

A COMPARATIVE TRIAL OF THE POLLEN SUBS: PART 4-WHY DID SOME SUBS OUTPERFORM OTHERS



A COMPARATIVE TRIAL OF THE POLLEN SUBS

Part 4:

Why did some Subs Outperform Others?

Beekeeper-Funded Research

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At first glance, one might conclude that the reason that two of the tested subs performed so well was because they contained natural pollen. But when I looked more deeply into the chemical analyses of the subs, I suspect that it was something else. It may be time to reexamine the protein and amino acid requirements in artificial diets for honey bees.

Since my sons and I no longer truck our bees out of state to better summer pastures, we've been dependent upon the feeding of pollen subs to get our colonies in shape for almond pollination. Thus, I've had great interest in the improvement of artificial diets for honey bees. Over the years researchers and beekeepers have "tested" (informally or in controlled trials) all sorts of combinations of feedstuff components in free-flying colonies in the field or flight cages, or with newly-emerged bees in incubators, but there has been little high-quality applied research performed along this line since its heyday in the 1970s at the Tucson Lab [\[11\]](#).

The testing of pollen sub formulations can be roughly divided into two categories: (1) the "by guess and by golly" throwing together of locally-available diet components to see how the colonies respond (very nicely reviewed by Paray [\[2\]](#)), or (2) the more analytical testing of specific diet formulations based upon phagostimulatory components and *mathematical nutrient models* (which calculate the relative proportions of nutrients required for each stage of an animal's development) [\[3\]](#) ^{[\[4\]](#)}.

Unfortunately, we still have much to learn about the nutritional requirements of honey bees [\[5\]](#) ^{[\[6\]](#)}.

Practical application: Other livestock industries are light years ahead of our own industry as far as the formulation of artificial diets. I suggest that it's time for the beekeeping industry to get serious about formulating pollen subs based upon nutrient models.

BACK TO MY 2020 CALIFORNIA TRIAL

I've already shown the in-field performance of the tested subs relative to each other. So the question is, ***why did some perform so much better than others?*** Since we had the nutritional composition of each sub analyzed, we can perhaps figure out the reasons that some stood out.

Some factors that did not appear to be important:

- **Phagostimulatory value:** Although some subs were clearly more phagostimulatory than others, the bees consumed all tested diets adequately.
- **Sugars and sugar content:** This did not appear to be a deciding factor. Research by Doulle [\[7\]](#) indicates that a mixture of sugars is well-accepted by bees.
- **Lipids and sterols:** The requirements for lipids in the diet are not clear, since insects are able to synthesize lipids from carbohydrates [\[8\]](#). Surprisingly, Robinson [\[9\]](#) found that **a colony is able to maintain apparently normal brood development for more than 50 days on a diet of pollen from which the lipids had been extracted by solvents.** To the point, the best-performing diets had some of the lowest lipid concentrations of all the diets in the trial. As far as sterols, bees are also able to convert other plant sterols into the 24-methylene cholesterol that they require, and I've previously found no benefit to supplementing canola oil with that sterol [\[10\]](#).
- **Vitamins and minerals:** We just don't know that much about this subject. I previously found no benefit to supplementing a diet with the critical trace element zinc [\[11\]](#).
- **Natural pollen and the gut microbiome:** We all love to think that "natural" is better, but two of the subs lacking natural pollen performed quite well. Not only that, but the community structures of the bee gut microbiomes were similar for all tested subs, whether they contained pollen or not. In fact, the bees consuming the best-performing sub had one of the lowest populations of gut bacteria of all. A recent study about the importance of symbiotic bacteria

for solitary bees [\[12\]](#) has beekeepers in a tizzy about gut microbes, but research by Chikaraishi [\[13\]](#) indicates that honey bees are not actually dependent upon their gut microbes for their nutrition.

- **Crude protein (CP) content:** Doug Somerville, in *Fat Bees, Skinny Bees* states that “When colonies collect very little pollen, whatever its CP% or when the CP% is below 20% whatever the volume collected, the colony will exhibit a protein deficiency by reducing the area of brood being reared.” And Herbert [\[14\]](#) determined that the optimal protein concentration of an artificial diet was around 25% [\[14\]](#). So it was a bit surprising that three of the best-performing subs had CP levels down in the 14-17% (wet weight) range. That fact got me looking more deeply into the details of the protein in the subs.

HAVE WE BEEN FOCUSING ON THE WRONG SUSPECTS?

Since none of the usual suspects seem to have made much difference, it leads me to question whether we’ve been barking up the wrong trees. What if the most important thing about pollen subs was something else? The thing in the lab analyses of the subs that jumped out at me were the proportions of the amino acids in the protein.

