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PURE Protein Products

Testing to ensure protein supplements
and functional foods meet label claims

Amount Per Serving

Calories

Calories From Fat

% Daily Value *

Total Fat	...g	...%
Saturated Fat	...g	...%
Cholesterol		...%
Sodium		...%
Total Carbohydrate		
Dietary Fiber		
Sugar		
Protein		



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The supplement and functional food industry is in the process of developing better protein tests and standards to help brand owners avoid the protein spiking of their products. **Gene Bruno** describes the proposed industry standard from the American Herbal Products Association (AHPA), and gives tips on how to ensure protein products aren't spiked.

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Trust or Scrutiny?

Is there nothing more common than the desire to get what you pay for? OK, if you're like me, you love to get more than you pay for, but it is safe to say a minimum requirement is to not get taken for a ride. How would you feel if the bartender gave you non-alcoholic beer instead of your favorite ale? Or if the barista left out your shot of espresso? Or if your car mechanic put in cheap overseas parts when you paid for high-quality American-made?



Some switcheroos are obvious—couldn't you tell the difference between well liquor and the top-shelf type you requested?—but in many cases, we can only trust the provider's word or what is on the label. Funny thing, trust. An opportunity to build a strong relationship for the honest, but a chance to exploit for the dishonest.

In the world of food and supplements, the dishonest spike products to fool tests and people into believing there is more of a prized ingredient than there really is. In the short-term, this cuts costs and increases profit, but the downside is a betrayal of consumers, who are paying for a stated level of a certain ingredient, such as protein.

For whatever bad public rep carbohydrates have suffered, protein has experienced the opposite, having enjoyed a steady growth in popularity across many segments, not just sports nutrition. Whole foods aside, protein mixes and supplements are increasingly valued for their protein types, but the overall protein content might be the most visible data on a label, making it the target of spiking.

In this Digital Pulse, dietary supplement scientist Gene Bruno explains the common testing practices for protein products and how dishonest ingredient suppliers are adding non-protein substances to generate tests that show a higher protein content than the true amount. He reviews common substances used to spike protein products, including why these compounds fool testing. He also touches on some of the difficulties in determining if amino acids were added to spike or for their own health benefits.

For whatever reason, if a product does not contain what it is labeled to contain, it can be considered misbranded by federal regulators; proven spiking would also bring adulteration to the table. Bruno tackles regulatory considerations for supplement brands relative to protein spiking and calculating protein content. In this gray area, he details industry efforts toward voluntary standards for protein labeling, including a guidance that helps define protein ingredients.

In the end, criminals will still try to commit crimes. Under this certainty, Bruno offers several recommendations for avoiding spiked protein products through the use of specific testing approaches and methods.

Trust or scrutinize? It's your call.

A handwritten signature in black ink that reads "Steve Myers".

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Pure Protein Products

Using tests and other verifications to ensure protein supplements and functional foods meet label claims

by Gene Bruno

Among consumers (especially bodybuilders and sports nutrition aficionados), the perception is the more protein per serving in a protein powder or supplement, the better. Of course, this can be problematic from price perspective, because the more protein per serving in a given product, the more that product is going to cost—particularly if a high-quality source of protein, such as whey protein isolate or hydrolyzed whey protein, is used. The challenge is to provide sufficient quality protein per serving to satisfy consumers, yet not make the MSRP so high that it is cost prohibitive for those same consumers, and at the same time, try to maintain a modicum of profit margin.

INSIDER'S Take

- A common test for measuring protein content, the Kjeldahl method, could indicate more protein in a product than there actually is.
- Industry leaders, such as the Association of Official Analytical Chemists (AOAC) and the American Herbal Products Association (AHPA), are developing new testing methodologies and standards.
- The Leucine Calculation Check may help brand owners test their own products to see if unnecessary leucine is added to products, but it is not foolproof.

Protein Spiking

“Protein spiking” is the practice of adding some non-protein substance to a protein powder in order to increase the overall nitrogen content of the powder. Proteins are the only nutrients that provide nitrogen (carbs and fat do not). According to John Travis, senior research scientist at NSF International, “The Kjeldahl method is commonly used to measure total nitrogen in protein products.” The method involves liberating reduced nitrogen as ammonia, and then measuring the ammonia. Since this test determines the nitrogen content, it is used to calculate the total grams of protein in a protein supplement. The Kjeldahl test could imply a given supplement contains more whole protein than it actually does if the test measures a non-protein substance simply by its nitrogen content.

