvae were weighted every week
- Experiments had a duration of three weeks

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Amino Acid requirements for *T. molitor*

Experiment 1: Effect of crude protein concentration on growth

Feed

- All feed consisted of five ingredients

HP 300 (Hamlet protein)
Wheat starch
Limestone
Monocalcium phosphate 16/22,7
Sodium chloride
Premix piglets

Set-up

- Six experimental diets (diet CP10, CP14, CP18, CP22, CP26 and CP30)
- Five groups of 100 larvae

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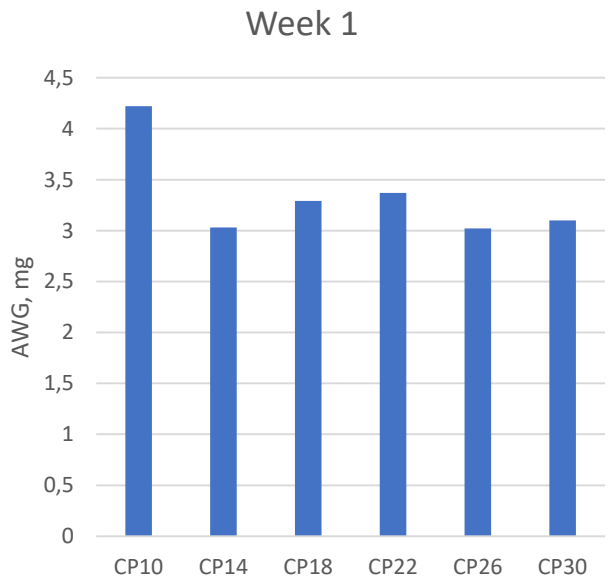
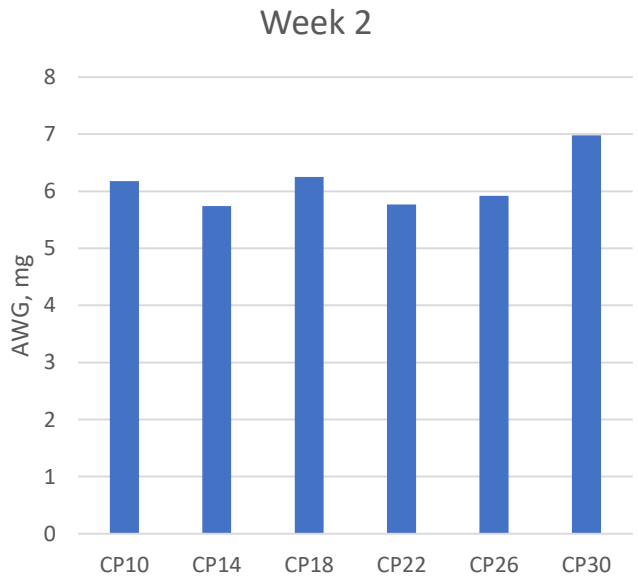
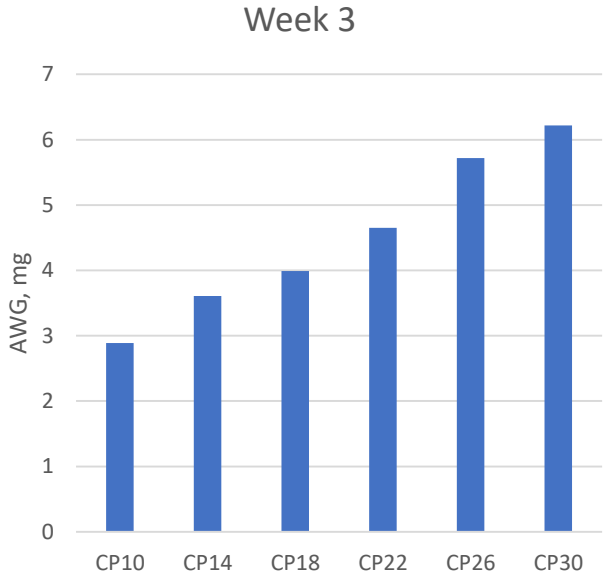
Amino Acid requirements for *T. molitor*

