

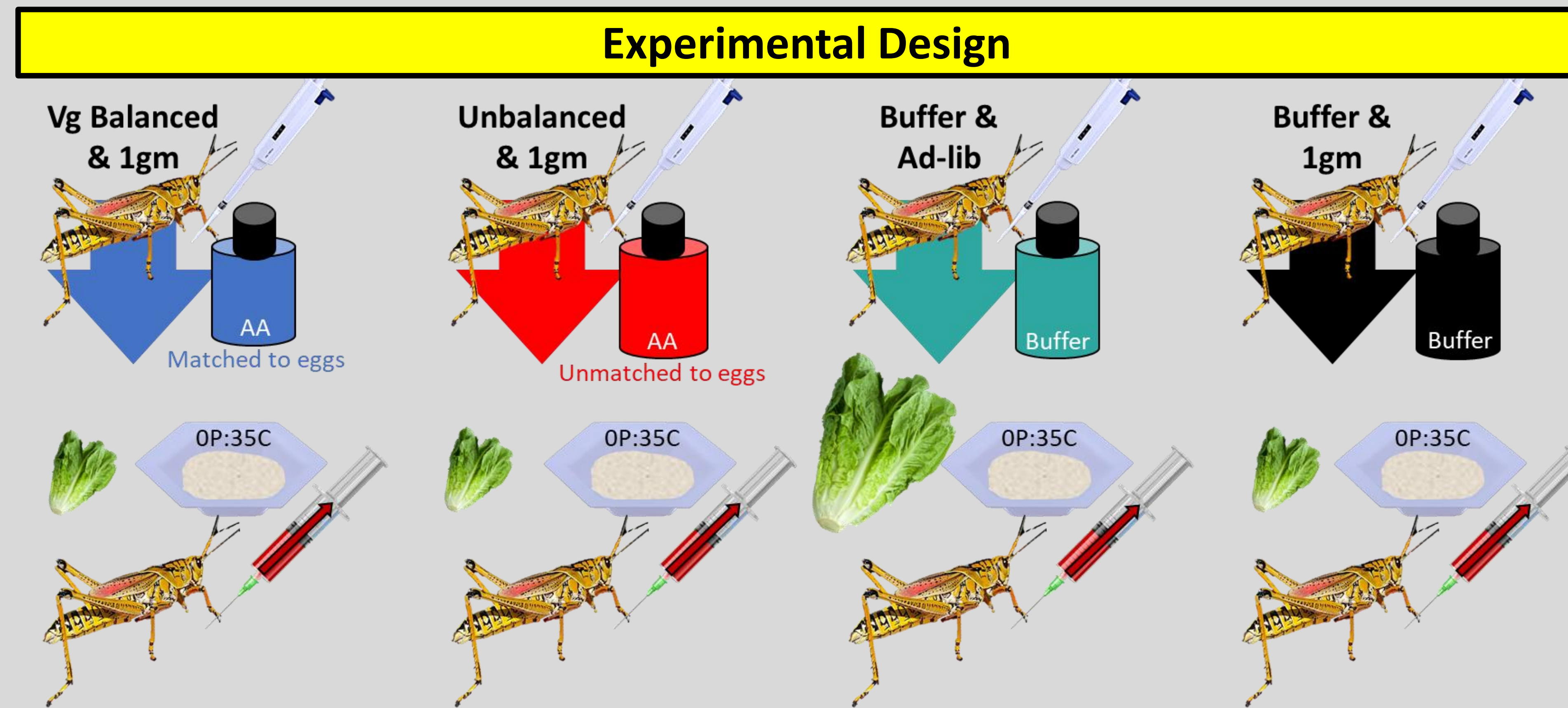
# Dietary and body mass thresholds for reproduction in grasshoppers

