Research Review of Feeder Fresh™ Nectar Defender™

Safety and Efficacy of Naturally Preserving Hummingbird Nectar with Micronutrient Copper

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Key Facts

- Nectar Defender is designed to naturally prolong the freshness of hummingbird nectar, and promote the health of hummingbirds by inhibiting spoilage due to the growth of harmful bacteria, algae, and mold.
- Nectar Defender keeps nectar fresh by using trace levels of micronutrient copper. Hummingbirds naturally consume copper in their daily diet of arthropods and nectar. It is something they must have for healthy growth and development.
- The micronutrient copper level in Nectar Defender is 6 ppm, which is within the natural diet range of wild hummingbirds, lower than the micronutrient requirement in long-term studies of hummingbirds, and far below the level that would cause any health risks to hummingbirds.
- Micronutrient copper has been proven to be safe for hummingbirds in long-term studies. For example, an official nectar diet containing 8 ppm copper was created and used by UC Davis Avian researchers for the long-term maintenance of hummingbirds. The copper level was selected by the researchers to satisfy hummingbirds' copper micronutrient requirement. Anna's and Costa's hummingbirds were maintained on this diet for several years, during which the hummingbirds thrived, and exhibited no problems. This diet has since been used by other zoos and wildlife rehabilitation organizations to successfully maintain the long-term health of their hummingbird colonies for years at a time.
- Hummingbirds are protected from absorbing more copper than they need due to their bodies' natural biochemical regulation. In fact copper absorption receptors are only triggered in a bird's digestive system, when it is in need of copper nutrient. This is due to copper homeostasis, a regulating system that precisely maintains a healthy copper balance even in when ingestion levels increase.
- Micronutrient copper will not accumulate in the hummingbird's system. Numerous studies with birds show that even large excess consumption is rapidly excreted from their system.
- A wild hummingbird's diet is estimated to range from 4 to 16 ppm copper due to the consumption of arthropods. The arthropods that hummingbirds eat can contain many times the copper level in Nectar Defender, and average about 20 ppm. Nectar Defender at 6 ppm is within the natural micronutrient range, and if Nectar Defender is combined with arthropods, the micronutrient level is still substantially within a hummingbird's natural nutrient range.
- In a real world situation, hummingbirds will have multiple sources of natural flower and feeder nectar, and as a result, when using Nectar Defender, the actual level of copper in their nectar diet will likely be less than the drinking water standard set by the EPA.
- Micronutrient copper in Nectar Defender is the same mineral supplement used for decades to provide essential trace minerals for birds, and approved by this use by the FDA and USDA. It is safety rated by the FDA as GRAS ("generally recognized as safe"), the safest rating given.
- Based on real world preference testing, hummingbirds prefer nectar with Nectar Defender just as much as nectar without.
- The safety of Nectar Defender has been approved by multiple avian experts, including Dr. Kirk Klasing, well-known University of California Avian Physiologist, and author of the book: "Comparative Avian Nutrition".

Introduction

We know that wild bird safety is critical to bird watchers, and it is our primary reason for creating products that protect the health and safety of wild birds for over 10 years. We love hummingbirds, and would never offer a product that was not tested and deeply researched to make sure it was completely safe and appealing for the birds you also love.

This research paper explores the safety issues of Feeder Fresh™ Nectar Defender™ . To investigate, we undertook an intensive study searching for everything available on copper physiology and safety in hummingbirds. This required diving deep into hummingbird biology , copper biochemistry, nutrient and toxicology research, regulatory databases, and consulting with university experts.

General

Some of the most harmful compounds on earth are created by molds, bacteria and other microbes, and can be harmful or even lethal to hummingbirds. Nectar Defender keeps nectar fresh by using an extremely low level of micronutrient copper sulfate, the same natural compound used as a mineral supplement for birds and humans. With Nectar Defender, nectar stays fresh for 1-2 weeks instead of days, making it easy to continuously maintain feeders in a fresh healthy condition[1].

General facts:

- Copper is plentiful in the environment, and naturally present in food, soil and water. It is essential for the normal growth and metabolism of all living organisms *(Schroeder, H. A., A. P. Nason, I. H. Tipton, and J. J. Balassa. 1966. Essential trace metals in man: copper. Journal of Chronic Diseases 19:1007-1034).*

- Hummingbirds consume micronutrient copper in their daily diet of arthropods and nectar. A mosquito, for example, has a copper content three times that of Nectar Defender nectar.

- Micronutrient copper in Nectar Defender is the same mineral supplement used for decades to provide essential trace minerals for birds, and approved by this use by the FDA and USDA. Copper sulfate is has been declared "GRAS" (Generally Recognized as Safe), the safest designation given by the FDA. *(21 CFR 184.1261).* Copper sulfate at low levels is even allowed in organic agriculture by the USDA, and in infant formula by the FDA. *(21 CFR 205.601).*

- Copper regulation (homeostasis) limits copper absorption from a bird's digestive system, unless they are in need of copper. Therefore moderate changes in copper level are inherently safe, since the birds will not absorb more copper than they need. In addition, copper homeostasis internally regulates a healthy copper balance, initiating excretion of any excess copper. Therefore copper will not accumulate in the hummingbird's system.

- Copper is an essential component of a number of enzymes which function in energy metabolism, maturation and stability of collagen and elastin, pigmentation, the antioxidant defense system, and iron removal, and other biological processes.

- Since copper is an essential nutrient, birds that do not consume an adequate level of copper, can encounter serious health issues. Too little copper may result in cardiovascular disorders, depigmentation, impaired keratinization, anemia, reduced growth, neonatal ataxia, bone abnormalities, and impaired immune responses.

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[1] We recommend cleaning your feeder at least weekly for proper avian hygiene

Expert approval for the safety of Nectar Defender in hummingbird nectar

Multiple avian experts have endorsed the safety of Nectar Defender for hummingbirds, and three are cited here for reference:

The safety of micronutrient copper in Nectar Defender has been personally approved by Dr. Kirk Klasing, Dr. Klasing is a well-known Avian Physiologist from the University of California, Davis, author of the reference book: "Comparative Avian Nutrition" and chair for the National Research Council's multiple-expert review book: "Mineral Tolerance of Animals", Second Revised Edition, 2005 .
When commenting on the 6 ppm level of copper in Nectar Defender, Dr. Klasing states that:

> "Given the available information and including a margin of safety for the unknowns, I recommend that copper levels in nectars that are between 3 and 20 ppm (as fed basis) should meet the copper needs of hummingbirds without causing toxicity when fed as the sole source of nutrition."