Experiment 1: Effect of crude protein concentration on growth







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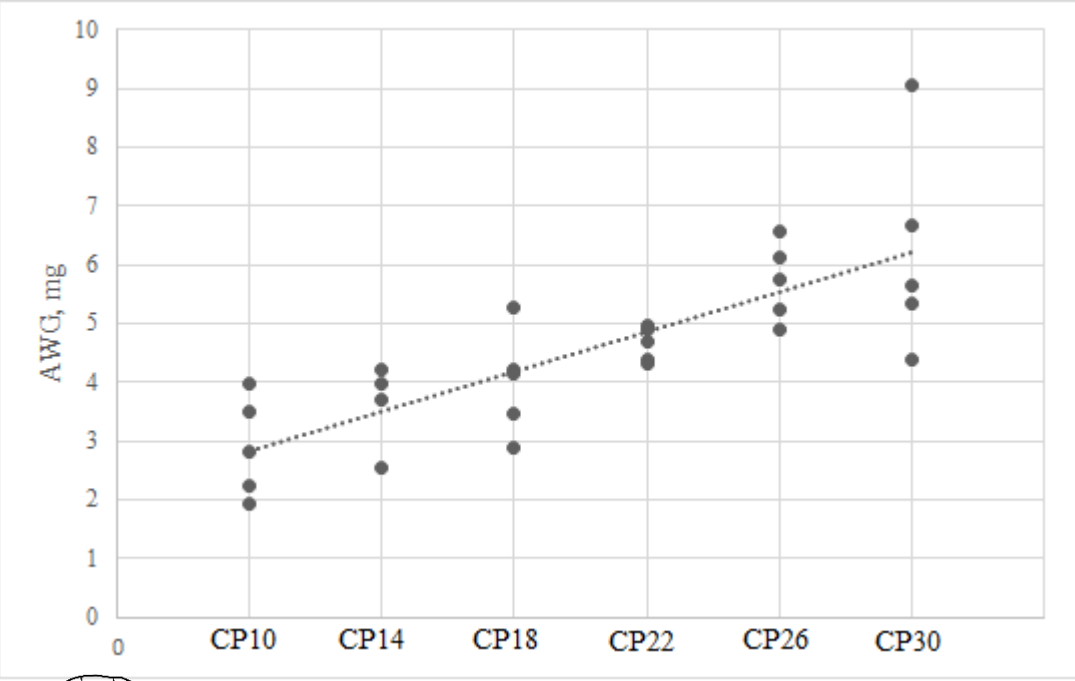
Amino Acid requirements for *T. molitor*

Experiment 1: Effect of crude protein concentration on growth

Results from week 3



- Linear model provided the best fit
- Appears to be flattening at the end, with a possible optimum at CP26
- Different responses week 1 and 2 may indicate that the requirement of crude protein depend of age of larvae

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Amino Acid requirements for *T. molitor*

Experiment 2: Screening of the effect of different amino acids on growth

Feed composition and Amino Acids level

10 essential amino acids tested (after John et al. 1979)

		NC	Ala	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	PC
Ala	g/kg	4.31	9.48	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	9.48
His	g/kg	2.55	2.55	4.59	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55	4.59
Ile	g/kg	4.90	4.90	4.90	10.9	4.90	4.90	4.90	4.90	4.90	4.90	4.90	10.9
Leu	g/kg	7.77	7.77	7.77	7.77	17.2	7.77	7.77	7.77	7.77	7.77	7.77	17.2
Lys	g/kg	5.47	5.47	5.47	5.47	5.47	12.1	5.47	5.47	5.47	5.47	5.47	12.1
Met	g/kg	1.32	1.32	1.32	1.32	1.32	1.32	2.89	1.32	1.32	1.32	1.32	2.89
Phe	g/kg	5.11	5.11	5.11	5.11	5.11	5.11	5.11	9.24	5.11	5.11	5.11	9.24
Thr	g/kg	3.91	3.91	3.91	3.91	3.91	3.91	3.91	3.91	8.62	3.91	3.91	8.62
Trp	g/kg	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	2.86	1.30	2.86
Val	g/kg	5.11	5.11	5.11	5.11	5.11	5.11	5.11	5.11	5.11	5.11	11.0	11.0

