# TECHNICAL BULLETIN

## HILUCIA™ PROTEIN: A HIGH-QUALITY INGREDIENT FOR PET FOOD

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## KEY POINTS

1. Hilucia™ Protein is a high-quality ingredient for pet foods, providing most of the indispensable amino acids in amounts required by both young and adult dogs and cats.

2. Both BSFL meal sources had superior or comparable amino acid composition and digestibility compared with chicken meal.

3. BSFL meal is a suitable substitute for chicken meal, one of the most common protein sources used in pet food.

## INTRODUCTION

The use of insects, such as black soldier fly larvae (*Hermetia illucens*; BSFL), as an alternative protein source for pet food has gained attention due to their high nutritional value, efficient resource utilization, low land and water requirements, and their highly efficient feed-to-protein conversion. In addition, BSFL contain unique components not commonly found in other foods, including chitin, lauric acid, and bioactive peptides. Innovafeed is a producer of Hilucia™ Protein, a defatted BSFL meal. Their mission is to build a more resilient food system by incorporating insects into the food chain. This study evaluated the chemical composition, amino acid digestibility, and protein quality of Hilucia™ Protein, using whole egg powder (WEP) and chicken meal (CM) as reference protein sources commonly found in pet foods.

## MATERIALS AND METHODS

### Trial Location

University of Illinois at Urbana-Champaign, Department of Animal Sciences & Division of Nutritional Sciences, College of Agricultural, Consumer and Environmental Sciences. **Principal Investigator:** Dr. Maria de Godoy

### Animals

A precision-fed rooster assay was conducted, using 20 cecectomized, single-comb White Leghorn roosters (five roosters per treatment). The precision-fed rooster assay has been validated as an effective canine in vivo digestibility model, demonstrating a high correlation with amino acid (AA) digestibility of ileal-cannulated adult dogs. The procedures and protocols involving animals in this study were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. All methodologies strictly adhered to the guidelines stipulated in the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals.

### Ingredients

Four ingredients were tested: two sources of black soldier fly larvae meal raised on corn substrate (BSFLM-C) or wheat substrate (BSFLM-W) as novel and alternative protein sources for pet food; and chicken meal (CM) and whole egg powder (WEP) were tested as conventional, high-quality protein ingredients commonly used in pet foods. The BSFLM-C was produced for this study in a pilot-scale facility using a dry processing method in which the larvae were dried and then the oil was extracted. The BSFLM-W was produced in a full-scale production facility using a wet processing method in which the larvae were ground, the oil was extracted, and then the meal was dried.

### Statistical Analysis

All data were analyzed in SAS using the Mixed Models procedure. Statistical significance was set at $P < 0.05$.

### Test Parameters

**Chemical composition:** The test ingredients were analyzed to determine dry matter, ash, organic matter, crude protein, gross energy, acid hydrolyzed fat, total dietary fiber, and amino acids.

**Digestible indispensable amino acid score-like values (DIAAS-like) were calculated as:**

$$\text{DIAAS-like (\%)} = \frac{\text{mg of digestible amino acid in 1g of dietary protein}}{\text{mg of amino acid in 1g of reference protein}} \times 100$$

The DIAAS-like values were calculated using standardized indispensable AA digestibility values obtained from the precision-fed rooster assays. The reference protein patterns (mg/g) were established by computing the quantity of each indispensable AA (mg) in 1 g of protein based on the canine and feline requirements during growth or maintenance from the Association of American Feed Control Officials (AAFCO). The indispensable AA (mg) present in 1 g of protein from each test ingredient was also calculated.

**Coefficients of standardized amino acid digestibility (SAAD) were calculated as:**

$$\text{SAAD (\%)} = \frac{\text{FAA} - \text{EAA} + \text{Endo AA}}{\text{FAA}} \times 100$$

FAA is the total amino acid fed; EAA is the total amino acid excreted after feeding; Endo AA is the endogenous loss of amino acid from fasted roosters.

## RESULTS

Table 1 shows the nutrient composition of the four protein sources, including crude protein and indispensable amino acids. CM contained the highest amount of protein (70.2%), WEP had the lowest protein content (51.2%), and the BSFLM-W and BSFLM-C contained 59.8% and 65.6% crude protein, respectively. The ash content of CM was highest among the protein sources as shown by the low organic matter content, whereas the BSFLM ingredients had the lowest ash levels.

Limiting amino acids are indispensable amino acids that are in the shortest supply in foods relative to requirements. Methionine is often the first limiting amino acid in pet foods. Methionine was highest in WEP at 1.62%, followed by 1.46% in CM, 1.09% in BSFLM-W, and 1.08% in BSFLM-C.

