A COMPARISON OF NITROGEN EXCRETORY PRODUCTS OF HONEY BEES MAINTAINED ON VARIOUS PROTEIN SOURCES

by

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W. F. McCaughhey

Date

Associate Professor of Biochemistry and Nutrition

May 10, 1960

W. F. McCaughhey
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INTRODUCTION

The natural diet of the honey bee (*Apis mellifera*) consists of honey and pollen, of which honey furnishes carbohydrate for energy, and pollen contains protein and other nutrients necessary for tissue building and the other life processes of this insect. Various protein-containing materials have been substituted for pollen in the diet of the honey bee: soy bean flour, egg albumin, and casein were among the more satisfactory of these substances (7, 11, 13, 24, 26, 28). However, the present criteria for diet efficiency (development of the pharyngeal gland, longevity, etc.) have shown that none of these substitutes is as effective as pollen (7, 26).

The research reported here was designed to demonstrate a relationship between pollen and other protein-containing diets and the end products of nitrogen metabolism in the excreta of the honey bee. The establishment of the quantitative values for the nitrogenous constituents of honey bee excreta would provide a means of gauging the efficiency of substitute diets in maintaining normal growth. Also, these values are of academic interest, since there has been no work
reported in the literature concerning the nitrogen excretion pattern of honey bees fed either natural or synthetic diets.
REVIEW OF LITERATURE

Urayov (30), in a review of the literature concerning insect nutrition and metabolism, concluded that insects required definite nitrogen food components. Haydak (10, 12) demonstrated an increase in nitrogen content and dry weight during the first five days after emergence of the honey bee. Normal development of the worker bees was observed when colonies were fed pollen substitutes. This conclusion was based on investigations which demonstrated that the body weight and nitrogen content of the honey bee showed increases similar to those observed when pollen was fed. Haydak (11, 13) described various feeding experiments with honey bees from which he concluded that soy bean meal was the most effective brood producing pollen substitute. Mykola (24) studied pollen substitutes in relation to brood production. A diet which consisted of soy flour, commercial casein, dried brewers yeast, dried skim milk, and dried egg yolk was more successful than pollen in brood production. De Groot (7) showed that soy flour does not contain adequate methionine for satisfactory growth of the honey bee. He also maintained caged bees on various diets and used mortality rate as a measure of the effect of diet composition on longevity. Although a
wide variety of protein sources was studied, pollen was consistently the best source of nutrients. There was a considerable variation in the nutritional value of pollen from different plant species.

Development of the pharyngeal gland, or brood food gland, has been used as a criterion for the nutritional value of pollen and other proteinaceous food (7). The ovaries and fat body may be used for the same purpose (20, 21). In this laboratory unpublished results have shown an inverse relationship between the quantity of excretory nitrogen and pharyngeal gland development. In these studies, an egg albumin diet was shown to be as effective as pollen. However, Haydak (11) noted that development of the pharyngeal gland could not serve alone as an indication of the suitability of any food as a pollen substitute, since such a gland may secrete a product that is lacking in a factor or factors essential to normal development. It is therefore necessary that other methods of growth determination be developed.

The present knowledge of amino acid and protein requirements of the honey bee is summarized by Albritton (1) and by De Groot (7), who showed the amino acids essential for honey bee growth to be arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine.

McEnroe and Forgash (22, 23) have established the synthesis of uric acid in the fat body of the American roach. Ludwig and Cullen
(19), in their work with the Japanese beetle, found that during starvation the uric acid content of the blood decreased, but the purine ring was not destroyed. It was concluded that, once formed, the purine ring of uric acid is unlikely to be destroyed in the insect. Trizian, Irrevirre, and Stahler (29) demonstrated that after emergence, mosquitoes fed sucrose showed a decrease of excreted uric acid with time. During the first week on this diet, the insects showed considerable loss of body nitrogen, which then remained constant. However, the uric acid content of the excreta continued to decrease from an initially high concentration. The lack of protein was demonstrated during the first week when the uric acid concentration of the excreta was high due to the loss of body nitrogen. Thus the uric acid concentration in the excreta can be an indication of the quality of diet, since in the event of a dietary nitrogen abnormality tissue nitrogen is utilized by the insects (18). The use of tissue nitrogen implies that more nitrogen is being excreted than is being utilized for tissue synthesis, with no growth as a result.