PROTEIN IN ANIMAL FEED FORMULATIONS

In order for any animal diet to allow for growth, it must contain protein; the same applies to honey bee *colony* growth. Protein is generally the most costly component of a diet, so manufacturers look for the least expensive sources. Formulated diets typically contain more than one protein source, combined to balance their amino acids in order to obtain the best *net protein utilization* by the animal. Allow me to quote from Wikipedia [\[15\]](#):

It is therefore a good idea to mix feedstuffs that have different weaknesses in their essential amino acid distributions. This limits the loss of nitrogen through deamination and increases overall net protein utilization.

Various attempts have been made to express the “quality” or “value” of various kinds of protein. Measures include the biological value, net protein utilization, protein efficiency ratio, protein digestibility-corrected amino acid score and complete proteins concept. These concepts are important in the livestock industry, because the relative lack of one or more of the essential amino acids in animal feeds would have a limiting effect on growth and thus on feed conversion ratio. Thus, various feedstuffs may be fed in combination to increase net protein utilization, or a supplement of an individual amino acid (methionine, lysine, threonine, or tryptophan) can be added to the feed.

Practical application: All the above concepts are important for the formulation of pollen subs. A common question is, why not feed concentrated protein to colonies? The answer is that it’s all about the protein to carbohydrate ratio [\[16\]](#). Too much protein in a pollen sub can result in greater worker body mass, but can also be metabolically toxic and reduce worker longevity, as evidenced by a well-performed field trial by Zheng [\[17\]](#). Since the manufacturer can’t know whether the beekeeper will be feeding supplemental sugar along with the pollen sub, it would be wise for them not to exceed ~25% protein on a dry matter basis. In general, it is likely good idea to feed sugar syrup to colonies along with pollen sub.

PROTEIN MIXING BY THE BEES THEMSELVES

Natural pollens vary greatly in their essential amino acid ratios, with only a few being in the optimal proportions for honey bee growth. Although some researchers have proposed that forager bees (which don't consume pollen themselves) can somehow detect the essential amino acid and fatty acid compositions of pollens, and then adjust which flower types to forage on in order to balance overall colony nutritional intake, I find this proposition to be largely based upon wishful thinking and confirmation bias. A more parsimonious behavioral method was posited by Eckhardt [[18](#)], who suggests that generalist bees simply haphazardly collect a variety of pollens, which are then likely to balance each other out by chance.

I and others have observed that some pollens, when offered in cups in the field are far more attractive to foragers than others. But despite that observation, looking at the incoming pollen loads of my colonies, I suspect that Eckhardt is correct, and that the honey bee has evolved the behavior for foragers to simply collect a diversity of pollen types when available (Figure 1).



Fig. 1 Although nectar foragers may focus upon the most “cost effective” nectar sources at the moment, the pollen foragers appear to seek out a diversity of pollens for the nurse bees to consume and convert into jelly.

I've also found that foragers clearly prefer some protein feed components over others (Figure 2).



Fig. 2 Some years ago I tested forager preferences for various dry pollen sub powders, and found that foragers exhibited clear preferences. Surprisingly, they really went for purified soy protein isolate, which had no odor that I could detect. However, I'm not convinced that forager preference has anything to do with nutritional value, especially since I also observe foragers enthusiastically gathering sawdust when nothing else is available.

Practical application: The “preferences” of foragers may not reflect the nutritional needs of the nurse bees that actually consume and digest the pollen or artificial diet. Vanessa Corby-Harris has shown the even the nurses either cannot or do not assess the nutritional value of the pollen that they choose to consume[\[19\]](#). So it's up to us to formulate artificial diets that are both attractive to the nurses, as well as nutritionally balanced.

CRUDE PROTEIN AND AMINO ACIDS

Since protein is the most important component of a pollen sub, this brings us to the subject of essential amino acids. Animals use some 20 naturally-occurring amino acids to create all the thousands of proteins used in their bodies. Depending on the species of animal, 10 or 11 of those amino acids are “indispensable” or “essential,” since the animal is unable to synthesize them de novo. We refer to them as essential amino acids (EAAs).

Note: Henceforth in this article I will refer to the ten essential amino acids as “EAAs.”

That said, animals don't grow well on a diet of EAAs alone — they require additional crude protein or other amino acids (such as serine, glycine, and proline) in their diet in order to maintain growth. Indeed, proline is the predominant free amino acid (not tied up in a protein) in honey bee haemolymph [\[\[20\]\]](#) and royal jelly [\[\[21\]\]](#)^[22].

Practical application: I've long been curious as to whether proline would be a useful addition to bee diets. Proline is readily available in the "collagen peptides" sold as a human protein supplement.