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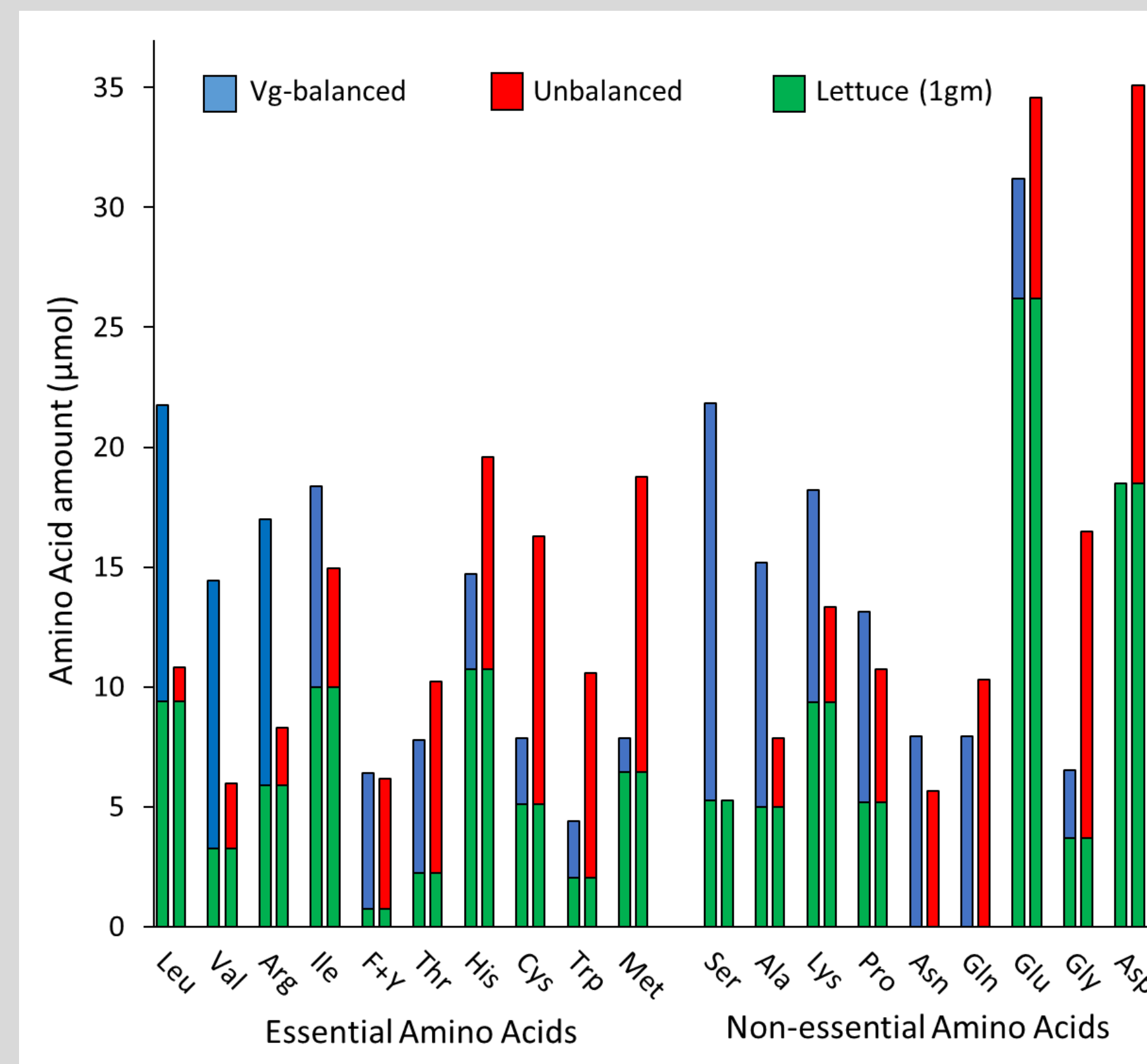
## Introduction