The simplest form in which nitrogen can be excreted is ammonia, which is a toxic substance. Therefore, in many insects free ammonia is excreted in the form of ammonia salts. Lennox (16) showed that 10% of the dried excreta of Lucilla was free ammonia. Brown (3) found that 0.6-0.7 mg. of ammonia nitrogen was excreted for every 10.6-15.6 mg. of uric acid nitrogen in the grasshopper. Wigglesworth (31) could find
no ammonia at any stage of growth in the urine of the reduviid bug. Staddon (27) demonstrated that ammonia constitutes 90% of the nitrogen excreted during starvation in larvae of *Sialis lutaria*. Craig (6) postulated in a review of the literature that if excretion is prevented, there is no accumulation of ammonia; and it may be transformed into some other nitrogenous compound.

Urea is present in the excreta of insects in trace quantities only (16, 31). Support for the existence of the urea cycle as the mechanism for urea formation in insects is enlightened by the finding of free ornithine and arginine in all stages of development of various insects. It is suspected that urea is utilized in the formation of uric acid, but as yet, this hypothesis is unsupported (6).

Small amounts of alpha-amino acid nitrogen have been reported in excreta of various species of insects. However, this is not generally found in excreta unless amino acids happen to be present in the blood in great excess (25). Brown (4, 5) determined that there was 0.35% amino nitrogen in dried excreta of the flesh fly. Wigglesworth (31) found small but undetermined quantities in the reduviid bug.

Creatine and creatinine are absent in the excreta of the flesh fly (4, 5), but creatine appears in very small quantities in the urine of the reduviid bug (31).
EXPERIMENTAL METHODS

The diets

The pollen and pollen substitute diets each contained 5% protein. The exact composition of each diet is indicated in Table 1. Nulomoline\(^1\) was the carbohydrate source, and in each of the pollen substitute diets, accounted for 75% of the total diet. Alphacel (a non-nutritive cellulose)\(^2\) and water were added to each diet in such a proportion that the consistency and texture was palatable to the honey bee. The pollen source was fresh bee-collected dandelion pollen (*Taratacum officinale*). The egg albumin\(^3\), soy hydrolysate\(^4\), and casein\(^5\) were procured from commercial sources. The negative control diet contained no protein.

\(^1\) Nulomoline consists of 24% water and 76% of a 1:1 mixture of dextrose and levulose. This carbohydrate source contains trace amounts of copper and iron. The Nulomoline Co., Los Angeles, Calif.

\(^2\) Nutritional Biochemicals Corporation, Cleveland, Ohio.

\(^3\) Ibid.

\(^4\) Ibid.

\(^5\) Local supply house.
<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein Source</th>
<th>Nulomoline</th>
<th>Alphacel</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen (9.74% Protein)</td>
<td>48.26</td>
<td>43.10</td>
<td>8.00</td>
<td>1.64</td>
</tr>
<tr>
<td>Egg Albumin (75% Protein)</td>
<td>7.00</td>
<td>75.00</td>
<td>8.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Soy Hydrolysate (52.6% Protein)</td>
<td>9.50</td>
<td>75.00</td>
<td>6.50</td>
<td>9.00</td>
</tr>
<tr>
<td>Casein (95% Protein)</td>
<td>5.30</td>
<td>75.00</td>
<td>10.50</td>
<td>9.20</td>
</tr>
<tr>
<td>Negative Control</td>
<td>---</td>
<td>75.00</td>
<td>13.00</td>
<td>12.00</td>
</tr>
</tbody>
</table>
Collection of the honey bees

Brood frames were taken from the hive. All cells containing honey and pollen were covered with bees wax. A screen wire was placed around the brood cells. Upon emergence the adult honey bee was confined to the screened area and could obtain no food. The brood frames were then incubated at $33 \pm 2^\circ C$. at a relative humidity of $25 \pm 3\%$. Each day the adult honey bees which had emerged from the brood cells in the preceding twenty-four hours were collected and put into individual cages to commence the diet period.