Some protein sources (such as chicken egg or cow's milk) are termed "complete proteins," since they (not surprisingly) contain all the amino acids, ***in the right proportions***, necessary for the full and rapid growth of developing chicks or calves, respectively.

Practical application: The protein extracts of egg or milk are albumen or casein, respectively. Both work well as protein sources for caged bees, and are sometimes incorporated into pollen subs (and in other formulated insect diets)[\[\[23\]\]](#). But both, being animal-derived, are more expensive than plant protein options. Unfortunately, the amino acid ratios of most plant proteins are not in the correct proportions for optimal animal utilization, and, as mentioned above, must be combined with other protein feedstuffs in order obtain the *optimal balance of EAAs*.

EAAS AS PERCENT OF CRUDE PROTEIN

In general, the EAAs should constitute roughly 30%-50% of the crude protein content of an animal diet (this is the same range as occurs in honey bee body protein, natural pollens, and royal jelly). All the tested pollen subs in my trial were at the upper end of this range (either these producers did their homework, or just got lucky) (Table 1).

But that 50% level is contingent upon the EAAs being ***in the right proportions relative to each other***. Since protein is generally the most expensive component in an artificial diet, the livestock and pet industries reduce diet cost by minimizing *total* crude protein and carefully balancing the essential amino acids in "precision diets," supplementing any deficient EAAs with concentrates [\[\[24\]\]](#).

Practical application: The "net protein utilization" of a diet is profoundly affected by the *limiting amino acid*— the one in shortest supply. However, the precise optimal proportions of the EAAs is not cut and dried, since some of the other amino acids can be "conditionally essential," or exhibit "sparing effects."

THE EAA REQUIREMENTS OF HONEY BEES

It wasn't until the early 1900s that researchers started to seriously study animal nutrition. By the late 1940s, the Committee on Animal Nutrition concluded that the most appropriate way to express a nutrient requirement was as the minimal dietary concentration required to support normal performance of the most demanding function [\[\[25\]\]](#). But even by the 1950's the study of animal nutrition was a nascent science, with researchers using painstaking chemical, radiolabeling, and other analytical methods to figure out critical nutrients, and the optimal proportions of them in animal diets. A number of labs (notably the USDA-ARS bee research lab at Tucson during the 1960s through 1980s) did extensive and informative studies, and Dr. Geraldine Wright has in recent years presented on her published and yet-unpublished findings.

In 2008, Behmer in his review of insect nutrition [\[\[26\]\]](#), explains that:

The qualitative nutritional requirements of insects are very similar to other animals as the basic chemical composition of their tissues and their metabolic processes are generally the same.

Much of what we know about insect nutrition comes from studies using artificial foods, and in general there are three main approaches to understanding insect nutritional requirements. The most common is the deletion method, which measures the effect of eliminating one specific component from a chemically defined artificial diet and then measuring whether the insect can develop on the modified diet under sterile conditions. Once the deletion method identifies which nutrients are essential, the substitution method can be used to test whether analogues of these nutrients also support development. Finally, radiolabeled precursors can be fed to insects to determine which nutrients insects are able to produce endogenously.

That said, most of what we know about the amino acid requirements of honey bees comes from a groundbreaking study by Antonius de Groot, in which he used the deletion method mentioned above [27]. Our beekeeping industry, ever on the cutting edge, generally formulates their diets to match those suggested by de Groot's oft-cited Table 25, published in 1953 (Figure 3).

Amino acid	Percentage in:			Minimal level required in honeybee
	Royal jelly	Pollen	Soybean flour	
arginine	5.1	5.3	7.7	3.0
histidine.	2.2	2.5	2.3	1.5
lysine	6.7	6.4	6.6	3.0
tryptophan	1.3	1.4	1.5	1.0
phenylalanine	4.1	4.1	5.1	2.5
methionine	1.9	1.9	1.4	1.5
threonine	4.0	4.1	3.9	3.0
leucine	7.7	7.1	8.0	4.5
isoleucine	5.3	5.1	5.3	4.0
valine	6.7	5.8	5.3	4.0

Table 25. Comparison of the essential amino acid content of royal jelly, pollen, and soybean flour with the approximate minimal levels for optimal growth in the honeybee (expressed as per cent of the protein).

Fig. 3 Based upon meticulous studies of caged newly-emerged worker bees, de Groot suggested the required *minimal levels* of the EAAs in a honey bee diet, standardizing them in ratios relative to tryptophan.

If we look at the EAA analyses for the diets I tested, they all exceed the minimal requirements indicated in the last column of de Groot's table (Figure 4).

