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Lebensmittelsicherheit

Guidelines

for the Prevention of the Occurrence of Hydroxymethylfurfural in Feed for Honey Bees

(as of 1 June 2018)

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Hydroxymethylfurfural (HMF), also 5-hydroxymethyl-2-furaldehyde, is a product formed by the degradation of simple sugars, especially fructose. HMF occurs in food and feed containing carbohydrates, e.g. in feed sugars used to feed honey bees in winter.

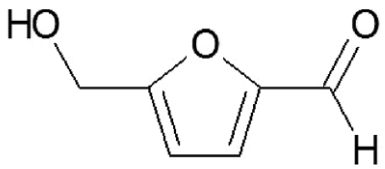

Figure I: Chemical structure of hydroxymethylfurfural[i]

Various studies suggest that increased HMF levels are toxic for honey bees, especially if a long feeding period (hibernation period) results in a long-term exposure to HMF. This fact sheet points out possibilities to minimise the development of HMF in industrially produced feed for honey bees during manufacture, transport and storage (by traders and beekeepers).

1. General requirements for feed for honey bees

1.1. Basic information on feed for honey bees

Feed for honey bees is usually provided during the hibernation period to replace the honey removed in summer by equivalent feed. Honey bees can only efficiently metabolise mono- and disaccharides, and, in certain amounts, their metabolites (e.g. citric acid, acetic acid). Ash, oligosaccharides, as well as products arising from caramelisation, conversion and decomposition of carbohydrates cannot be used by honey bees and may even be harmful to them. The intake of these should therefore be kept as low as possible. These substances can otherwise result in the excrement placing excessive strain on honey bees' rectums. Under unfavourable weather conditions, this may also lead to defecation in the hive and thus possibly to the loss of the colony.

1.2. Requirements for sugar syrup as feed for honey bees

Industrially produced sugar syrups for honey bees should meet the following requirements[1)]:

- **Suitable types of sugar:** They must consist of saccharides that can be metabolised well by the honey bees. Above all, these are the monosaccharides fructose (fruit sugar), glucose (dextrose) and the disaccharides saccharose (cane or beet sugar) and, to a limited degree, maltose (malt sugar, see point 4.1).
- **Microbiologically stable:** They must be microbiologically stable. This requires highly concentrated solutions that also have a low a_w value (water activity). This reduces the danger of their deteriorating (e.g. development of slime moulds, fermentation).

- **Avoiding crystallisation:** The highly concentrated syrups must not crystallise. Glucose, in particular, tends to crystallise in the combs. On the one hand, honey bees are not able to dissolve – and thus use – crystallised feed. On the other hand, it can be assumed that, as a result of the crystallisation of glucose, the HMF present accumulates in the parts of the feed that are still liquid.[2)] Fructose is required to obtain highly concentrated, non-crystallising syrups. However, fructose is particularly prone to converting into HMF.
- **Minimising indigestible substances:** They should contain as little ash and oligosaccharides as possible, and as little caramelisation and decomposition products of carbohydrates as possible as well. This includes syrups produced under the influence of acids or high temperatures. Partially refined (brown) raw materials should not be used either.

1.3. Composition of feed syrup

The composition of industrially produced feed syrups for honey bees is based on the sugar spectrum of nectar and blossom honeys. Fructose, glucose and saccharose each making up around a third constitutes a good ratio for the mixture of these sugar types in the syrup. To reduce the risk of crystallisation, it is beneficial to ensure that the fructose content is above the glucose content.

Based on the above-mentioned requirements for feed for honey bees, recommendations for manufacture, transport, trade and beekeeping are listed under point 4.

Figure II: It may require nine months of winter feeding until honey bees fly again in spring.[ii]

2. Safety of feed for honey bees containing hydroxymethylfurfural

2.1. Effects of HMF in feed syrup on the mortality of honey bees

Studies conducted to date have produced different results as regards the dose-dependent effects of HMF on the mortality of honey bees.

EU maximum levels of undesired substances for a feedstuff are given for a humidity level of 12% which corresponds to a dry matter content of 88%. As feed syrups with such high dry matter contents crystallise, the following HMF levels refer to the standard commercial dry matter content for feed syrup of 72%. Some feed syrups used in experimental situations had different dry matter contents. In order to allow a direct comparison of research findings, the HMF levels in the cases dealt with here have been converted to a dry matter content of 72% in the feed syrup. It must therefore be take into account that the HMF levels given in this fact sheet may be different from those indicated in literature sources.