The cages

The basic design of the cages was developed by Standifer$^1$. The dimensions of the inside of the cages were 6.5 x 5.5 x 6.5 inches. On two sides of the cage there were two sliding glass panels (2.5 x 7 inches) to allow for observation, changing of diet, and removal of dead honey bees. In each corner of the top were openings (1.5 x 1.5 inches) for insertion of water vials. Water was contained in glass vials with a small hole in the cap from which the bees could obtain an ample supply. The floor of the cage was lined with a waterproof paper. The

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$^1$ Bee Culture Laboratory, Entomological Research Division, U.S. Dept. of Agr., Tucson, Arizona.
diets were contained in glass vials 9 x 30 mm. in size. Eighteen of
these vials were set in a wooden block in the top center of the cage.

**Maintenance of honey bees on diets**

Between 150 and 250 newly-emerged adult bees were put in
each cage which was deposited in a dark incubator maintained at
33 ± 2°C, and a relative humidity of 25 ± 3%. Water and diet were
provided *ad libitum* at all times. The honey bees were observed daily
for activity and mortality. The seven-day period of incubation was
decided upon for two reasons: (1) at this time the rectum of the pollen-
fed honey bee is filled to capacity, and (2) normally, this is the time in
a worker bee's life when she is fully developed to perform her duties.

**Collection of excretory material for analysis**

The honey bee naturally excretes waste products in flight,
therefore they were very reluctant to defecate in the cages. Removal
of the excreta-containing rectum was therefore the most practical
method for the collection of materials for analysis. It was assumed
that nitrogen content of the rectal tissue would be constant for the
various diets. Immediately after evisceration the recta were com-
posited and oven-dried overnight at 80°C. The dried material was then
ground to a fine powder in a mortar.
Analytical methods

Preparation of Extracts

After the excretory material was oven-dried, 0.25 g. was ground in a mortar together with 1 ml. of saturated lithium carbonate solution and diluted to 25 ml. with distilled water (5). This extract was diluted, if necessary, for each particular analytical method.

Total Nitrogen Determinations—Microkjeldahl Method (15)

Fifty mg. of dried excreta was digested in a Kjeldahl flask in 2.0 ml. sulfuric-phosphoric acid (3:1 by volume). The digested sample was transferred to the distillation flask, and 10 ml. of 40% sodium hydroxide was added. The liberated ammonia was distilled into 10 ml. of 2.0% boric acid which contained a modified methyl red indicator. After a color change from purple to green, the distillation was continued for three minutes. The distillate was then back-titrated with 0.01 N hydrochloric acid to the original purple color.

Uric Acid Determinations—Direct Colorimetric Method (2)

One ml. of the excreta extract was diluted to 5.0 ml. with water. Exactly one ml. of this solution and 5.0 ml. of a 5.0% sodium cyanide solution, followed by 1 ml. of improved uric acid reagent (Folin's modification of arsenophosphotungstic acid), were diluted to 50 ml. in a volumetric flask. The contents of the flask were mixed
by gently shaking, and at the end of 5 minutes diluted to the 50 ml.
mark with water and mixed. The blue solution was read in an Evelyn
colorimeter at 520 m\(\mu\). The standard curve for uric acid is shown in
Figure 1.

**Alpha-Amino Nitrogen Determination**  
**Ninhydrin Method (14, 32)**

Exactly one ml. of excreta extract was added to 0.5 ml. of
citrate buffer, 0.2 ml. of 5.0% ninhydrin-methylcellosolve, and 1.0 ml.
of 0.0002 M potassium cyanide-methylcellosolve. The solutions were
heated at 100°C. for 15 minutes and cooled under tap water for 15
minutes. To the solutions, 7.0 ml. of 60% ethyl alcohol was then added.
The optical density was determined at 620 m\(\mu\). The standard curve for
alpha-amino nitrogen, using leucine as a standard, is shown in Figure 2.