This raises the question, “Does this violate the regulations associated with calculating protein?” Unfortunately, the answer is not entirely clear since the method measures total nitrogen in protein products, not the individual components that provide the nitrogen.

The industry could use a defined standard of what substances (e.g., whole protein, amino acids, etc.) should be included in measuring nitrogen content, according to the American Herbal Products Association (AHPA). Michael McGuffin, president of AHPA, explained that he been working with a committee in an attempt to do this: “AHPA’s Sports Nutrition Committee has been discussing the issue of labeling of protein in protein products with an intention to ensure clarity for consumers. The AHPA board recommended a voluntary industry standard. Ultimately, the objective is to work with other industry groups to obtain a consensus on that standard.”



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In March 2014, AHPA issued a voluntary guidance on labeling of protein in food and dietary supplement products and suggested a six-month time frame to adhere to the guidance. AHPA's guidance recommended product manufacturers define protein as “a chain of amino acids connected by peptide bonds” for labeling purposes. Further, AHPA recommended non-protein nitrogen-containing substances not be counted toward total protein content on product labels. The non-protein nitrogen-containing substances should be accounted for and subtracted from the total nitrogen content when protein is measured by nitrogen content, according to AHPA's guidance.

According to Neil Thanedar, CEO of LabDoor, protein spiking comes in two main types: “One type involves the use of melamine or urea,” he said. “This is primarily found in proteins from China. The other type of protein spiking is the use of amino acids such as glycine and sometimes taurine. This is the more common type.”

In addition, Tim Ziegenfuss, CEO of the Center for Applied Health Sciences (a clinical research organization that conducts studies on dietary supplements) said arginine and creatine are also used for protein spiking: “Arginine has approximately three times more nitrogen than whey protein, and creatine has approximately one and a half times more nitrogen at less than half the price. Spiking protein with these nutraceuticals is a cheap way to drive up the nitrogen level of a protein powder without adding more high-quality protein.”



Although amino acids are the building blocks of protein, they do not have the same beneficial effects of whole protein. Part of the reason for this has to do with protein digestion and absorption.

Ziegenfuss explained another protein spiking issue regarding nutrient content claims. Specifically, nutrient content claims are voluntary, unlike listing protein in a Nutrition Facts Box or Supplement Facts Box. Even if a brand believes it is in compliance with regulations with nitrogen extrapolation to generate the number of protein grams in the Nutrition/Supplement Fact Box, using a nutrient content claim on other parts of the label is a different matter. When a nutrient content claim is made, the nutrient must meet accepted standards of identity. The 2005 dietary reference intake (DRI) guidance clearly defines protein as macromolecules with links of amino acids, and does not mention amino acids or creatine.

Although amino acids are the building blocks of protein, they do not have the same beneficial effects of whole protein. Part of the reason for this has to do with protein digestion and absorption. Research indicates that protein fragments, mainly small peptides from protein digestion, are absorbed intact and transported to systemic circulation to peripheral tissues. Furthermore, several studies have shown that protein hydrolysates, made up mostly of di- and



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tripeptides, are absorbed more rapidly than amino acids.¹ This considerably greater absorption rate of amino acids from dipeptides appears to be the result of a system that has a greater transport capacity than free form amino acid carrier systems. Ultimately, the di- and tripeptides from proteins are absorbed intact, hydrolyzed intercellularly, and released as free-form amino acids or released into the circulation. Less than 1 percent of total protein that passes through the gastrointestinal (GI) tract is lost in stools. Free-form amino acids just don't have this same high level of absorption when used in large amounts.

Nevertheless, there is a legitimate reason to add the solo amino acid glycine to a protein powder that has nothing to do with protein spiking. According to Marc Stover, director of marketing for sports nutrition products at Twinlab, "Glycine is often added to improve the mouthfeel of high-quality whey protein powder, making its chalky texture more creamy and satisfying. This is important since consumers buy a protein powder the first time based on price, but come back and buy again based on taste." At the same time, Stover added, "But it is Twinlab's position that amino acids like glycine shouldn't be used to increase nitrogen levels and make it look like there is more protein present than there actually is."