Negative control (NC) and positive control (PC)

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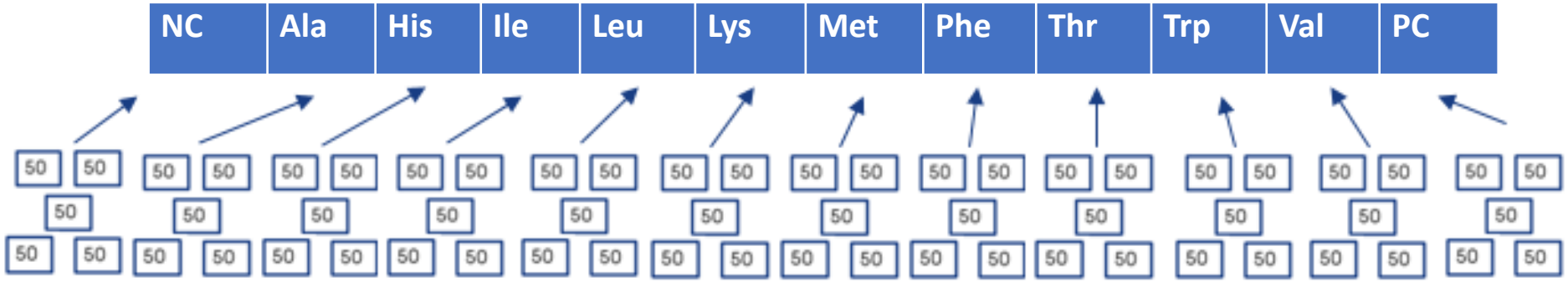
Amino Acid requirements for *T. molitor*

Experiment 2: Screening of the effect of different amino acids on growth

Set-up

- 12 treatments (10 essential amino acids plus NC and PC)
- Five groups with 50 larvae per treatment



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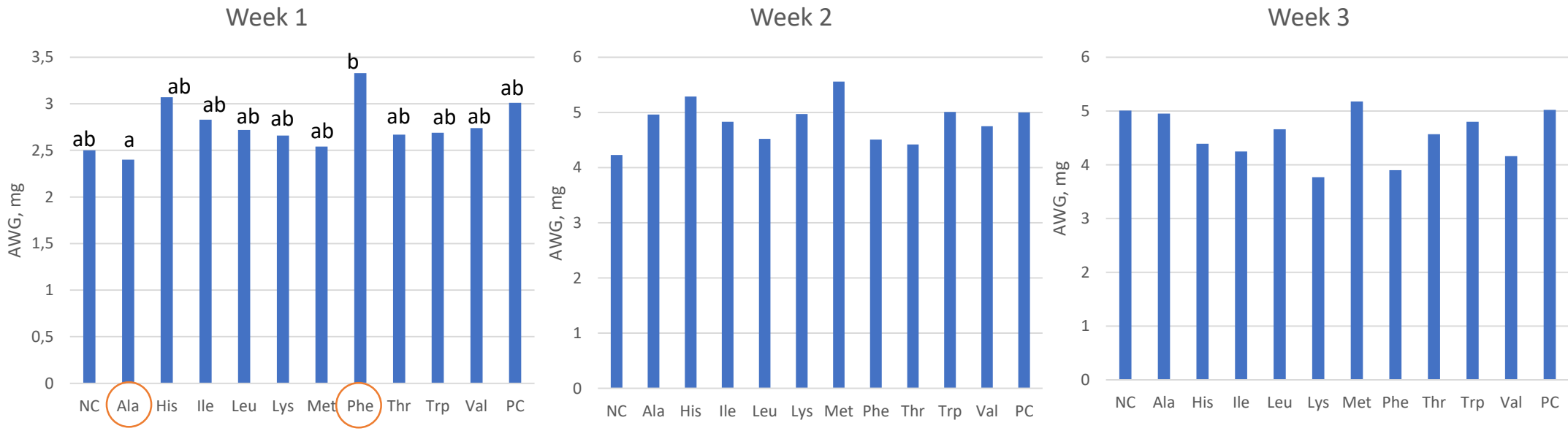
Amino Acid requirements for *T. molitor*