**Determination of Creatinine and Creatine**  
**Colorimetric Method (9)**

Creatinine

Exactly three ml. of excreta extract was pipetted into a 100-ml.
volumetric flask. Twenty ml. of 1.0% picric acid and 1.5 ml. of 10%
sodium hydroxide were added to the extract with swirling, and allowed
to stand for 15 minutes. The solution was diluted to volume with water,
and the optical density was read at 490 m\(\mu\) and compared with a standard.
Figure 1. Calibration curve for uric acid by the direct colorimetric method.
Figure 2. Calibration curve for alpha-amino nitrogen by the ninhydrin method.
Creatine

Three ml. of extract was pipetted into a 250 ml. beaker; 20 ml. of 1.0% picric acid and 150 ml. of water were added to the extract. The solution was boiled gently for 45 minutes and then more vigorously until the volume was reduced to approximately 3 ml. This volume, with rinsings, was decanted into a 100 ml. volumetric flask, and 1.5 ml. of 19% sodium hydroxide was added. The solution was swirled gently and allowed to stand for 15 minutes. After dilution to 100 ml. with water the optical density was read at 490 m\(\mu\) compared with a standard. The standard curve for creatinine or creatine is shown in Figure 3.

Urea and Ammonia Determination
Urease Colorimetric Method (8)

Into a test tube containing 2.8 ml. of water, 3.0 ml. of the extract and 3.0 ml. of the urease-glycine solution were pipetted. After incubation of the mixture at 50°C. for 15 minutes, 0.4 ml. of 0.3 N barium hydroxide and 0.4 ml. of 5% zinc sulfate were added, thoroughly mixed, and centrifuged at 1500 R.P.M. for five minutes. Five ml. of the supernatant was pipetted into a tube containing 3.0 ml. of water. Two drops of iodine solution were added followed by 1.0 ml. of Nessler solution. The optical density was determined at 480 m\(\mu\) and compared with a standard urea solution. The standard curve for urea and ammonia is shown in Figure 4.
Figure 3. Calibration curve for creatine or creatinine by the Folin method.
Figure 4. Calibration curve for urea and ammonia nitrogen by the Nesslerization method.
Statistical method

Five series of caged bees were maintained on the five diets for a seven-day period. A series consisted of five cages, each containing one of the diets. The eviscerated recta were composited from each cage.

Confidence intervals were calculated for each nitrogenous product from each diet. The confidence intervals were determined with a 95% confidence coefficient.(17).
RESULTS AND DISCUSSION

Uric acid nitrogen

Uric acid is the chief end product of nitrogen catabolism in the honey bee. The quality of the test diets varied inversely with the uric acid concentration of the excreta. The egg albumin excreta contained the least amount of uric acid, which was not significantly different from that of the pollen excreta as shown in Table 2. From these determinations it is suggested that there was much catabolism of nitrogenous products in bees fed the egg albumin diet as there was in bees fed the pollen diet. It would appear that egg albumin protein is as efficient as pollen protein for maintenance of normal growth in the honey bee.

The casein and soy hydrolysate excreta were not significantly different from each other, but had a significantly higher uric acid nitrogen content than the pollen excreta. A comparison of the negative control excreta indicates that only a part of the dietary nitrogen was used for the anabolism of body tissue.
Table 2
Uric Acid Nitrogen in Honey Bee Excreta.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Uric Acid Nitrogen</th>
<th>Total Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g./100 g. Excretal</td>
<td>g./100 g.</td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>1.55 ± 0.13</td>
<td>47.69</td>
</tr>
<tr>
<td>Pollen</td>
<td>1.60 ± 0.16</td>
<td>61.30</td>
</tr>
<tr>
<td>Casein</td>
<td>2.22 ± 0.15</td>
<td>96.52</td>
</tr>
<tr>
<td>Soy Hydrolysate</td>
<td>2.48 ± 0.13</td>
<td>62.00</td>
</tr>
<tr>
<td>Negative Control</td>
<td>3.09 ± 0.13</td>
<td>96.27</td>
</tr>
</tbody>
</table>

A continuous line beside two or more values indicates that they are not significantly different.
The total nitrogen accounted for by the uric acid determinations showed that egg albumin excreta was extremely low. Soy hydrolysate and pollen excreta were somewhat higher with little difference in these values. The casein excreta was almost the same as the negative control, which was very high.

It has been demonstrated in the Japanese beetle that once the purine ring of uric acid is formed, it will not be broken (22, 23). When a large amount of nitrogen is excreted, rather than incorporated into body tissues, the uric acid content of the excreta will reflect this nitrogen imbalance. When the uric acid content of the excreta of the bees fed a pollen substitute is higher than for bees fed pollen, it is reasonable to assume that the diet was deficient in certain nitrogenous constituents and that tissue nitrogen is utilized. If this is the case in honey bees, the quantity of uric acid in the excreta is an excellent indication of tissue catabolism. It is therefore likely that a comparison of the quality of protein diets can be made on the basis of the uric acid content of the excreta.