Table 2 shows the standardized amino acid digestibility for each of the four protein sources from the cecectomized rooster assay. The digestibility of all indispensable amino acids in WEP was significantly higher than that in BSFLM-W, BSFLM-C, and CM ($P < 0.05$). Standardized digestibility of arginine, histidine, and valine did not differ ($P > 0.05$) between BSFLM-C and WEP. In general, BSFLM-C had higher amino acid digestibility than BSFLM-W and CM. BSFLM-W and CM both displayed similar digestibility for indispensable amino acids. Overall, the AA digestibility for all indispensable amino acids exceeded 75% for all treatments, except for lysine for CM (73.6%), and methionine + cysteine for BSFLM-W (72.6%) and CM (72.3%).

**Table 1. Nutrient composition of protein ingredients**

| | BSFLM-W | BSFLM-C | CM | WEP |
|---|---|---|---|---|
| **INGREDIENT[1]** | | | | |
| Dry Matter, % | 96.1 | 96.9 | 94.4 | 94.4 |
| **%, DM basis** | | | | |
| Organic Matter, % | 93.6 | 93.3 | 86.2 | 95.6 |
| Crude protein, % | 59.8 | 65.6 | 70.2 | 51.2 |
| Acid-hydrolyzed fat, % | 16.0 | 10.8 | 15.9 | 43.9 |
| Total dietary fiber, % | 28.7 | 22.3 | ND[2] | ND[2] |
| Soluble dietary fiber | 1.6 | 2.3 | ND[2] | ND[2] |
| Insoluble dietary fiber | 27.2 | 20 | ND[2] | ND[2] |
| Gross energy, kcal/g[2] | 5.8 | 5.5 | 5.4 | 7.2 |
| **% OF TOTAL AMINO ACIDS, DM BASIS** | | | | |
| **Indispensable AA** | | | | |
| Arginine | 2.75 | 2.76 | 4.78 | 3.20 |
| Histidine | 1.64 | 1.94 | 1.59 | 1.24 |
| Isoleucine | 3.00 | 2.87 | 2.99 | 2.86 |
| Leucine | 4.58 | 4.31 | 5.11 | 4.32 |
| Lysine | 3.70 | 4.10 | 4.74 | 3.93 |
| Methionine | 1.09 | 1.08 | 1.46 | 1.62 |
| Phenylalanine | 2.68 | 2.82 | 2.87 | 2.72 |
| Threonine | 2.28 | 2.35 | 2.73 | 2.39 |
| Tryptophan | 1.11 | 1.05 | 0.67 | 0.89 |
| Valine | 4.32 | 3.74 | 3.59 | 3.44 |
| **Dispensable AA** | | | | |
| Alanine | 4.11 | 4.05 | 4.59 | 2.81 |
| Aspartic acid | 5.07 | 5.76 | 5.82 | 4.81 |
| Cysteine | 0.48 | 0.56 | 0.72 | 1.12 |
| Glutamic acid | 5.34 | 6.58 | 8.94 | 5.24 |
| Proline | 3.85 | 3.61 | 4.31 | 1.88 |
| Serine | 2.17 | 2.02 | 2.39 | 3.29 |
| Tyrosine | 3.77 | 3.86 | 2.49 | 2.09 |

*[1]BSFLM-W: black soldier fly larvae meal wheat substrate; BSFLM-C: black soldier fly larvae meal corn substrate; CM: Chicken Meal; WEP: Whole egg powder*
*[2]ND: Not determined*

**Table 2. Standardized amino acid digestibility of black soldier fly larvae meal reared on wheat substrate (BSFLM-W), black soldier fly larvae meal reared on corn substrate (BSFLM-C), chicken meal (CM), and whole egg powder (WEP) calculated using cecectomized rooster assay[1]**

| | BSFLM-W | BSFLM-C | CM | WEP |
|---|---|---|---|---|
| **Indispensable AA** | | | | |
| Arginine | 84.03[b] | 89.98[a] | 84.00[b] | 91.85[a] |
| Histidine | 78.31[b] | 80.13[ab] | 77.58[b] | 83.72[a] |
| Isoleucine | 82.68[c] | 87.11[b] | 81.20[c] | 94.71[a] |
| Leucine | 82.68[c] | 87.57[b] | 81.94[c] | 94.81[a] |
| Lysine | 79.74[b] | 78.11[bc] | 73.55[c] | 91.02[a] |
| Methionine | 84.96[c] | 88.40[b] | 83.42[c] | 96.24[a] |
| Methionine + Cysteine | 72.58[b] | 76.50[b] | 72.33[b] | 91.50[a] |
| Phenylalanine | 81.41[c] | 86.70[b] | 81.31[c] | 94.77[a] |
| Phenylalanine + Tyrosine | 83.38[c] | 88.03[b] | 80.72[c] | 93.89[a] |
| Threonine | 80.46[b] | 81.56[b] | 76.64[b] | 91.58[a] |
| Tryptophan | 89.22[b] | 88.95[b] | 82.30[b] | 94.85[a] |
| Valine | 86.46[b] | 90.21[ab] | 80.17[c] | 94.31[a] |
| **Select dispensable AA** | | | | |
| Alanine | 84.28[b] | 86.18[b] | 79.64[c] | 93.49[a] |
| Aspartic acid | 72.00[c] | 79.92[b] | 62.03[d] | 90.57[a] |
| Cysteine | 44.31[b] | 53.35[b] | 49.81[b] | 84.66[a] |
| Glutamic acid | 79.05[c] | 83.97[b] | 77.84[c] | 91.62[a] |
| Glycine | 46.74[b] | 61.47[ab] | 72.32[a] | 59.94[ab] |
| Proline | 82.04[ab] | 83.19[a] | 75.62[b] | 89.09[a] |
| Serine | 76.28[bc] | 80.14[ab] | 72.76[c] | 83.22[a] |
| Tyrosine | 84.78[b] | 88.99[a] | 80.05[c] | 92.74[a] |