- A natural diet does not perfectly match nutritional needs.
- To acquire the sufficient amounts of some amino acids, organisms overconsume other amino acids. This may be detrimental to health.
- An exome-matched diet was designed to provide exactly the required quantities of amino acids for flies (Piper et al. 2017). This allowed for simultaneous faster development and longer lifespan.
- Theory suggests that thresholds of nutrition or storage need to be attained to commit to major developmental events (e.g., initiation of reproduction) (Juliano et al. 2004).
- For adult female grasshoppers (*Romalea microptea*), production of vitellogenin (the precursor of egg yolk protein, found in blood) is a major demand of dietary amino acids (Borst et al. 2000; Hatle et al. 2001).
- We hypothesize that grasshoppers on a diet with an amino acid composition that matches vitellogenin will reproduce earlier and more than grasshoppers on an isonitrogenous diet with an amino acid profile that is unmatched to vitellogenin.**



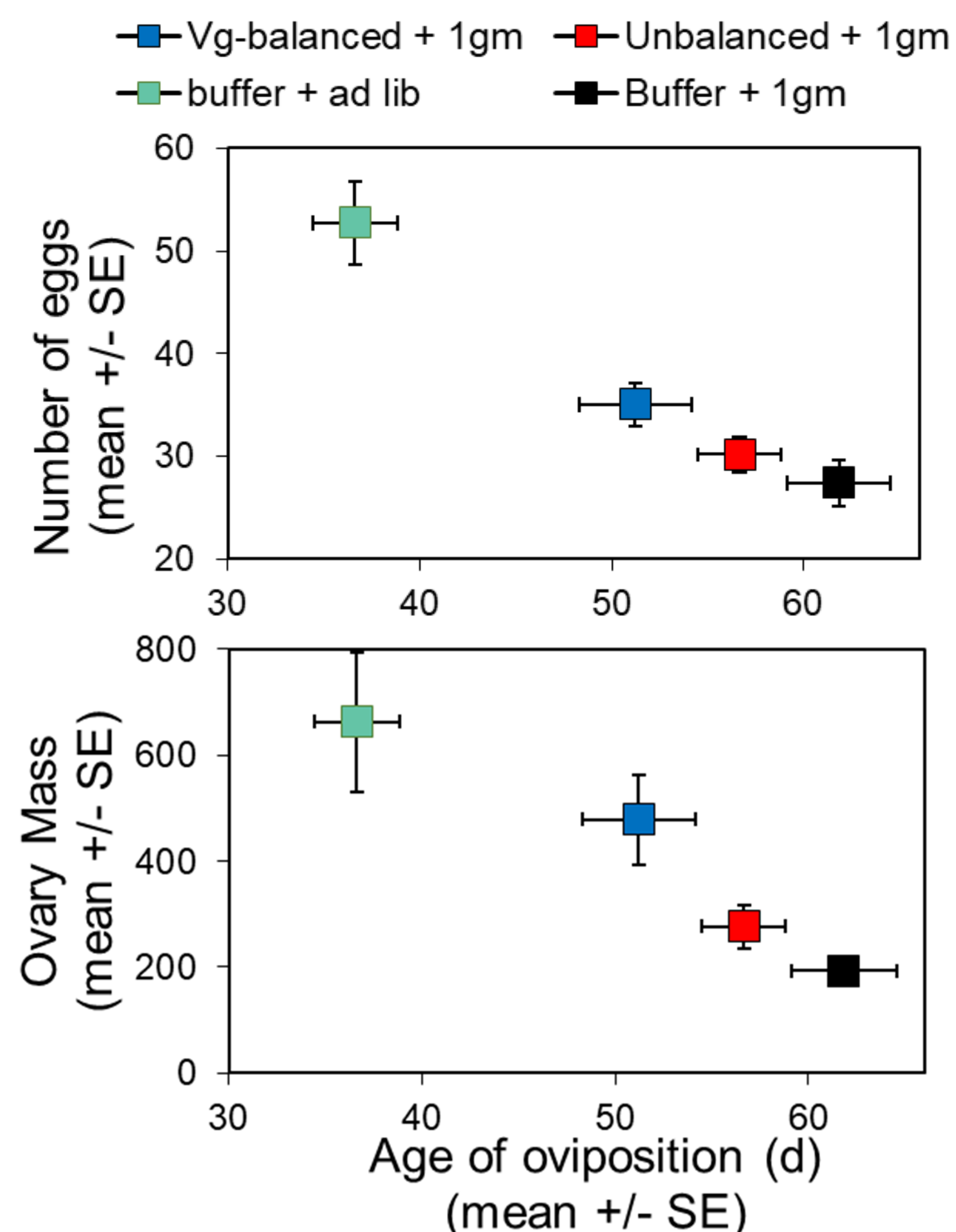
**Figure 2. Experimental diets.** Each treatment group was fed three items daily: a solution force-fed via micropipette, an amount of lettuce, and a dry artificial diet. Smaller lettuce icon indicates 1gm of lettuce daily, which is not sufficient for normal reproductive development. Larger lettuce icon indicates ad libitum lettuce (~5-8gm / day). Solutions were: Vg-balanced = amino acids matched to egg yolk protein (see Figure 3); Unbalanced = amino acids approximately reciprocal to those in egg yolk protein (see Figure 3); buffer = no amino acids. Weigh boat contains dry artificial diet of 0 protein : 35 carbohydrates offered continuously. Grasshoppers were weighed daily.