**Alpha-amino nitrogen**

The values for the alpha-amino nitrogen determinations are shown in Table 3. By a comparison of the alpha-amino nitrogen in the
Table 3
Alpha-Amino Nitrogen in Honey Bee Excreta.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Alpha-Amino Nitrogen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g./100 g. Excreta</td>
<td>g./100 g. Total Nitrogen</td>
</tr>
<tr>
<td>Negative Control</td>
<td>.0437 ± .0156</td>
<td>1.36</td>
</tr>
<tr>
<td>Casein</td>
<td>.0674 ± .0195</td>
<td>2.93</td>
</tr>
<tr>
<td>Pollen</td>
<td>.1596 ± .0163</td>
<td>6.11</td>
</tr>
<tr>
<td>Soy Hydrolysate</td>
<td>.1674 ± .0285</td>
<td>4.18</td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>.2144 ± .0229</td>
<td>6.60</td>
</tr>
</tbody>
</table>

1 A continuous line beside two or more values indicates that they are not significantly different.
pollen excreta and the negative control, it is observed that the quality of diet varies with the amino acid concentration in the excreta.

Casein was not significantly different from the negative control; therefore, this diet is the least efficient diet of the pollen substitutes. Soy hydrolysate was found to be as effective as pollen because these values were not significantly different. Egg albumin was the best of the pollen substitutes because the amino acid concentration in the excreta was high.

The ease of protein hydrolysis could be responsible for the variation of the amino acids in the excreta. If this were the case, it is indicated that egg albumin was more readily hydrolyzed to amino acids than the proteins of the other diets fed. Since the proteins of soy flour were previously hydrolyzed, it is assumed that the proteolytic enzyme systems were not needed for the digestion of this diet. The pollen excreta was not significantly different in alpha-amino nitrogen content from the soy hydrolysate. If it is assumed that the same amount of diet was consumed in each of the above cases, the results would show that pollen, soy hydrolysate, and egg albumin are completely hydrolyzed. Since the alpha-amino nitrogen value for casein was not significantly different from the negative control, casein was apparently difficult for the bee to degrade.
Completely contrary to the above proposition would be the hypothesis that the high content of alpha-amino nitrogen in the excreta would indicate an excess of protein which would induce a protein poisoning caused by undigested material accumulated in the gut (9). If this were the case, egg albumin was probably the least effective diet. Soy hydrolysate was normal because it was not significantly different from pollen.

It has been shown by the work of Ramsey (25) that urine from the Malpighian tubes of C. morosus contains amino acids which are reabsorbed in the rectal region and returned to the blood. This dynamic action would give an indication of the availability of amino acids for metabolism. On this basis egg albumin would be the most effective diet for supplying amino acids to the body. Soy hydrolysate and pollen would be good sources of amino acids, and casein would be very poor.

Creatine and creatinine nitrogen

In mammals the urinary output of creatinine is independent of the protein intake and is related directly to tissue creatine; however, it is possible that the honey bee shows a more direct relationship between protein intake and creatine or creatinine excretion. According to the literature already cited (4, 5, 16), creatine and creatinine are found very infrequently in the excreta of insects, and then only in trace
amounts. In this investigation these two substances accounted for 9% of the total nitrogen in the pollen excreta and 33% of the total nitrogen in the negative control excreta. According to data in Table 4, there were marked variations in the amounts of either creatine or creatinine per 100 g. of excreta of honey bees fed the pollen substitute diets. Egg albumin excreta contained the highest concentration of creatinine which was not significantly different from the amounts in the soy hydrolysate and negative control excreta. Creatinine was lowest in the pollen excreta and was significantly lower than that in casein excreta.

The pollen excreta contained the least creatine. The negative control contained about six times as much creatine as the pollen excreta. Soy hydrolysate was not significantly different than pollen in creatine content. Casein and egg albumin contained increasingly larger amounts, and about 20% of the total nitrogen of the negative control was creatine nitrogen.