*[1]n = 5 cecectomized roosters per select protein*
*[a-c]Means within a row with different superscripts differed at $P < 0.05$*

A DIAAS-like value greater than 100% is considered to be of high quality, a value between 70% and 100% is considered good quality, a value between 70% and 50% is considered to be of moderate quality, and a value below 50% is considered to be of poor quality. The DIAAS-like values using AAFCO nutritional guidelines for puppies, adult dogs, kittens, and adult cats are presented in Table 2 (i-iv).

The limiting amino acid is the indispensable amino acid with the lowest DIAAS-like value. For BSFLM-W and BSFLM-C, the limiting amino acids were methionine + cystine for puppies, adult dogs, and kittens, while for adult cats, the limiting amino acids were phenylalanine + tyrosine. For chicken meal, threonine was the limiting amino acid for puppies, methionine + cystine for adult dogs and kittens, and phenylalanine + tyrosine for adult cats. For WEP, threonine was the limiting amino acid for puppies, but there were no limiting amino acids for adult dogs, kittens, and adult cats, as DIAAS-like values exceeded 100.

**Table 3. Digestible indispensable amino acid (DIAAS) values for black soldier fly larvae meal reared on wheat substrate (BSFLM-W), black soldier fly larvae meal reared on corn substrate (BSFLM-C), chicken meal (CM), and whole egg powder (WEP) compared with AAFCO requirements for (i) puppies (ii) adult dogs (iii) kittens (iv) adult cats**

| (i) DIAAS values for puppies | BSFLM-W | BSFLM-C | CM | WEP |
|---|---|---|---|---|
| **AMINO ACID** | | | | |
| Arginine | $85^{b}$ | $86^{b}$ | $129^{a}$ | $130^{a}$ |
| Histidine | $108^{b}$ | $122^{a}$ | $90^{c}$ | $104^{b}$ |
| Isoleucine | $128^{b}$ | $121^{c}$ | $110^{d}$ | $169^{a}$ |
| Leucine | $108^{b}$ | $101^{c}$ | $104^{bc}$ | $141^{a}$ |
| Lysine | $121^{b}$ | $123^{b}$ | $125^{b}$ | $176^{a}$ |
| Methionine | $98^{c}$ | $94^{c}$ | $112^{b}$ | $197^{a}$ |
| Phenylalanine | $97^{b}$ | $101^{b}$ | $91^{c}$ | $138^{a}$ |
| Threonine | $65^{b}$ | $64^{b}$ | $65^{b}$ | $93^{a}$ |
| Tryptophan | $174^{b}$ | $161^{c}$ | $86^{d}$ | $183^{a}$ |
| Valine | $202^{b}$ | $171^{b}$ | $136^{c}$ | $212^{a}$ |
| Methionine + Cysteine | $60^{c}$ | $62^{c}$ | $73^{b}$ | $159^{a}$ |
| Phenylalanine + Tyrosine | $89^{b}$ | $90^{b}$ | $71^{c}$ | $116^{a}$ |