Experiment 2: Screening of the effect of different amino acids on growth

Results



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Amino Acid requirements for *T. molitor*

Experiment 3: Effect of graded methionine concentrations on growth

Feed

Based on CP10 supplemented crystalline AAs to reach same level of methionine as in diet CP30

Nutrient	Unit	Met1.3	Met1.8	Met2.4	Met2.9	Met3.4	Met3.9
DM	%	89.2	89.2	89.2	89.2	89.2	89.2
GE	MJ	15.1	15.1	15.1	15.1	15.1	15.1
CP	%	12.9	12.9	12.9	13.0	13.0	13.0
CF	%	0.47	0.47	0.47	0.47	0.47	0.47
Ash	%	4.97	4.97	4.97	4.97	4.97	4.97
Ca	g/kg	8.80	8.80	8.80	8.80	8.80	8.80
P	g/kg	5.80	5.80	5.80	5.80	5.80	5.80
Lys	g/kg	12.1	12.1	12.1	12.1	12.1	12.1
Met	g/kg	**1.32**	**1.84**	**2.37**	**2.89**	**3.41**	**3.94**
Cys	g/kg	1.46	1.46	1.46	1.46	1.46	1.46

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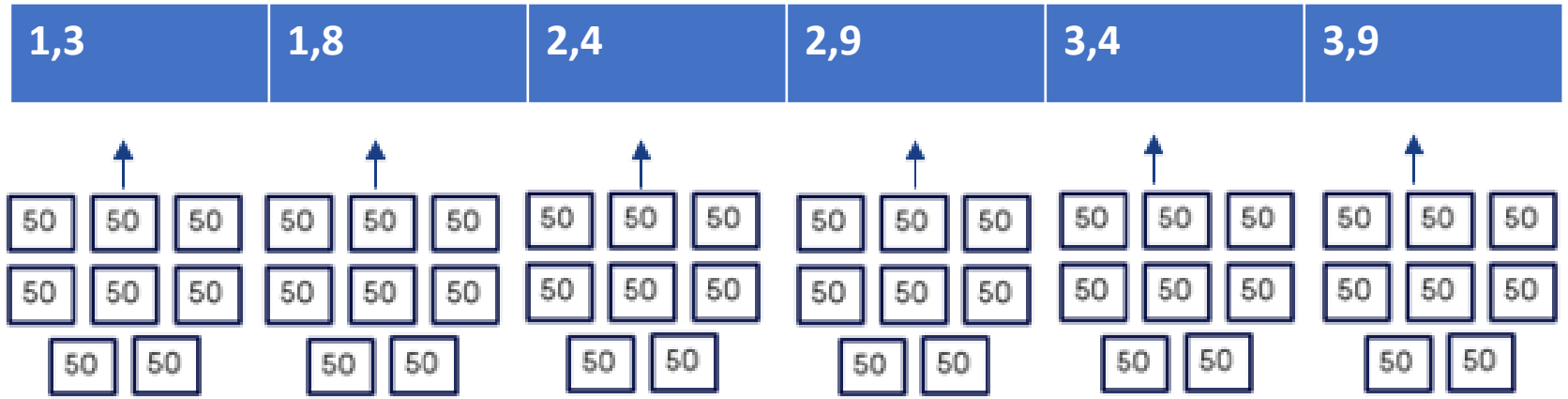
Amino Acid requirements for *T. molitor*