The safety of Nectar Defender has also been personally approved by Dr. Ellen S. Dierenfeld, Dr. Dierenfeld has been in the Department of Wildlife Nutrition, Wildlife Conservation Society, The Bronx Zoo, the head of the Nutrition Department at the St. Louis Zoo, and was part of the team who solved the hummingbird iron toxicosis problem at the hummingbird aviary of the Sonora Desert Museum. She is co-author of the book: "Comparative Animal Nutrition and Metabolism", 2010. Dr. Dierenfeld has a deep understanding of the micronutrient need of hummingbirds and other animals.
Dr. Dierenfeld states:

> "Based on years of experience feeding a variety of nectarivorous species, including hummingbirds, the level of copper (6 mg/kg) in Feeder Fresh Nectar Defender is of no concern of toxicity to hummingbirds and is, in fact, within a range considered optimal for long-term health of this species regarding its dietary micronutrient copper requirements."

The safety of micronutrient copper in Nectar Defender has also been approved by Dr. Dan Brown, Cornell University Professor of Animal Science. Dr. Brown's focus is Nutritional Toxicology of animals, studying the effects of nutrients and toxins in the food chain and their metabolism. He was previously a faculty member of the Animal Science department at UC Davis for 11 years, and has been a faculty member at Cornell University for 21 years.
Dr. Brown states:

> "Based on my expertise in nutritional toxicology, animal science and previous research, the level of copper micronutrient in Feeder Fresh Nectar Defender (6-7 ppm) will be safe for hummingbirds, and actually within the micronutrient range considered optimal for the long-term health of hummingbirds."

Long-term studies show the safety of micronutrient copper in a hummingbird's diet

The micronutrient copper level in Nectar Defender is 6 ppm, which is within the natural diet range of wild hummingbirds, lower than the micronutrient requirement of hummingbirds, and far below the level that would cause any health risks to hummingbirds.

A hummingbird diet study performed by University of California avian scientists,which provided protein-supplemented nectar containing 8 ppm copper (25 ppm dry basis) to hummingbirds for 3 months. This level of copper was selected by the researchers to satisfy the copper micronutrient requirement of hummingbirds, in this case Costa's hummingbirds. There were no reported signs of copper related problems or toxicities by the researcher, and the birds displayed their normal behavior of preening and singing. *(Protein Requirements of Costa's Hummingbirds Calypte costae, Ann T. Brice and C. Richard Grau, Department of Avian Sciences, University of California, Davis, Physiological Zoolology 64 (2):611-626, 1991)*

In a longer study, an official nectar diet containing 8 ppm copper (25 ppm dry basis) was used for the long-term maintenance of hummingbirds by University of California avian scientists. Anna's and Costa's hummingbirds were maintained on this diet for several years. On this diet and copper level, the hummingbirds thrived, with no signs of problems. The birds molted normally and the males displayed singing. The birds ate the diet readily, and they drank little supplementary water, from which it was concluded that the percentage of solids in the diet was not excessive.

Birds were weighed routinely to confirm health, and body weights varied little over time. The researchers concluded that the hummingbirds' acceptance of the diet, and health of the hummingbirds has provided a means to determine their requirements for nutrients. The diet has since been used by other zoos and wildlife rehabilitation organizations for the long-term maintenance other species such as Allen's (Selasphorus sasin), black-chinned (Archilochus alexandri), and ruby throated (A. colibri) hummingbirds. *(Hummingbird Nutrition: Development of a Purified Diet for Long-Term Maintenance, A.T. Brice and C.R. Grau, Department of Avian Sciences, University of California, Davis, Zoo Biology 8:233-237 (1989))*

Another long-term hummingbird diet that was developed on a rational scientific basis using physiological and ecological studies on birds in captivity and in the wild, contains 8 ppm copper. *(Nutritional requirements and diets for humminbirds and sunbirds, M. Peaker, International Zoo Yearbook, Dec 1990).*

Whole-diet hummingbird nectar products for the long term care of captive hummingbirds contains copper in the single digit ppm range as a micronutrient, so the acceptance by avian nutritionists that copper is needed near that nutrient level, appears to be universal.

In order to ensure that the very low micronutrient level in Nectar Defender is absolutely and inherently safe, it was designed to be even lower than the recommended level of copper in the official hummingbird diet and other long-term testing recommendations.

Real world level of Nectar Defender in a hummingbird's diet

For the purposes of this safety evaluation, we are being conservative and in most cases assuming that all of the nectar a hummingbird consumes comes from a single feeder containing Nectar Defender. However, in a real world situation hummingbirds have multiple sources of feeder and flower nectar. Experts estimate they have the ability to visit thousands of sources per day.

If a hummingbird has even four other sources of nectar, then the 6 ppm of copper in the treated nectar becomes 1.2 ppm copper the hummingbird's nectar diet. The recommended limit of copper in drinking water is <1.3 ppm copper. In other words with the likely situation of a handful of other nectar sources, the result is a level below even the safe level in drinking water set by the EPA.

Birds do not absorb more copper than they need, due to copper homeostasis

Birds, and most animals, have robust systems for regulating copper input and excreting any excess copper. Experts on the biochemistry of copper are in agreement that birds do not accumulate copper, because they have sophisticated and efficient copper homeostasis mechanisms that regulate uptake, distribution, sequestration and export of copper.

Copper levels are kept stable by multiple biochemical controls (homeostasis), including copper importers, copper chaperones, transcription factors, small metal binding proteins (metallothioneins), and copper exporters. For this reason, almost all animals can tolerate a range of copper consumption with little effect. It is also the reason that copper does not accumulate in birds, even at elevated dietary copper conditions.

The basic mechanisms of copper regulation are understood, and universal across multicellular organisms from fruit flies, to hummingbirds, to humans. The first level of control is in the intestine, where copper will not be absorbed, unless copper transporters allow the movement of copper into the system. The amount of these transporters are regulated by the copper level in the organism. Therefore copper does not even enter the system from the intestines if it is not needed by the organism. Within the system, any excess copper is controlled by copper uptake proteins or metallothioneins, regulated by the metal-responsive transcription factor . Unused copper is temporarily stored in the liver, and any excess is removed by another copper transporter into the bile. The bile is then excreted via the normal waste removal process.

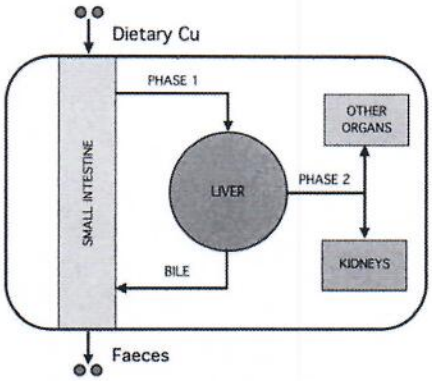

Figure A: Basic process for absorption of needed copper, and removal of excess. In insects and lower animals, a fat-body will be the storage organ instead of a liver. (from Southon et. al.)