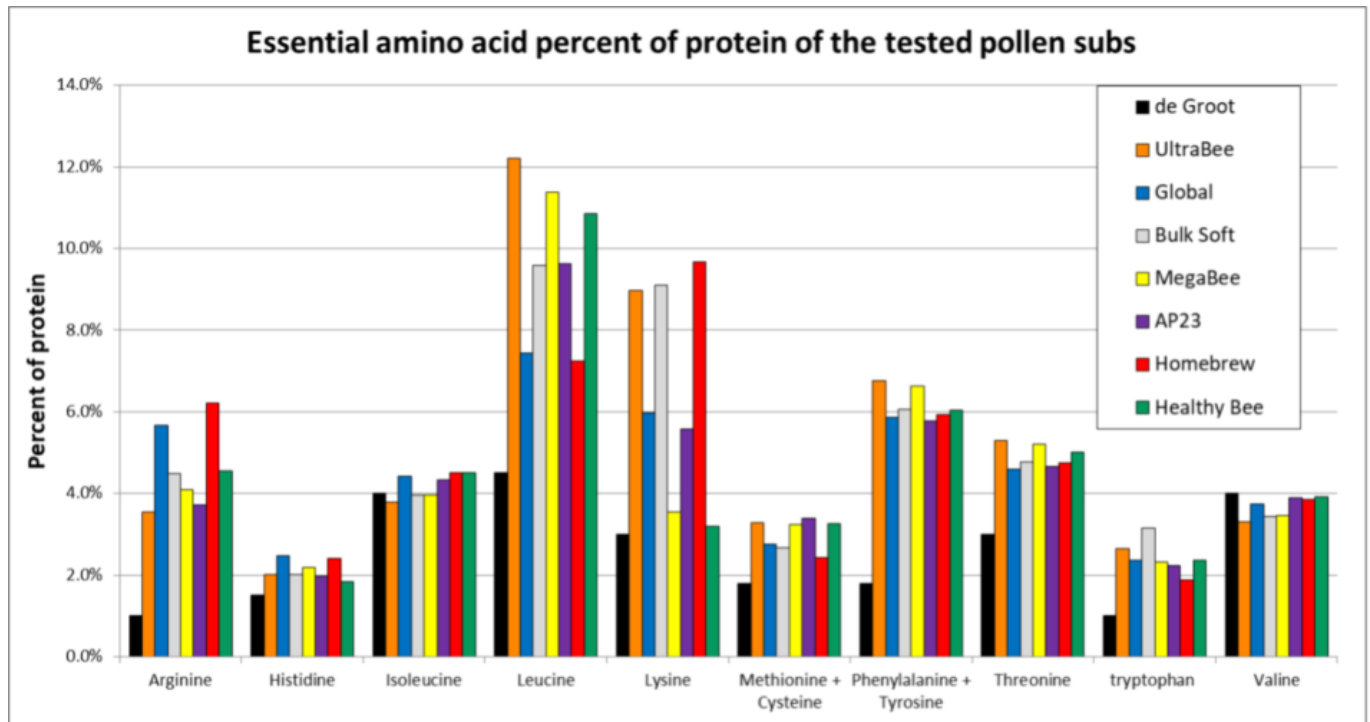


Fig. 4 Relative to de Groot's *minimal* recommendations (black columns), all the tested subs had very good EAA profiles, with none appearing to be appreciably deficient in any EAA. ***But also note that all the diets contained about twice as much tryptophan and leucine as suggested by de Groot.***

Practical application: The striking excess of two important EAAs was a red flag to me, and raised a question — did all the diets contain excesses of tryptophan and leucine, or were they instead deficient in the other EAAs, *relative to tryptophan or leucine*?

Also note the discrepancy with regard to the current widely-accepted recommendation that EAAs constitute 50% of total protein — the EAAs in de Groot's table add up to only 25.6% of the protein in the diet. Not only that, but *de Groot himself pointed out that the test diets that he used contained too little protein for maximum growth.*

Practical considerations: The above observations should give us all pause when making the jump from *optimal ratios* of EAAs to *percentages in the diet*. I suspect that for optimal nutrition, we'd need to double the minimal levels indicated in de Groot's Table 25.

Should we question de Groot's recommendations? I've studied de Groot's monumental and detailed research at length; let me say that I'm in awe of the amount of painstaking and detailed research that he performed over a period of years. His paper is some 80 pages long, and I suspect that most who have read it didn't give it the scrutinization that it deserves. So next month we'll do a deep dive into it, and I will reinterpret his findings with regard to the performances of the pollen subs that I tested.

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[27] de Groot, A. (1953) Protein and amino acid requirements of the honey bee. Physiol. Comp. Oecol. 3, 197-285. *Unfortunately, this paper, which is required reading for anyone seriously interested in honey bee nutrition, is not readily available online.*