It was shown in these studies that the creatine and creatinine of the excreta were affected by the diets. It is possible that the protein intake did not affect the excretion of these nitrogen excretory products, and that this variation may be due to undetermined abnormalities in the substitute diets.
Table 4

Creatine and Creatinine Nitrogen in Honey Bee Excreta.

<table>
<thead>
<tr>
<th>Diets</th>
<th>g./100 g. Excreta 1</th>
<th>g./100 g. Total Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Creatine Nitrogen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen</td>
<td>.097 ± .027</td>
<td>3.71</td>
</tr>
<tr>
<td>Soy Hydrolysate</td>
<td>.120 ± .046</td>
<td>3.25</td>
</tr>
<tr>
<td>Casein</td>
<td>.260 ± .037</td>
<td>11.30</td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>.535 ± .008</td>
<td>16.46</td>
</tr>
<tr>
<td>Negative Control</td>
<td>.666 ± .029</td>
<td>20.75</td>
</tr>
<tr>
<td><strong>Creatinine Nitrogen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen</td>
<td>.138 ± .017</td>
<td>5.29</td>
</tr>
<tr>
<td>Casein</td>
<td>.257 ± .001</td>
<td>11.17</td>
</tr>
<tr>
<td>Soy Hydrolysate</td>
<td>.376 ± .010</td>
<td>9.40</td>
</tr>
<tr>
<td>Negative Control</td>
<td>.398 ± .037</td>
<td>12.40</td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>.424 ± .044</td>
<td>13.04</td>
</tr>
</tbody>
</table>

1 A continuous line beside two or more values indicates that they are not significantly different.
Urea and Ammonia Nitrogen

There was no indication of any ammonia or urea in the excreta of bees fed either pollen or pollen substitute diets. It is very probable that free ammonia had formed some insoluble salts. Because of the accumulation of excreta in the rectum and the small amount of voluntary defecation of the honey bee during the period of which the diets were fed, Craig's (6) postulate that if excretion is prevented there is no accumulation of ammonia, is substantiated in this study. There was no analysis for free ornithine or arginine in the honey bee excreta, but the absence of free urea indicates the urea cycle is absent in the honey bee.

Total nitrogen

Total nitrogen in the excreta was determined by the micro-kjeldahl procedure as previously described. Since it was more practical to excise the entire rectum containing the excreta than to collect the defecated excreta, it was necessary to determine the amount of total nitrogen contributed by the rectal tissue. A preliminary experiment showed this to be negligible, amounting to only 0.08% of the total nitrogen in the excreta. Although this was determined only for bees fed a pollen diet, it can be assumed that rectal tissue of bees fed the non-pollen diets would also show a negligible nitrogen content.
In Table 5 are listed the total nitrogen values of excreta from bees fed the various test diets. Also included in this table are the accounted nitrogen values which represent a summation of the individual nitrogenous constituents determined in this work. The difference between total and accounted nitrogen then represents either undetermined nitrogen or analytical error.

The total excreted nitrogen of honey bees fed the soy hydrolysate, egg albumin, and the negative control was higher in each case than for bees fed pollen. This would indicate that nitrogen in the excreta of bees fed the non-pollen diets came from either or both of two abnormal sources: (1) non-metabolized dietary nitrogen and (2) tissue nitrogen. Since the negative control did not contain a protein source, the nitrogen in the excreta must have come from the tissue nitrogen. The observation that the negative check excreta and the egg albumin excreta were not significantly different in total nitrogen concentration does not imply that the egg albumin excretory nitrogen also comes from the body tissues. The previous nitrogen excretory product determinations indicate that the proteins were readily hydrolyzed to amino acids; thus, it is most probable that a significant portion of the egg albumin excretory nitrogen came from non-metabolizable nitrogenous substances. Soy hydrolysate was previously hydrolyzed; therefore, by the above proposition, the excreta of this diet also contained a considerable portion of
Table 5

Total and Accounted Nitrogen in Honey Bee Excreta.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Total Nitrogen</th>
<th>Accounted Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g./100 g. Excreta</td>
<td>g./100 g. Total Nitrogen</td>
</tr>
<tr>
<td>Casein</td>
<td>2.30 ± .18</td>
<td>121.92</td>
</tr>
<tr>
<td>Pollen</td>
<td>2.16 ± .09</td>
<td>76.41</td>
</tr>
<tr>
<td>Negative Control</td>
<td>3.21 ± .23</td>
<td>130.78</td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>3.25 ± .13</td>
<td>83.79</td>
</tr>
<tr>
<td>Soy Hydrolysate</td>
<td>4.00 ± .13</td>
<td>78.83</td>
</tr>
</tbody>
</table>