As stated by Zhou et. al.: "Cells have evolved sophisticated mechanisms to control copper homeostasis at the level of uptake, distribution, sequestration, and export. The Ctr1 proteins are a family of high affinity copper transporters, characterized in fungi, plants, amphibians, and mammals, that are predominantly localized to the plasma membrane and that are thought to transport reduced copper... ...Upon delivery of copper by Ctr1 proteins to the interior of cells, it is transferred to copper-dependent enzymes, or the compartments where these enzymes are assembled, via the action of three copper-binding chaperones... "

("Ctr1 and its role in body copper homeostasis", Paul A. Sharp, The International Journal of Biochemistry & Cell Biology, Volume 35, Issue 3, March 2003, Pages 288–291; " A Copper-regulated Transporter Required for Copper Acquisition, Pigmentation, and Specific Stages of Development in Drosophila melanogaster", September 8, 2003, Hao Zhou, Ken M. Cadigan, and Dennis J. Thiele, THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 48, Issue of November 28, pp. 48210–48218, 2003; " Copper homeostasis in Drosophila by complex interplay of import, storage and behavioral avoidance", Balamurugan, K., et. Al. Institute of Molecular Biology, University of Zurich, The EMBO journal, vol 26,no 4, 2007.)

See Appendix A for more detail on copper homeostasis, and studies showing proof of copper protection in birds.

Avian copper dietary studies show that birds do not accumulate copper, or absorb it even at elevated levels

Actual dietary studies with birds demonstrate the protective nature of copper homeostasis. When fed increasing levels of copper, domestic birds do not show accumulation or any significant increase in the copper content of their blood or liver. The studies show the bird's excrement contains the increase in copper, proving that the biocontrols keep the excess copper in the gut, and restricted from enter the bird's system. The studies also show copper in the bile, supporting an active copper removal process. Appendix A explains this topic in detail, and provides experimental proof that copper increases in a bird's diet, are not reflected in a bird's system.

For all of these physiologic reasons, it is difficult for hummingbirds to absorb more copper than they need. Nectar Defender is within the range of natural copper consumption, but a further protection to hummingbirds is the universal copper regulation. Even if an increase was present, hummingbirds would not see it reflected within their system, and definitely would not accumulate it.

(Poult Sci. 1998 Mar;77(3):445-8, "Studies on the feeding of cupric sulfate pentahydrate, cupric citrate, and copper oxychloride to broiler chickens". Ewing HP, Pesti GM, Bakalli RI, Menten JF. , Department of Poultry Science, The University of Georgia, Athens 30602-2772, USA.)

Source and level of copper in a wild hummingbird's diet

Copper is present in the foods hummingbirds consume in their natural diet. A substantial part of a hummingbird's diet is arthropods (insects and spiders), and can reportedly range from 10% to 80% of their diet depending on the time of year. Arthropods provide their main source of protein, vitamins, oils, fiber and minerals. Hummingbirds will eat flies, spiders, bugs, aphids, mites, leafhoppers, and flying ants. These arthropods contain a moderate

amount of copper. For example mosquitos, a common part of a hummingbird's diet, has a copper content of 23 ppm, three times higher than that of Nectar Defender. Other arthropods consistently contain copper, even up to 9 times higher than that of Nectar Defender. The level of copper in arthropods is not surprising, since their circulatory system contains unique copper-bearing proteins (mainly hemocyanin supergroup structures) used for immunity, wound healing and oxygen transport.

Since arthropods can be a large part of a hummingbirds diet, the range of copper in a wild hummingbirds natural diet can be significant. As seen in Table 1, arthropods range from 8 to 50 ppm copper, averaging roughly 20 ppm. Even with no other source of copper, we can estimate that a 10% to 80% arthropod consumption means a wild hummingbird natural diet ranging from 2 to 16 ppm. This means that Nectar Defender at 6 ppm is well within the natural range of copper found in a wild hummingbird's diet.

Also, the consumption of Nectar Defender plus arthropods is substantially within the natural dietary range. If Nectar Defender was about half of the nectar consumed, due to one other source, the resulting range of insects plus nectar for 10% to 80% arthropod consumption would be 5 to 17 ppm, essentially within the 2 to 16 ppm natural range in the wild. Even if Nectar Defender was the sole source of nectar, the resulting estimated range of insects plus nectar for 10% to 80% arthropod consumption would be 7 to 17 ppm, overlapping the 2 to 16 ppm natural range in the wild. In other words:

Range of a hummingbird's diet, no Nectar Defender (est.) : 2 to 16 ppm Cu
Range of a hummingbird's diet with 50% Nectar Defender (est.): 5 to 17 ppm Cu
Range of a hummingbird's diet with 100% Nectar Defender (est.): 7 to 17 ppm Cu

This means that Nectar Defender or Nectar Defender combined with arthropods is substantially within a wild hummingbird's natural dietary range.

("A nesting hummingbird feeding solely on arthropods". MONTGOMERIER, . D. AND C. A. REDSELL. 1980. Condor 82:463464. "Frequency of arthropods in stomachs of tropical hummingbirds". REMSEN, J. V., JR., F. G. STILES, AND P. E. Scorr. 1986. Auk 103:436-441.)

Table 1 Copper Content of Arthropods

Insect	Copper ppm (as fed basis)
Mosquito, adult (Aedes sp.)	23
Mosquito, larvae (Aedes sp.)	19
Garden Spider (Araneus diadematus)	21
Wolf Spider (Pirata piratucus)	55
Wolf Spider (Pardosa lugubris)	28
Orb-weaving Spider (Metellina segmentata)	14
Funnel-web Spider (Agelena labyrinthica)	37
Fruit Fly, adult (Drosophila melanogaster)	8
House fly larvae (Musca domestica)	17
House fly pupae (Musca domestica)	18

Citations for Table 1: "Feeding Captive Insectivorous Animals, Nutritional Aspects of Insects as Food" Nutritional Advisory Group, Nutrition Advisory Handbook, Bernard, J, Allen, M. and Ullrey, D., Michigan State University, East Lansing and National Zoological Park, Smithsonian Institution, Washington D.C.
Iowa State University Entomology Department ,
"Retail insect food mineral composition" Voedingswaarde Table 2 Engels_55
"Complete Nutrient Content of Four Species of Feeder Insects", Mark D. Finke, Zoo Biology 00: 1–15 (2012)
"Nutrient Content of Insects", Mark D. Finke, Encyclopedia of Entomology, 2008, pp 2623-2646
"Spider Ecophysiology", Wolfgang Nentwig, Universität Bern Springer Science & Business Media, Feb 15, 2013

Micronutrient copper in other avian diets

The primary component of Nectar Defender (copper sulfate) has been studied and used in domestic and wild bird feed for decades as a micronutrient mineral supplement. General avian recommendations range from 4-50 ppm copper. Since copper is an essential nutrient, the greatest risk to birds is lack of copper in the diet.