## Amino acid compositions of liquid foods force-fed to grasshoppers daily.



**Figure 3. Amino acid composition of diets that were fed to grasshoppers daily.** The Vg-balanced solution (blue) plus 1gm lettuce daily exactly matches the amino acid composition of vitellogenin. The Unbalanced solution (red) plus 1gm lettuce daily does not match the amino acid composition of vitellogenin; it has insufficient branched-chain amino acids and arginine and excessive histidine, tryptophan, cysteine, and methionine.

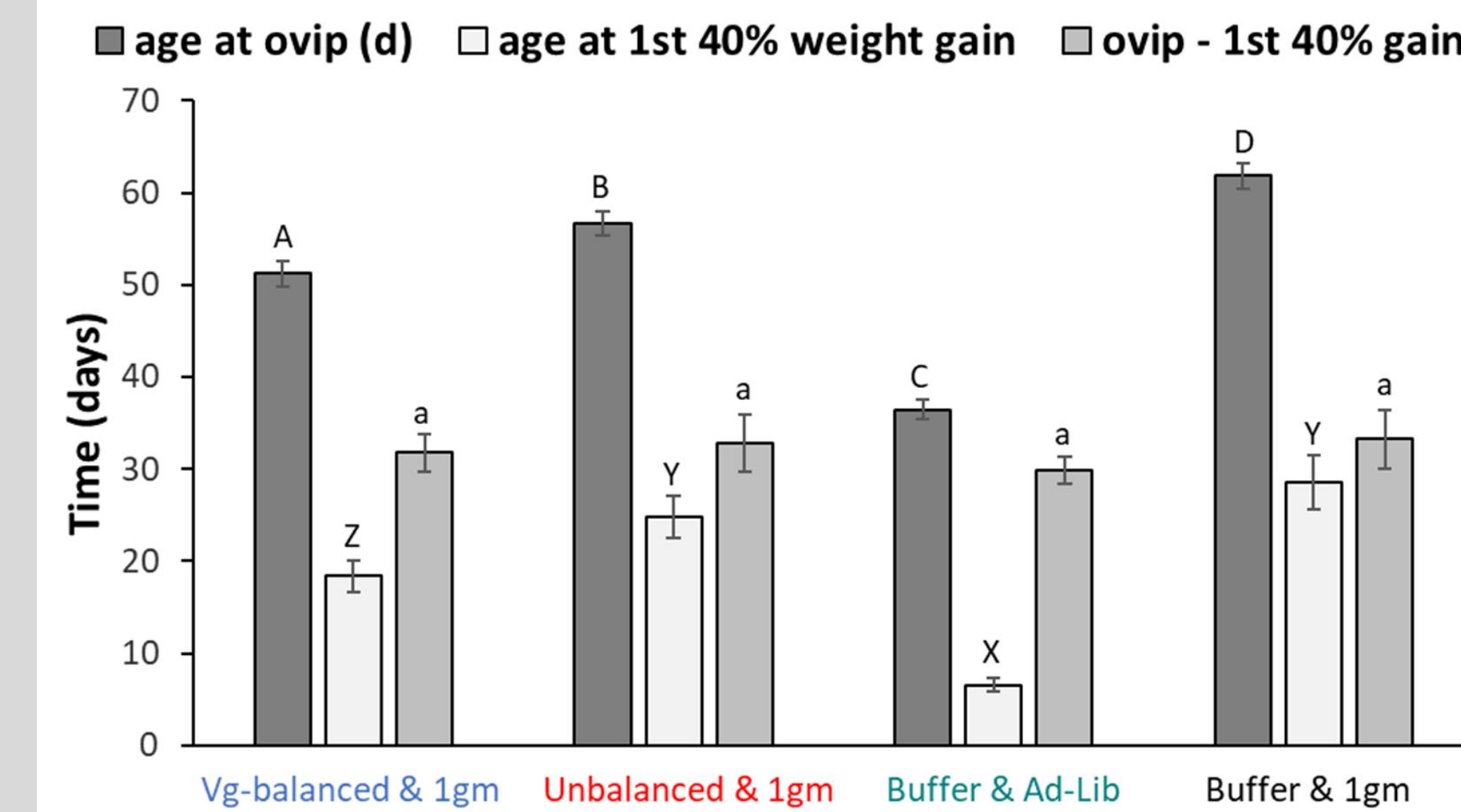
## Vg-balanced & 1gm group reproduced earlier and more than the Unbalanced & 1gm group.