Experiments conducted in the 1970s examined, over a period of 20 days, the mortality of honey bees that had ingested feed syrup with a dry matter content of 50% and undetectable HMF levels, as well as with HMF concentrations of 30 mg, 150 mg and 750 mg HMF per kg of feed syrup. In relation to a dry matter content of 72% for comparison, these values would be equivalent to approx. 43 mg, 216 mg and 1080 mg HMF per kg of feed syrup. At 58.7%, the mortality of honey bees that had been given 216 mg HMF/kg feed syrup was significantly higher than the mortality of honey bees that had ingested the syrups with undetectable HMF levels (mortality: 12.5%) or with concentrations of 43 mg HMF/kg feed syrup (mortality: 15.0%). If given feed syrup with HMF levels of 1080 mg/kg feed syrup, the mortality of the honey bees even reached 98.8%.[3)]

Up-to-date values were established by the Institute for Apiculture of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) in a study with three sub-studies carried out in 2015 and 2016 for the Federal Ministry of Food and Agriculture. This study used pure saccharose with a dry matter content of 50% in an aqueous solution in order to prevent an introduction of HMF through the sugar used. Due to the saturation concentration value of saccharose, it was not possible to use a saccharose solution with a dry matter content of 72%.

The test concentrations were between 20 and 3840 mg HMF/kg. Mortality was significantly higher than in the control group at concentrations from 480 mg/kg. The calculated EC_{10}* was at 440.25 mg HMF/kg (Weibull Analysis).[4)] This corresponds to **635 mg HMF/kg of feed syrup** with a dry matter content of 72%.

2.2. Derivation and recommendation of a reference value for HMF in feed for honey bees

How much HMF a honey bee can ingest without adverse health effects is probably dependent on the environmental conditions and on the overall tolerability of the feed. The studies mentioned under 2.1 examined the impact of increased HMF levels over a period of 20 and 30 days. As various studies suggest a life span of 170 to 243 days for winter bees[5)], thus implying that winter bees can survive up to nine months from late summer until spring, it can be expected that, in practice, a honey bee colony is exposed to increased HMF levels over several months or that the HMF levels continue to rise during this period (Table 1). In addition, margins of safety for the unavoidable formation of HMF during transport and storage until feeding must be taken into account.

* EC_{10}: Calculated mean effective concentration of HMF in feed syrup that will probably result in the death of 10% of the exposed honey bees during the trial period.

Based on the up-to-date results of the examinations under 2.1 (EC_{10} = 635 mg HMF/kg feed syrup), and taking into account a safety factor of 10, a **reference value of a maximum of 60 mg HMF/kg feed syrup** (with a dry matter content of 72%) at the time of dispatch of the feed is recommended.

3. Identification and determination of hydroxymethylfurfural (HMF) in feed for honey bees

The official collection of food analysis methods recognises two methods for identifying and quantitatively determining HMF levels in honey:

- **HPLC** (high-performance liquid chromatography) in accordance with DIN 10751 L40.00 10/3
  The current view is that HPLC is the most specific method. It was assessed by the Association of German Agricultural Testing and Research Institutes (VDLUFA) as a means of evaluating the HMF levels in sugar syrups for use as feed material for honey bees and found to be suitable. The relative expanded uncertainty for HPLC measurements was 6%. Most laboratories give 2 mg/kg as the detection limit and 5 mg/kg as the determination limit.

  In the case of the HPLC method, the test substance, together with a so-called mobile phase, is pumped through a separation column, the so-called stationary phase. Depending on how long a component of the test substance interacts with the stationary phase, the time at which it appears at the end of the separation column, and is detected there, differs. While the examination of honey requires a protein precipitation/clarification with Carrez solution, this is not necessary for the analysis of pure sugar syrup.

- **Photometric method** ("Winkler method") in accordance with DIN 10751 L40.00 10/1
  This photometric method was often used until the 1980s. It can be conducted more quickly than the HPLC but requires the use of carcinogenic reagents (*p*-Toluidine) which is the reason why this method is not applied any more.

At present, there is no rapid test available that beekeepers could use to test the HMF level of their sugar syrup on-site before feeding.

When storing feed samples, care should be taken that they are not exposed to temperatures above 25°C (better: room temperature or cooler) and that they are stored in the dark until analysed in order to prevent or reduce an increase in the HMF level subsequent to the taking of the sample (see point 4.2).

4. Possible preventive measures that can be taken by the responsible links in the chain with regard to reducing hydroxymethylfurfural (HMF) in feed for honey bees

HMF develops through the dehydration of sugars under the influence of heat or of the acids used for sugar inversion. In addition to this, HMF forms through non-enzymatic reactions between reducing sugars and amino acids (so-called Maillard reaction).