Avian food containing 8 ppm of Cu is recommended by The Association of Avian Veterinarians for psittacine and passerine birds *(Exotic Bird Nutrition Expert Panel Nutrition and Management Committee of the Association of Avian Veterinarians, 1996)* .

In the standard reference " Mineral Tolerance of Animals" the dietary micronutrient range recommended for birds is between 4 and 12 ppm. (The National Academies Press, Second Revised Edition, 2005). It also states that higher levels of copper (125 to 250 ppm) can improve growth.

Another classic reference for bird nutrition recommends that the minimum copper level in feed for Japanese Quail, Bobtail Quail, pheasants, duck, geese, chickens, and turkeys from 4 to 8 ppm. Maximum levels of copper are estimated to be 800 ppm. *(Nutrient Requirements of Poultry, Ninth Revised Edition, 1994, Subcommittee on Poultry Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council, NATIONAL ACADEMY PRESS, Washington, D.C.),*

The summary statement on copper put out by the Extension Toxicology Network of the National Pesticide Information Center states that: "Copper sulfate is practically nontoxic to birds. It poses less of a threat to birds than to other animals. The lowest lethal dose (LDLo) is 1000 mg/kg in pigeons and 600 mg/kg in ducks. The oral LD50 for Bordeaux mixture [copper containing spray] in young mallards is 2000 mg/kg (Extoxnet 1996)" [however this has been revised to a rating of low-toxicity] *(Tucker, R. and Crabtree, D. G. Handbook of Toxicity of Pesticides to Wildlife. U.S. Department of Agriculture, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, U.S. Government Printing Office, Washington, DC, 1970.10-28)*

There have been a few cases of acute copper toxicity in birds, but these have been at levels a hundred times more that the levels being considered in this review. And, the harm was not due to absorption or accumulation of copper, but the acute effects of copper on the mucosa at those levels.

Copper is an essential component of a number of enzymes including cytochrome oxidase, lysyl oxidase, superoxide dismutase, tyrosinase, ceruloplasmin, and dopamine β-monooxygenase. Copper-dependent enzymes function in energy metabolism, maturation and stability of collagen and elastin, pigmentation, the antioxidant defense system, and iron removal, as well as other biological processes.

Since copper is an essential nutrient, birds that do not consume an adequate level of copper, can encounter serious health issues. Too little copper may result in cardiovascular disorders (cardiac failure or rupture of the aorta), depigmentation, impaired keratinization, anemia, reduced growth, neonatal ataxia, bone abnormalities, and impaired immune responses.

Appendix B summarizes many of the numerous studies showing the safety and benefit of copper in birds and poultry.

Appendix C summarizes the recommended levels, and maximum recommended levels of copper for various birds.

Iron accumulation versus copper elimination

Copper is completely different both chemically and biochemically from iron. However some novice birders have confused copper metabolism with iron metabolism. Iron can be an issue for hummingbirds, because it is the only common metal that cannot be removed and excreted from the system, and therefore can accumulate in the avian system. Birders must avoid using water or sugar that is too high in iron.

However, as detailed above, copper is very different and homeostasis keeps copper balanced. The bird's system uses the copper it needs for health, and excretes what it doesn't need. It therefore does not accumulate like iron. As a consequence, copper toxicity is rare in birds, even when exposed to elevated levels.

(Avian Iron Storage Disease: Variations on a Common Theme, Klasing, K., Dierenfeld, E., Koutsos, E., Journal of Zoo and Wildlife Medicine, September 2012, Vol 43, no. 3, pp S27-S34.) (Analysis of Nectar Replacement Products and a Case of Iron Toxicosis in Hummingbirds; Fredrick, H., et.al, 2003, Veterinary Proceedings of the Fifth Conference on Zoo and Wildlife Nutrition, AZA Nutrition Advisory Group, Minneapolis, MN).

Palatability of Nectar Defender

Based on field preference testing by ourselves and other groups, hummingbirds do not have a preference between plain nectar and nectar with Nectar Defender. This is not surprising, since nectar with Nectar Defender does not taste any different than untreated nectar to human subjects, who have much more sensitive taste perception.[2]

For example, a hummingbird preference test was performed using 8 small feeders in different locations within a 50' by 60' suburban area. The feeders were monitored for consumption over two 5 day intervals. Half of the feeders were filled with plain sugar nectar (1:4 ratio) and half were filled with plain sugar nectar plus Nectar Defender. To eliminate location and feeder differences, after 5 days the feeders were rinsed, and the contents switched to the other nectar. The mean consumption of the plain sugar nectar was 21.3 mls, while the mean consumption of the Nectar Defender nectar was 22.2 mls. There was no statistically significant difference between the two nectars. This study's conclusions were the same as others: there is no preference or aversion to Nectar Defender.

Lab and real-world performance of Nectar Defender

This section details the surprising ability of Nectar Defender to keep hummingbird nectar fresh by naturally inhibiting microbial growth.

The following experiments show the efficacy of the product at different micronutrient levels, in lab and real world conditions. Similar to petri dishes, the clear nature of the nectar allowed us to directly observe any microbial colonies growing in the lab or field samples, and make comparisons of the extent of microbial growth between samples. To inoculate the solutions with microbes, an inoculation medium (designated as SHF-1) was prepared by mixing the contents of several spoiled hummingbird feeders, to represent a variety of microorganisms including the black mold typical of spoiled hummingbird feeders.

Experiment 1 – Initial Screening Study: A stock solution of sucrose nectar was prepared by mixing cane sugar and water in a 1:4 ratio, and stirred until completely dissolved. The water used was room temperature tap water to replicate a typical consumer preparation. 100 ml of sucrose nectar was placed in transparent cups and inoculated with 5 drops of SHF-1. Varying amounts of copper sulfate 0.1% solution were added to the tubes to achieve trace concentrations ranging from 0 ppm to 50 ppm. The compositions were left at 70 degrees for four weeks.