Experiment 3: Effect of graded methionine concentrations on growth

Set-up

- Six treatments
- Eight groups with 50 larvae per treatment



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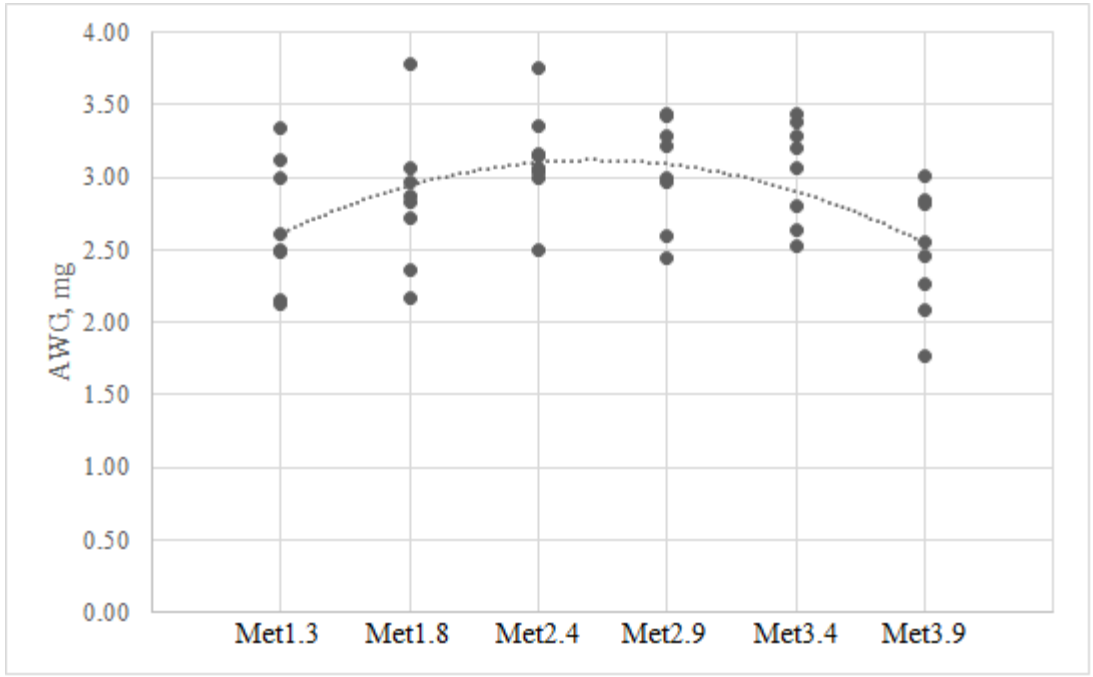
Amino Acid requirements for *T. molitor*

Experiment 3: Effect of graded methionine concentrations on growth

Results week 2



Optimum estimated for Met
= 2.56 g/kg

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Amino Acid requirements for *T. molitor*

Observations and complications

- Deficit of other nutrients? Minerals?
- Application of water was difficult. We tried different methods but often larvae drowned

Conclusion

- The method was all right but had several sources of error
- Different growth responses with different amino acids was observed; however, it is uncertain still if methionine is the first limiting
- Nutrient requirements may well vary depending on larval age

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Thank you for your attention!

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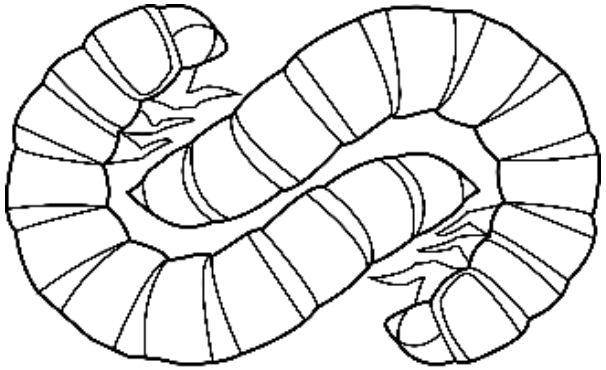

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