Melamine

While the regulatory status of protein spiking with amino acids is one thing, spiking with melamine is downright dangerous and sometimes deadly. Melamine is a nitrogen-rich chemical used to make plastic and sometimes used as a fertilizer. Melamine has been found in wheat gluten, rice protein concentrate and, in South Africa, corn gluten, all imported from China, and commonly meant for use in pet food.² At the same time, research from China has shown human infants consuming melamine spiked infant formula experience kidney and urinary tract injury, in some cases causing irreversible damage.³ Infant fatalities have even resulted. However, protein spiking with melamine does not seem to be a problem in the natural products industry.

While the regulatory status of protein spiking with amino acids is one thing, spiking with melamine is downright dangerous and sometimes deadly.



Testing for Purer Protein Products

Although the Kjeldahl method is the currently standard for measuring total nitrogen in protein products, the Association of Official Analytical Chemists (AOAC) announced it was in the process of developing new testing methodology during an August 2013 meeting, according to Travis. This new method might more easily identify proteins that have been artificially spiked.

Meanwhile, Travis explained testing programs allow manufacturers to verify that they have not been adulterated: "If a product has an NSF Certified for Sport mark, that is verification that the free-form aminos are not counted as part of the protein content," he said.

Likewise, Thanedar indicated that LabDoor often measures the amino acid levels in a protein via high-performance liquid chromatography (HPLC), thereby clearly ascertaining if an amino acid has been used for spiking purposes.

It should be noted that higher profit margin for a supplement brand may not always be the motivation for protein spiking. It’s possible that a dietary supplement company could have a spiked protein product, but not realize it.

A dietary supplement company that uses a contract manufacturer may ask it to make a whey protein that provides 30 grams of protein per serving at a specific cost. Knowing that the cost of whey protein is too expensive to make it for X dollars per unit, the manufacturer may spike the protein with an amino acid in order to meet the requested cost.

Tips for Avoiding Adulterated, Spiked Protein in Your Products

If a brand’s protein product is produced by a contract manufacturer, how can it tell if it has been spiked, or if an amino acid has been added for a legitimate reason? Although there is no fool-proof method, the Leucine Calculation Check (created by the author of this article) as one possible way to do a quick check.

The Leucine Calculation Check works upon the basis that whole proteins natural contain a specific percentage of the amino acid leucine. Knowing the percentage of leucine in the product can lead to a simple mathematical calculation to see if it is present in a considerably lower levels then it should be. If so, then there is a good chance that the protein has been spiked with an amino acid.

In order to do this test, a brand must request a complete amino acid profile of the product from the contract manufacturer or get it tested, in order to compare it against the total grams of protein. Note: this method won’t work if there are multiple types of protein combined together in the same product.

Depending upon the research, the percentage of leucine in various proteins varies somewhat. Nevertheless, the following percentages include the lower levels of leucine that should be possible in each protein:

Protein Type	Percentage of leucine
Whey protein hydrolysate	10% or more ^{5,6}
Whey protein isolate	10% or more ^{5,6, 7}
Soy protein isolate	8% or more ^{5,6, 7}
Casein	8% or more ^{5,6}
Egg protein	8% or more ^{6, 7}

With this in mind, here's how a supplement brand can do the calculation, using a hypothetical whey protein product:

- 1** The product lists the leucine content per serving as 2593 mg, or 2.593 g, and the protein content per serving as 25 g
- 2** Divide 2.593 g by 25 g, resulting in 0.10372
- 3** Move the decimal to places to the right, resulting in 10.372 percent
- 4** This means the protein provides more than 10-percent leucine, so it probably isn't spiked

While the Leucine Calculation Check may work in many situations, Travis warned it is not foolproof and won't help in situations where intentional spiking is an issue. "One could add leucine along with other nitrogen sources and still fool this test," he said.

Travis added a more reliable method to determine protein adulteration is to have the finished product or raw material tested twice. "The first test is to measure free amino acids in the material," he said. "The second test is to measure total amino acids in the material. The amount of free amino acids is subtracted from the amount of total amino acids. The result of this calculation provides the amount of amino acids attributable to the protein content."

The development of new testing methods and standards holds promise for identifying spiked proteins, and but in the meantime, supplement brands must select the protein test that can best help them determine the purity of protein in their products. ■

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