Sample Number	Sucrose Nectar (1:4 ratio)	Copper level (ppm)	Results – two weeks	Results – four weeks
A	100 ml	0	Spoiled - Cloudy with white and black spots	Spoiled – Cloudy, fibrous white mass, and black spots
B	100 ml	5	Clear, no spoilage	Clear, one black spot
C	100 ml	10	Clear, no spoilage	Clear, no spoilage
D	100 ml	20	Clear, no spoilage	Clear, no spoilage

Experiment 2 – Optimization: A stock solution of sucrose nectar was prepared by mixing cane sugar and tap water in a 1:4 ratio, and stirred until completely dissolved. 100 ml of sucrose nectar was placed in transparent plastic tubes, and inoculated with 5 drops of SHF-1. Varying amounts of copper sulfate 0.1% solution were added to the tubes to achieve trace concentrations ranging from 0 ppm to 4 ppm, and the tubes were mixed and capped. The tubes were left outside for four weeks at ambient (seasonal range was 50-90 deg F) .

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[2] There seems to be a common presumption that hummingbirds don't taste. However recent research has shown that, although hummingbirds have lost their taste receptors for sweetness, they can perceive sugar by use of their unmami taste receptors. *(Evolution of sweet taste perception in hummingbirds by transformation of the ancestral umami receptors, M. Baldwin, et.al., Sceince 22, August 2014, vol 345, no 6199, pp 929-933)*

Sample Number	Rep	Sucrose Nectar (1:4 ratio)	Copper level (ppm)	Results – two weeks	Results – four weeks
M	1	100 ml	0	Spoiled - Cloudy with white spots	Spoiled – Cloudy and white spots
M	2	100 ml	0	Spoiled - Cloudy with black spots	Spoiled – Cloudy with black spots
N	1	100 ml	2	Clear, no spoilage	Cloudy
N	2	100 ml	2	Clear, no spoilage	Clear, no spoilage
O	1	100 ml	4	Clear, no spoilage	Clear, no spoilage
O	2	100 ml	4	Clear, no spoilage	Clear, no spoilage

Experiment 3 – Real World Confirmation: A stock solution of sucrose nectar was prepared by mixing cane sugar and tap water in a 1:4 ratio, and stirred until completely dissolved. Sucrose nectar was placed in small hummingbird feeders and inoculated with 5 drops of SHF-1. Varying amounts of copper sulfate 0.1% solution were added to the feeders to achieve trace concentrations ranging from 6 ppm to 10 ppm. The feeders were left outside, used by hummingbirds and exposed to seasonal temperatures ranging from 60 to 100 deg F .

Sample Number	Sucrose Nectar (1:4 ratio)	Copper level (ppm)	Results – two weeks	Results – four weeks
X	100 ml	6	Clear,no spoilage	Clear, one spot
Y	100 ml	8	Clear, no spoilage	Clear, no spoilage
Z	100 ml	10	Clear, no spoilage	Clear, no spoilage

It is clear from these studies that Nectar Defender is effective at naturally inhibiting microbial growth even at trace micronutrient levels.

Microbiological confirmation of Nectar Defender performance

To confirm that the clear appearance of nectar protected with Nectar Defender is truly due to inhibiting spoilage, we studied the microbiological environment of nectar as it ages in a real world setting. To do so, clean hummingbird feeders were filled with nectar and placed outside on curved pole supports in an open location at a height of ~2 meters. One feeder was filled with plain sucrose nectar (cane sugar and tap water in a 1:4 ratio) and the other with sucrose nectar protected with Nectar Defender at a 6 ppm Cu level. Hummingbirds had free access to the feeders for 16 days. The location was free of any contaminating foliage above, or ~7 meters to the side. Barriers on the feeder supports protected the feeders from ants or other crawling insects. The temperature during this period ranged from 50 to 80 degrees F. Nectar samples were taken from of each feeder at 8 and 16 days, and submitted for microbiological testing. Certified test methods were performed to determine the level of bacteria[3], fungus, mold and yeast[4] in each sample.

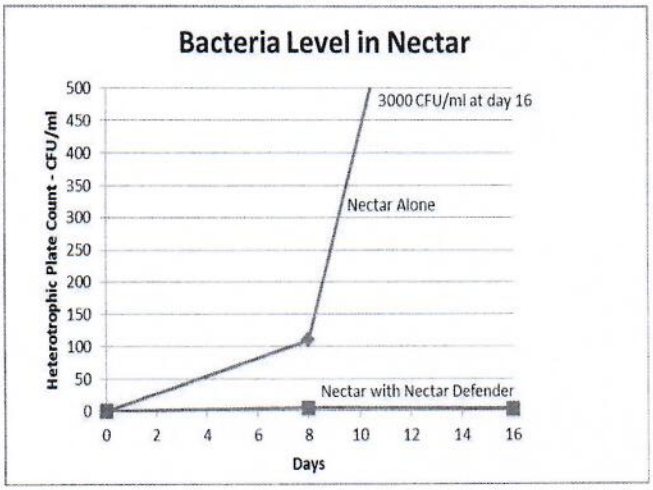

Figure B

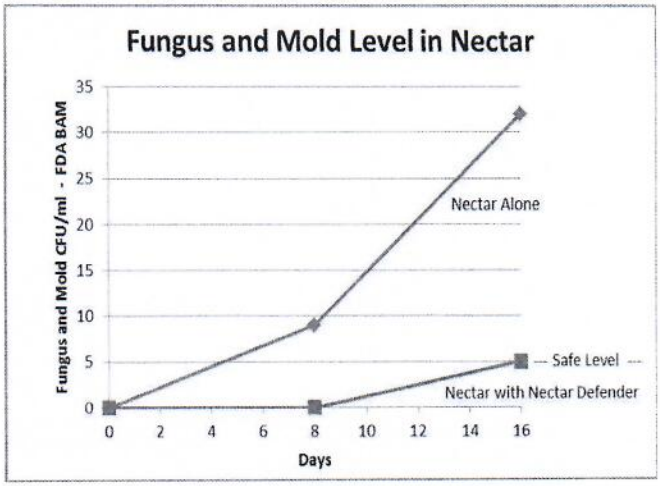

Figure C

[3] Bacteria determined by Heterotropic Plate Count tested by McCampbell Analytical Inc. using method SM9215B (HPC),
[4] Fungus, mold and yeast determined by FDA BAM, tested by McCampbell Analytical Inc.

As Figure B shows, plain nectar experienced an exponential growth of bacteria over the time period to an unhealthy level of 3000 CFU/ml [5], while the Nectar Defender nectar maintained a healthy baseline level during the entire 16 days.