*$^{a-d}$Means within a row with different superscripts differed at $P < 0.05$*

| (ii) DIAAS values for adult dogs | BSFLM-W | BSFLM-C | CM | WEP |
|---|---|---|---|---|
| **AMINO ACID** | | | | |
| Arginine | $133^{b}$ | $135^{b}$ | $203^{a}$ | $204^{a}$ |
| Histidine | $200^{b}$ | $226^{a}$ | $167^{c}$ | $194^{c}$ |
| Isoleucine | $192^{b}$ | $181^{c}$ | $164^{d}$ | $253^{a}$ |
| Leucine | $164^{b}$ | $153^{c}$ | $158^{bc}$ | $214^{a}$ |
| Lysine | $138^{b}$ | $140^{b}$ | $142^{b}$ | $201^{a}$ |
| Methionine | $83^{c}$ | $80^{c}$ | $95^{b}$ | $168^{a}$ |
| Phenylalanine | $143^{b}$ | $150^{b}$ | $134^{c}$ | $203^{a}$ |
| Threonine | $112^{b}$ | $110^{b}$ | $112^{b}$ | $162^{a}$ |
| Tryptophan | $174^{b}$ | $161^{c}$ | $86^{d}$ | $183^{a}$ |
| Valine | $224^{a}$ | $190^{b}$ | $151^{c}$ | $235^{a}$ |
| Methionine + Cysteine | $52^{c}$ | $53^{c}$ | $63^{b}$ | $137^{a}$ |
| Phenylalanine + Tyrosine | $125^{b}$ | $126^{b}$ | $100^{c}$ | $163^{a}$ |

*$^{a-d}$Means within a row with different superscripts differed at $P < 0.05$*

| (iii) DIAAS values for kittens | BSFLM-W | BSFLM-C | CM | WEP |
|---|---|---|---|---|
| **AMINO ACID** | | | | |
| Arginine | $91^{b}$ | $92^{b}$ | $139^{a}$ | $140^{a}$ |
| Histidine | $191^{b}$ | $217^{a}$ | $160^{c}$ | $186^{b}$ |
| Isoleucine | $217^{b}$ | $205^{c}$ | $186^{d}$ | $286^{a}$ |
| Leucine | $145^{b}$ | $136^{c}$ | $140^{bc}$ | $189^{a}$ |
| Lysine | $121^{b}$ | $123^{b}$ | $125^{b}$ | $176^{a}$ |
| Methionine | $73^{c}$ | $71^{c}$ | $84^{b}$ | $149^{a}$ |
| Phenylalanine | $206^{b}$ | $216^{b}$ | $193^{c}$ | $294^{a}$ |
| Threonine | $123^{b}$ | $121^{b}$ | $123^{b}$ | $177^{a}$ |
| Tryptophan | $270^{b}$ | $151^{c}$ | $134^{d}$ | $284^{a}$ |
| Valine | $267^{a}$ | $227^{b}$ | $180^{c}$ | $280^{a}$ |
| Methionine + Cysteine | $51^{c}$ | $52^{c}$ | $62^{b}$ | $135^{a}$ |
| Phenylalanine + Tyrosine | $80^{b}$ | $81^{b}$ | $64^{c}$ | $105^{a}$ |

*$^{a-d}$Means within a row with different superscripts differed at $P < 0.05$*

| (iv) DIAAS values for adult cats | BSFLM-W | BSFLM-C | CM | WEP |
|---|---|---|---|---|
| **AMINO ACID** | | | | |
| Arginine | $94^{b}$ | $95^{b}$ | $144^{a}$ | $145^{a}$ |
| Histidine | $177^{b}$ | $200^{a}$ | $148^{c}$ | $171^{b}$ |
| Isoleucine | $203^{b}$ | $191^{c}$ | $173^{d}$ | $267^{a}$ |
| Leucine | $130^{b}$ | $121^{c}$ | $125^{bc}$ | $169^{a}$ |
| Lysine | $151^{b}$ | $154^{b}$ | $156^{b}$ | $221^{a}$ |
| Methionine | $197^{c}$ | $191^{c}$ | $227^{b}$ | $399^{a}$ |
| Phenylalanine | $221^{b}$ | $232^{b}$ | $207^{c}$ | $315^{a}$ |
| Threonine | $107^{b}$ | $105^{b}$ | $107^{b}$ | $154^{a}$ |
| Tryptophan | $251^{b}$ | $233^{c}$ | $124^{d}$ | $264^{a}$ |
| Valine | $286^{a}$ | $243^{b}$ | $193^{c}$ | $300^{a}$ |
| Methionine + Cysteine | $121^{c}$ | $125^{c}$ | $147^{b}$ | $321^{a}$ |
| Phenylalanine + Tyrosine | $87^{b}$ | $88^{b}$ | $70^{c}$ | $114^{a}$ |

*$^{a-d}$Means within a row with different superscripts differed at $P < 0.05$*

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Dr. Jennifer Adolphe is responsible for providing technical support, nutrition training, formulation services and new product development for ADM's customers in the pet food industry. She graduated with her PhD in companion animal nutrition from the Western College of Veterinary Medicine at the University of Saskatchewan. She has a Master of Science degree in human nutrition and previously worked as a registered dietitian. Dr. Adolphe has more than 20 years of experience in both human and companion animal nutrition and previously held positions at two leading pet food companies in Canada. In addition to her role at ADM, she is an adjunct professor at the University of Saskatchewan and is pursuing a Master of Business Administration through Penn State University