1 A continuous line beside two or more values indicates that they are not significantly different.

2 Accounted nitrogen is the summation of the individual nitrogenous constituents determined.
non-metabolizable nitrogen. To further substantiate the hypothesis that soy hydrolysate and egg albumin contained non-metabolizable nitrogen, it is observed in Table 5 that the accounted nitrogen in the pollen substitute excreta in this study was much lower than that of the negative control excreta and approximately the same as the pollen excreta. This shows that although the proteins were hydrolyzed with ease, nitrogenous products were formed by the honey bee that were not included in the products determined in this study. Thus, the total nitrogen of soy hydrolysate and egg albumin excreta was a primary factor of the diets and not tissue nitrogen.

The total nitrogen content of the casein excreta was lower and significantly different from the pollen excreta. This shows that the casein diet was the most efficient of the diets tested; but if the accounted nitrogen is observed, it is indicated that the nitrogen excretory products which were not accounted for in the rest of the excreta were absent in casein excreta. Therefore, casein excreta shows an abnormality in the nitrogen metabolism.
SUMMARY AND CONCLUSION

Analysis of the excreted nitrogenous compounds from honey bees fed a pollen diet for seven days yielded the following results, expressed as grams of nitrogen per 100 g. of total nitrogen excreted: uric acid 61.30, creatine 3.70, creatinine 5.29, alpha-amino nitrogen 6.11, urea 0.00, and ammonia 0.00. The uric acid percentage was somewhat lower than that reported for certain species of moths (15a). Bees fed the negative control showed an abnormally high creatine and creatinine nitrogen content, amounting to 33% of the total nitrogen.

When uric acid in pollen excreta is compared with that in the negative control excreta, this waste product is shown to be a good indication of dietary protein suitability or efficiency. The uric acid analysis of the honey bee excreta showed egg albumin to be as effective a protein source as pollen. Soy hydrolysate was as good as casein, not as good as pollen, but better than the negative control. This is in agreement with results from the pharyngeal gland development studies (7, 20, 21). Thus, uric acid concentration in the excreta can provide a measure of the efficiency of a protein source.
A comparison of the alpha-amino nitrogen in the pollen and in the negative control excreta showed this factor to be directly related to the diet suitability. Egg albumin was the far superior diet. Soy hydrolysate was as good as pollen, and the casein was no better than the negative control.

Creatine and creatinine nitrogen usually are excreted only in trace amounts by insects; however, the results reported here showed values of 9% of the total nitrogen for bees fed pollen and 33% for those fed the negative control. Creatinine in the casein excreta was higher and significantly different from that in the pollen excreta. Egg albumin excreta, soy hydrolysate excreta, and the negative control excreta were not significantly different and were the highest in creatinine content. The creatine content varied considerable from this result. Pollen and soy hydrolysate were not significantly different, yet contained a low concentration of creatine. Casein, egg albumin, and the negative control were significantly different and increased respectively.

Variation of the total nitrogen values of the excreta was shown to be inconsistent with variations of the individual excreted products. Thus, total nitrogen in the excreta would appear to be of little value in measuring protein efficiency. Casein contained the least amount of total nitrogen and was significantly different from the pollen excreta.
which also had a low concentration of nitrogen. The negative control and egg albumin excreta were not significantly different and contained a large amount of total nitrogen. Soy hydrolysate excreta was very high in total nitrogen.

The amount of nitrogen which was accounted for in this research indicated that in the excreta of pollen, egg albumin, and soy hydrolysate, there was a significant amount of nitrogen which was not included in the analyses reported. The casein and negative control excreta were completely accounted for, though it is not understood why the accounted nitrogen was higher than the total nitrogen.

No ammonia or urea could be determined in the excreted material.
LITERATURE CITED


McNally, Joseph Bryan, 1937–

A comparison of nitrogen excretory products of honey bees maintained on various protein sources.


vi, 35 l. illus. 29cm.

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