As Figure C shows, plain nectar experienced a mild growth in fungus and mold over the time period, while the Nectar Defender nectar maintained a safe level during the 16 days. Yeast concentration in both nectars were very low (not graphed).

The table below shows the results of visual inspection over the same samples. The plain nectar showed cloudiness and abundant spots over the 16 days, while the Nectar Defender nectar was clear over the same interval.

Visual Inspection of the Nectar

	Day 8	Day 16
Plain Sucrose Nectar	Slightly Cloudy with two white spots	Cloudy, with a multitude of black and white flocculent clumps
Nectar with Nectar Defender	Clear	Clear

This study shows that in a real-world setting aged nectar can generate major microbial growth, and Nectar Defender inhibits that microbial growth. The study also shows that when the nectar is clear due to Nectar Defender, it is clear due to lack of microbial growth, as has been suspected.

Nectar Defender and aquatic species

Aquatic species are more susceptible to copper than birds and mammals, but the low level of copper present in nectar treated with ND would not present a risk to natural bodies of water. Copper sulfate has been approved by the EPA as an algaecide when raising fish for human consumption (Straus and Tucker 1993), and is used in aquariums to treat a variety of fish diseases and parasites. If a pint of nectar treated with Nectar Defender contacted a body of water even one meter in diameter, the dilution effect would reduce the copper content to that of natural water, below any risk level to aquatic species.

Human safety of Nectar Defender

Nectar Defender is safe for adult users to handle when used in the nectar, and as the concentrate. As with any product, it should be kept out of the hands of children, to avoid accidental ingestion.
Copper sulfate is has been declared "GRAS" (Generally Recognized as Safe) by the FDA and the USDA, the safest designation given by the FDA. *(21 CFR 184.1261)*. Copper sulfate at low levels is even allowed in organic agriculture by the USDA, and in infant formula by the FDA. *(21 CFR 205.601)*.

Copper and its compounds are not carcinogenic, mutagenic, or teratogenic at environmentally realistic concentrations. The EPA has stated that there is no evidence linking copper to cancer development in higher animals that can regulate copper in their bodies. *(U.S. Environmental Protection Agency (USEPA). 1980. Ambient water quality criteria for copper. U.S. Environmental Protection Agency Report 440/5-80-036. 162 pp.; Aaseth, J., and T. Norseth. 1986. Copper. Pages 233-254 in L. Friberg, G.F. Nordberg, and V.B. Vouk, editors. Handbook on the toxicology of metals. Second edition. Volume II: specific metals. Elsevier, New York)*

According to the Linus Pauling Institute, copper toxicity is rare in humans. This is because, as described earlier, most higher organisms absorb the copper they need, and excrete what they don't need. The U.S. Food and Nutrition Board (FNB) set the tolerable upper level of intake for copper for humans at 10 mg/day from food and supplements. This is approximately the copper in one tablespoon of ND concentrate, or 1 quarts of prepared nectar.

Very high concentrations of copper can be harmful, but ND only provides trace levels of copper. The standard way of assessing toxicity is by the LD50 rating, the median lethal dose. A range of human LD50 for copper sulfate has been reported,from 450 to 790 mg/kg of body weight (EPA data). As Table 2 shows, if we assume the most conservative level of risk (450 mg/kg), the LD50 of Nectar Defender concentrate would be 225000 mg/kg body weight, less hazardous than salt or sucrose. Even if a small child (25 kg) consumed an entire bottle of ND

[5] CFU/ml values are Colony Forming Units/ml

concentrate, or an entire quart of prepared nectar, it would result in <10 mg copper sulfate/kg body weight, 45 times less than the minimum LD50 risk level.

Table 2 Human Safety of Nectar Defender

	Highest LD50 Copper Sulfate Risk Reported (450 mg/kg body weight)	Lowest LD50 Copper Sulfate Risk Reported (790 mg/kg body weight)
LD50 of ND Concentrate	>50,000 mg/kg body weight	>50,000 mg/kg body weight
LD50 of Sucrose	30,000 mg/kg body weight	30,000 mg/kg body weight
LD50 of Table Salt	3,000 mg/kg body weight	3,000 mg/kg body weight

To put the amount of copper into perspective, a prepared quart of nectar contains less copper than 1000^{th} of a copper penny. The entire bottle of ND contains less copper than about 2 100ths of a copper penny.

How Nectar Defender works

Copper sulfate is soluble, and dissociates into copper (Cu^{+2}) ions and sulfate ($SO4^{-2}$) ions. The copper ion is the active ingredient (sulfate is a common ion which is also safe and does not play a role).

The copper in Nectar Defender acts naturally to inhibit microbes from growing, to keep the nectar fresh. The preserving action of copper is attributed to its ability to target the cellular proteins of mold, bacteria and algae, denaturing them, and deactivating their enzyme systems. The mode of action might be that copper ions bind to various groups including sulfidal groups, imidazoles, carboxyls, phosphate groups and thiol groups, and this binding causes non-specific denaturing of proteins . *(Caldwell B, Brown Rosen E, Sideman E, Shelton AM and Smart CD. 2006. Resource guide for organic insect 1163 and disease management. Cornell University NYS Agricultural Experiment Station.)*

Conclusion

This review has documented several facts and scientific principles on the safety of micronutrient copper in nectar.

- One of the most important facts, is that copper will not be absorbed from a bird's digestive system, unless it is in need of copper. Therefore moderate changes in copper level are inherently safe, since the birds will not absorb more copper than they need.
- In addition copper homeostasis in birds, including hummingbirds, internally regulates a healthy copper balance, initiating excretion of any excess copper. Therefore copper will not accumulate in the hummingbird's system.
- The copper level in Nectar Defender is within the natural diet range of wild hummingbirds, lower than the micronutrient requirement of hummingbirds, and far below the level that would cause any health risks to hummingbirds. The safety of Nectar Defender has been approved by university avian scientists
- In a real world situation, when using Nectar Defender with other sources of nectar, the average level of copper in their nectar diet will likely be less than the drinking water standard set by the EPA.
- Field preference testing shows that hummingbirds prefer nectar with Nectar Defender just as much as nectar without.

Based on these facts, we conclude that Nectar Defender is safe for hummingbirds, safe for handling by humans, and beneficial to hummingbirds by naturally maintaining healthy nectar feeders.

Appendix A

1. Details of the Common Copper Homeostasis Mechanism Across the Animal Kingdom

Most animals, including birds, have a robust system for regulating copper input and excreting any excess copper. Figure 2 below shows the cellular regulatory process for copper in mammals and fruit flies (Drosophila). Though a few of the individual biochemical paths may vary, the universality of the copper homeostasis process is seen by the remarkable similarity of the cellular process for these two diverse species.