**Figure 4. The Vg-balanced & 1gm group performed better than the Unbalanced & 1gm group for all responses;** age at oviposition, egg number, and ovary mass (estimate of 2<sup>nd</sup> clutch). The buffer & 1gm group is not different from Unbalanced & 1gm group in egg number or ovary mass.

## Diets affected

**time from molt to 40% weight gain, but not time from 40% gain to ovip.**



**Figure 5. Time to 40% weight gain serves as a threshold for commitment to oviposition.** The age at which each individual attained a 40% increase over weight at molt was identified (open bars); these times were affected by dietary amino acid composition. However, the time from this 40% increase to oviposition (light gray bars) was not affected by diet.

## Conclusions

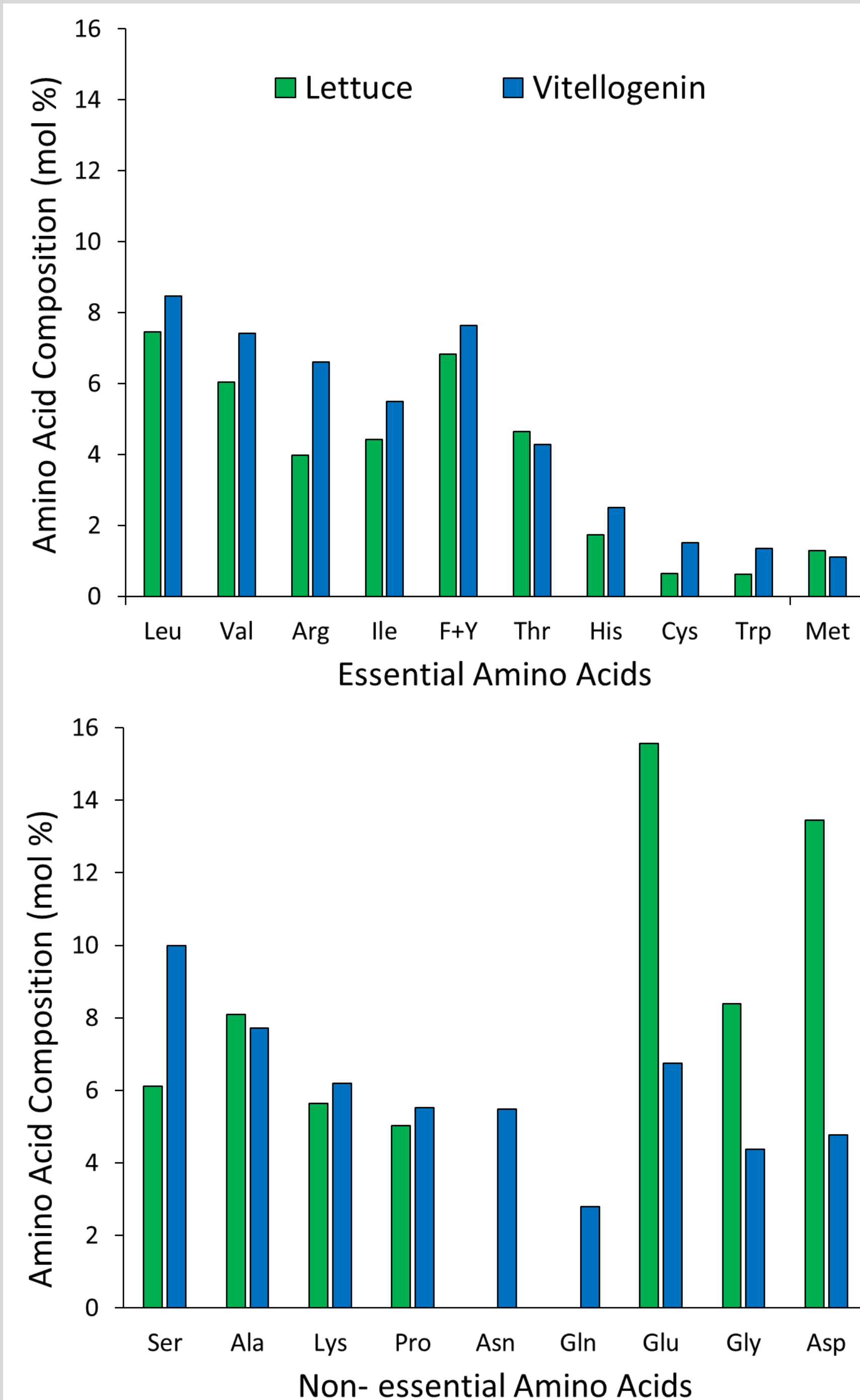
- Amino acid composition of the diets strongly effected reproductive tactics.
  - The Vg-balanced & 1gm group and the Unbalanced & 1gm group were isonitrogenous, so these groups consumed exactly the same total molar amount of amino acids.
- The nutrition of the Vg-balanced & 1gm diet was not sufficient to match the nutrition of the Buffer & ad libitum diet, which was allowed to show compensatory feeding.
- The Buffer & 1gm group laid despite our prediction that they would not, perhaps due to nutrients from the juvenile period.
- Body mass and fat body mass, both after oviposition, did not differ across the groups fed 1gm lettuce daily (data not shown). That is, somatic mass was not affected by the amino acid diets.