Fructose in solution is stable only to a limited degree and the main source for the formation of HMF. But fructose in honey bee feed fulfils the essential function of preventing the crystallisation of the feed in the combs. The formation of HMF in feed for honey bees can therefore not be completely avoided. With a view to minimising HMF levels, it is important to optimise the production, transport and storage conditions of sugar syrups.

The main factors contributing to the formation of HMF are source material, temperature, pH value and time. The storage temperature, in particular, is of major importance in the formation of HMF. The temperature should therefore be below 25°C or, even better, below 20°C. Even at room temperature fructose degrades, although very slowly. A slight increase in HMF can even be observed at a temperature of 4°C. At ambient temperatures of more than 40°C, HMF levels rise considerably (Table 1).

4.1. Manufacture of feed for honey bees

Saccharose is the basic component in the manufacture of feed syrup and must be split into fructose and glucose. Industrial production with state-of-the-art technology takes place at low pH values and temperatures between 60°C and 80°C with the help of **ion-exchange** resins. In addition, saccharose can also be inverted **enzymatically** with the help of invertase or (at a lower price) by **exposure to acids** (e.g. oxalic acid, hydrochloric acid). The addition of acid, however, reduces the pH value and therefore leads to increased HMF formation.

As an alternative, feed syrup can be produced on the basis of starch hydrolysate (wheat, corn). In addition to fructose and glucose, this syrup contains high levels of maltose which is split into glucose by the enzyme invertase contained in the honey bees' saliva. This results in undesirable high glucose contents in stored feeds which should be taken into consideration in their handling and feeding (see point 4.3).

Immediately after manufacturing, HMF levels are usually between 15 and 25 mg/kg. At delivery, feed for honey bees should have an HMF level below 60 mg/kg feed syrup with a dry matter content of 72%.

The following recommendations for practical application can help producers to ensure these levels:

- ✓ Ensure a defined composition of the sugar types. Manufacturer-related variations should be less than 10% of the dry matter content. Check deviating compositions before introduction on the market.
- ✓ Ash content below 0.5 g/kg dry matter content in the syrup (calculated from the conductivity).
- ✓ Produce honey bee feed without added acids.
- ✓ Avoid heating during the production process.
- ✓ Supply every batch of feed for honey bees with a date of minimum durability (usually 18 months).
- ✓ Provide information on transport and storage conditions (dry, dark, temperatures below 25°C).

4.2. Trade, transport and storage of feed for honey bees

The composition and the HMF level of the feed syrup changes with time and also depends on the temperature (Table 1): The glucose and fructose content increases gradually while the saccharose content decreases. The HMF level can rise from normal, low starting values of 25 mg/kg to more than 350 mg/kg syrup. These changes should be minimised if possible.

Table 1: Increase in HMF levels in saccharose-based feed syrup during storage at different ambient temperatures.

Temperature	Duration of storage	Increase in HMF level	Data source
4 °C	5 weeks	Increase by 4.0%	VDLUFA validation ring trial 166/2015/M
25 °C	5 weeks	Constant (Δ=-0.4 %)	VDLUFA validation ring trial 166/2015/M
41 °C	5 weeks	Increase by 134%	VDLUFA validation ring trial 166/2015/M
20 °C	12 months	Typical additive increase of approx. 10 ± 5 mg HMF/kg [1]	Verein der Zuckerindustrie e. V.
30 °C	12 months	Typical additive increase of 50 to 100 mg HMF/kg [1]	Verein der Zuckerindustrie e. V.
10-25 °C [2]	24 months	Additive of approx. 10 ± 5 mg HMF/kg [1]	Verein der Zuckerindustrie e. V.

[1] depending on package size and material

[2] measured internal temperature profile in a well-insulated, non-temperature controlled hall

According to this, the sugar syrup should, if possible, not be stored in tanks or containers outside of buildings, as the sunlight could warm the syrup and thus increase its HMF level. It was also found that storage stability depended on the package size, with the HMF level increasing slightly more in smaller package sizes than in larger ones.

The following recommendations apply to the trade, transport and storage of feed for honey bees:

- ✓ Check the product specification of the sugar factory for low HMF starting levels.
- ✓ Choose short transport routes, if possible.
- ✓ Optimise storage conditions: dry and temperature-controlled (if possible below 25°C).
- ✓ Avoid direct sunlight.
- ✓ Avoid long storage periods.
- ✓ Comply with the shelf life of the products (usually 18 months).
- ✓ Ensure careful handling if the syrup is poured from its original packaging into other containers (handling in closed buildings, cleaning of the filling nozzle after each use).
- ✓ At the time of delivery, HMF levels should be below 60 mg/kg feed syrup (72% dry matter substance).