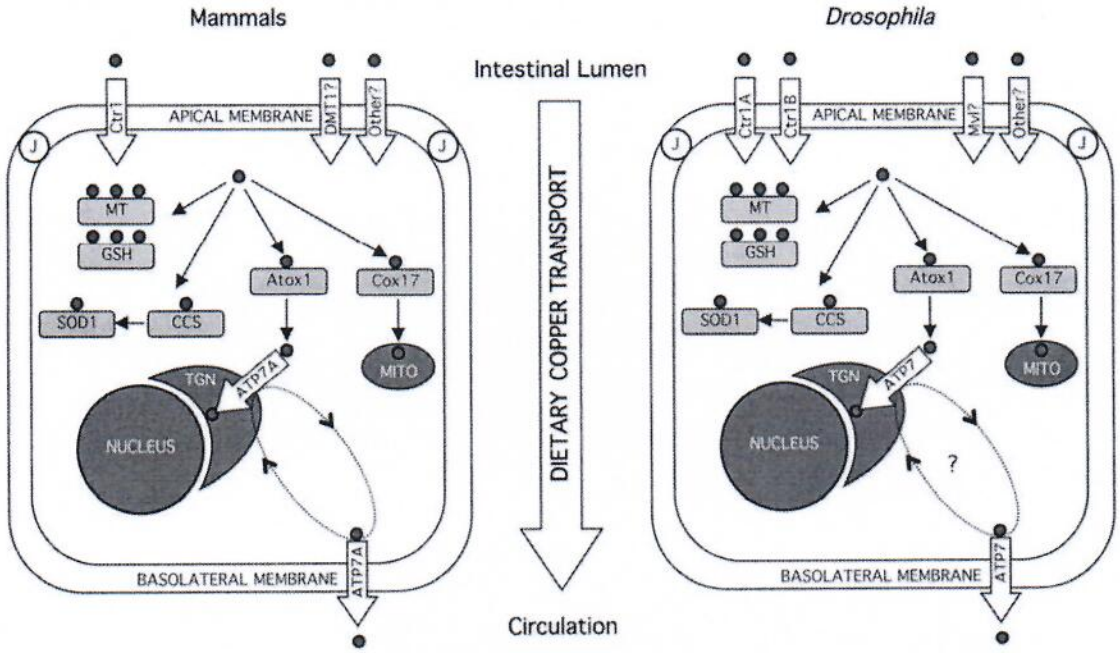

Fig. 2 Schematic of cellular Cu transport in mammals and *Drosophila*.

(From Adam Southon, Richard Burke, and James Camakaris, What Can Flies Tell Us about Copper Homeostasis, Metallomics Vol 5, no 10, 2013).

The sequence of the process is described here:

1. Dietary Cu enters the enterocytes (gut cells) from the intestinal lumen.
2. Cu uptake at the apical surface occurs via the Cu-specific transporter CTR1 (Ctr1A or Ctr1B in fruit flies).
3. The copper chaperones Atox1, CCS and Cox17 deliver Cu to the trans-Golgi network (TGN), SOD1 or the mitochondria, respectively.
4. Metallothionein (MT) or glutathione (GSH) sequester intracellular Cu
5. If the organism is copper-poor, Ctr Cu transporters are increased by the system, so more Cu is allowed across the apical membrane.
6. If the organism has enough copper, the system decreases the Ctr transporters, so less Cu is allowed across the membrane. This regulation is done by the Metal Transcription Factor (MTF-1), which regulates the gene expression for the Ctr transporters.
7. ATP7A (or ATP7) transports Cu both within the system, and out of the system. The ATP7A is also thought to be responsible for transporting Cu out of the liver into the bile, for removal by excretion.

2. Proof of Efficient Copper Homeostasis in Birds

Diet studies with birds demonstrate the effective nature of avian copper homeostasis (biochemical regulation). When fed increasing levels of copper, birds do not show an accumulation, or even a proportional increase of copper in their tissue. Instead, birds absorb only what is needed, even in under conditions of high copper consumption, and excrete the excess.

Copper Homeostasis Study 1

In a study by Ewing et. al., poultry fed with relatively high levels of copper in various forms did not show a corresponding absorption or accumulation of copper in their system. In fact a doubling of the copper level did not significantly affect plasma or liver copper levels.

Cu Addition (ppm)	Copper Form	Resulting Plasma Cu level (Mean ug/100 ml)	Resulting Liver Cu level (Mean ug/g DM)
0		12.5	10.8
63	Citrate	16.6	12.3
125	Citrate	16.0	14.9
125	Sulfate	15.9	13.6
125	Oxychloride	16.1	13.5

No significant statistical difference in response between the low and high copper additive levels (Duncan's multiple range test, 95%). 56 days. (abbreviated table)

Ewing et.al. concluded, "The plasma copper and liver copper levels of the birds fed cupric citrate were not different from those of birds fed the other sources at the same supplementation level or at half the level of the other sources. Adding more than the 63 ppm copper from cupric citrate did not
result in any increase in plasma copper, suggesting homeostatic mechanisms either decreased copper absorption, transport to, and storage in some other tissue, excretion, or some combination of these. "

The chickens with copper also saw significantly increased weight gain by up to 4.9%. Mortality was not affected by the addition of copper in the experiment.
("Studies on the Feeding of Cupric Sulfate Pentahydrate, Cupric Citrate, and Copper Oxychloride to Broiler Chickens", H. PETTIT EWING, GENE M. PESTI,2 REMZI I. BAKALLI, and JOSE FERNANDO M. MENTEN, Department of Poultry Science, The University of Georgia, Athens, Georgia 30602-2772, 1998 Poultry Science 77:445–448)

Copper Homeostasis Study 2

In a study by Bao et. al., poultry fed with a copper additive did not show a corresponding absorption or accumulation of copper in their system[6] , but does have a proportional increase in the copper excreted by the bird. The compiled chart below shows the results of a copper sulfate additive on blood, liver and excreta. The fact that the copper input was tripled and the excretion level tripled, confirms that homeostasis is limiting the absorption of copper to what the bird actually needs.

Feed Cu Level (ppm)	Resulting Plasma Cu level (Mean ug/ml)	Resulting Liver Cu level (Mean ug/g DM)	Excreted Cu mg/bird per day
4.2 (control)	0.25	8	0.28
12.2	0.21	4	0.86

29 days of feeding

[6] *a reduction actually occurs due to growth reasons explained in the paper*

Referring to excretion correlation, the author states: " it is well known that changes in trace mineral absorption and excretion in the gastrointestinal tract are primary mechanisms for maintaining trace mineral homeostasis."