- The 40% weight gain serves as a threshold for the commitment to reproduction.

## Future directions

- Analyze storage proteins and hexamerins in hemolymph samples to test for a physiological threshold of reproduction.
- Use a similar experimental design to test: a) speed of development of juvenile males, and b) extension of lifespan without reducing reproduction (as in Piper et al. 2017).

## References

- Piper et al. 2017. Matching dietary amino acid balance to the in silico-translated exome optimizes growth and reproduction without cost to lifespan. *Cell Metabolism* 25: 610-621
- Juliano et al. 2004. Plasticity and canalization of insect reproduction: testing alternative models of life history transitions. *Ecology* 85: 2986-2996.
- Borst et al. 2000. Quantification of juvenile hormone III, vitellogenin, and vitellogenin-mRNA during the oviposition cycle of the lubber grasshopper. *Insect Biochem Mol Biol* 30: 813-19.
- Hatle et al. 2001. Maximum titers of vitellogenin and total hemolymph protein occur during the canalized phase of grasshopper egg production. *Physiol Biochem Zool* 74: 885-893.



**Figure 1. Amino acid composition of Romaine lettuce does not match the amino acid composition of vitellogenin (precursor to egg yolk protein) in grasshoppers.** Concentrations of amino acids in Romaine lettuce and vitellogenin (Vg). Lettuce contains 42% of the required Trp but 280% of the required Asp. F+Y = Phe & Tyr. For Asn and Gln, the content in lettuce is unknown.