("Effect of Organically Complexed Copper, Iron, Manganese, and Zinc on Broiler Performance, Mineral Excretion, and Accumulation in Tissues", Y. M. Bao, M. Choct, P. A. Iji,1 and K. Bruerton, School of Environmental and Rural Science, and Australian Poultry Cooperative Research Centre , 2007 Journal. Of Applied Poultry Research. 16:448–455)

Copper Homeostasis Study 3

A study by Liu et. al. , shows that even when birds are given high levels of copper, moderate tissue copper levels are maintained, with only a very slight increase in liver Cu concentration. However, bile Cu levels are tripled, showing that and active removal system is playing a role in controlling internal Cu levels and inhibiting accumulation.

Added Cu (ppm)	Feed Cu Level (ppm)	Resulting Plasma Cu level (Mean ug/ml)	Resulting Liver Cu level (Mean ug/g DM)	Bile Cu ug/g
0 (Control)	6	0.076	3.9	22.6
125	128	0.078	4.1	36.9
250	258	0.080	4.5	66.4

42 days of feeding

("Copper in Organic Proteinate or Inorganic Sulfate Form is Equally Bioavailable for Broiler Chicks Fed a Conventional Corn-Soybean Meal Diet", Songbai, Liu; Lin, Lu; Sufen, Li; Jingjing, Xie; Liyang, Zhang; . Biological Trace Element Research , Volume 147 (1) – Jun 1, 2012)

Appendix B

Additional Studies on Micronutrient Copper in Bird Diets

Domestic chicks fed diet a copper-adequate diet of 8.7 ppm copper for 60 days resulted in good survival and growth. Chicks fed a low copper diet of 2.7 ppm copper for 60 days had normal growth but a high frequency of vascular problems. Chicks fed diet a copper deficient diet of only 1.5 ppm copper for 60 days resulted in only 5% survival due to copper deficiency. *(Carlton, W. W., and W. Henderson. 1964b. Studies on the copper requirement of growing chickens. Avian Diseases 8:227-234.)*

Domestic chicks (1 day old Gallus spp.) were fed a copper-adequate diet of 8.0 ppm and a copper deficient diet of 0.7 ppm copper for 4-6 weeks. Chicks on copper-adequate diet had negligible mortality, no histopathology, and normal growth. Chicks fed copper-deficient diet had >50% mortality and high frequency of cardiovascular and skeletal lesions., *(Carlton, W. W., and W. Henderson. 1963. Cardiovascular lesions in experimental copper deficiency in chickens. Journal of Nutrition 81:200-208. Carlton, W. W., and W. Henderson. 1964a. Skeletal lesions in experimental copper deficiency in chickens. Avian Diseases 8:48-55.)*

Chickens were fed either a control diet or the control diet supplemented with copper sulfate or copper citrate. Feeding 125 or 250 ppm copper increased growth (4.9%) and decreased feed conversion ratios (3.4%), total plasma cholesterol (40.2%), and breast muscle cholesterol (37.0%). Feeding 375 ppm copper was without further beneficial effect. *(Poult Sci. 1996 Sep;75(9):1086-91, Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Pesti GM, Bakalli RI., Department of Poultry Science, University of Georgia, Athens 30602-2772, USA.)*

In turkeys, natural diets with as much as 800 mg Cu/kg ration have no adverse effects on growth or survival. But diets with no copper at all are toxic to turkeys in three weeks, and diets with only 50 ppm copper still have adverse effects from copper deficiency *(Waibel et al. 1964).*

A study on Mallards suggests that they prefer drinking water that contains 100 ppm copper to distilled water containing no copper. For 15 days adult mallards were given a choice of distilled water or water with 30, 60, or 100 ppm copper. Ducks consumed significantly more water treated at 100 ppm copper than distilled water. *(Rowe, B. L., and H. H. Prince. 1983.. Bulletin of Environmental Contamination and Toxicology 30:505-510.)*

Appendix C

Recommended Copper Levels

This appendix provides a summary of the maximum recommended level of copper with various birds. The maximum levels in the last three columns are based on research showing safe use at those levels, footnoted below. Researchers have not tested the upper level of copper that can be tolerated by hummingbirds, but due to the commonality of avian physiology, the upper level can be inferred from the copper requirements and tolerances of other birds[7].

Feeder Fresh Nectar Defender		Hummingbirds	Quail, Pheasants, Ducks, Geese	Chickens	Turkeys
6 ppm	Recommended Copper Level in the Diet →	8 ppm[8]	4-8 ppm[9]	8 ppm[10]	>50 ppm[11]
	Maximum Recommended Copper Level in the Diet →	128 ppm[12] (conservative estimate) (ratio of 16)	800 ppm[13] (ratio of 100)	375 ppm[14] (ratio of 47)	800 ppm[15] (ratio of 16)

Based on this, the level of copper in nectar prepared with Nectar Defender will be even lower than the recommended level, and far below the maximum level for copper.

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[7] On the record statement by Dr. Kirk Klasing, Avian Science Department, UC Davis,.

[8] *Hummingbird Nutrition: Development of a Purified Diet for Long-Term Maintenance, A.T. Brice and C.R. Grau, Department of Avian Sciences, University of California, Davis, Zoo Biology 8:233-237 (1989)*

[9] *Nutrient Requirements of Poultry, Ninth Revised Edition, 1994, Subcommittee on Poultry Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council, NATIONAL ACADEMY PRESS, Washington, D.C.*

[10] *Carlton, W. W., and W. Henderson. 1963. Cardiovascular lesions in experimental copper deficiency in chickens. Journal of Nutrition 81:200-208. Carlton, W. W., and W. Henderson. 1964a. Skeletal lesions in experimental copper deficiency in chickens. Avian Diseases 8:48-55*

[11] Waibel et al. 1964

[12] The ratio of maximum recommended copper level and recommended copper level spans values from 16 to 100 in other birds. The estimated maximum level for hummingbirds was determined by taking the most conservative ratio of 16, and applying it to the hummingbird recommended level (16x8=128).

[13] *Nutrient Requirements of Poultry, Ninth Revised Edition, 1994, Subcommittee on Poultry Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council, NATIONAL ACADEMY PRESS, Washington, D.C.*

[14] Poult Sci. 1996 Sep;75(9):1086-91, Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Pesti GM, Bakalli RI., Department of Poultry Science, University of Georgia, Athens 30602-2772, USA.

[15] Waibel et al. 1964