4.3. Handling and feeding of feed syrup by beekeepers

HMF levels can further increase in the beehive. The following factors can have an influence on how much HMF is formed and how much is ingested by the honey bees:

- **Duration of winter feeding**: Depending on the length and severity of the winter, honey bees may have to rely on the winter feed for up to nine months. Depending on the circumstances, they are therefore exposed to feed containing HMF over a very long period of time. During this time, HMF levels in the feed may even further increase in the hive.
- **Starting time of the winter feeding**: If additional feeding is already required in August/September, the honey bees are given this feed while still breeding. Breeding activities may, to a lesser degree, last until longer-lasting frost and restart as early as around the turn of the year. The high temperatures in the breeding area can then lead to increasing HMF levels. Outside temperatures of above 25°C can also induce a rise in HMF levels.
- **Addition of other substances**: HMF levels can also rise if other substances, e.g. pre-heated honey, are added to the feed sugar.
- **Varroa treatment**: A varroa treatment in the bee hive can also have an indirect impact on the HMF level. The acids used for such treatments (e.g. lactic acid, oxalic acid, formic acid) can reduce the pH value of the stored feed which can result in increased HMF levels.
- **Feed syrup on the basis of starch hydrolysate:** If, in addition, the high-glucose nectar of late-flowering plants (e.g. mustard, oil radish, ivy) gets into this stored and converted feed or if stored honey is transferred and mixed with the feed syrup, the glucose content can further increase and crystallisation nuclei can enter the stored feed. Especially in long winters, this feed can strongly crystallise, leaving no sufficient liquid fraction available to the bees.[5)]

This results in the following recommendations for beekeepers:

- ✓ Ask the supplier for an analysis of sugar composition, the HMF level and the ash content of the feed.
- ✓ Take care that the HMF level in the original feed is as low as possible in order to have a safety factor: Reference figure: **not above 60 mg/kg syrup**.
- ✓ Observe the instructions for use on the packaging.
- ✓ Do not store the feed syrup for longer periods of time and feed it as fresh as possible.
- ✓ Do not mix fresh syrup with old honey bee feed that has spent a long time in storage.
- ✓ Observe the recommendations under point 4.2 even if the feed is only stored for a short period.

4.4. Reducing HMF levels in sugar syrup by way of ultraviolet-C (UV-C) treatment

There are some treatment methods that can reduce the already existing HMF levels in sugar syrup. One study examined the effect of UV-C treatments on HMF levels in sugar syrup caused by thermal treatment.[7)] The syrups examined were commercial high-fructose corn syrup (HFCS; 55% fructose) and a sugar solution with 55% fructose. In fact, the UV-C treatment reduced the HMF level in fructose syrup (with an initial content of 12.75 mg and 6.16 mg HMF per litre) by 43% and in HFCS (with an initial content of 29.03 mg HMF/l) even by 63%. But the composition of the sugar syrups used in this study differs from the composition of syrups suitable for the feeding of honey bees. The practicability of using this method in honey bee feeding and, should the occasion arise, the right point in time for such use are debatable as, in the case of bad practice, HMF levels can also rise considerably shortly before feeding. The best way to keep HMF levels in feed syrups as low as possible until feeding is therefore to prevent the formation of HMF during production, transport and storage (see points 4.1 to 4.3).

In a nutshell:

The higher the temperature or the lower the pH value and the longer the time of exposure, the greater the increase of the HMF level.

5. Legal basis for feed for honey bees and HMF levels

Honey bees are food-producing animals. Feed for honey bees is therefore subject to the relevant European and German feed legislation, especially to the feed safety requirements. Honey bee feed that is unsafe for animal health must not be placed on the market or fed to honey bees.

Feed syrup gained from saccharose is a feed material within the meaning of Regulation (EC) No 767/2009 and is listed under 4.1.12 of Part C of the Annex to Regulation (EU) No 68/2013 as "sugar syrup" and described as "*Product obtained by processing of sugar and/or molasses*". In contrast, feed syrup gained from starch hydrolysate (corn, wheat) and pollen patties are compound feed.

HMF in feed is to be considered an undesirable substance within the meaning of Section 3 number 18 of the Food and Feed Code. No legally binding maximum limit has yet been established. When determining HMF levels in feed through analytical tests, it must therefore be examined on a case by case basis whether the feed safety requirements under Article 15 of Regulation (EC) No 178/2002 are met.

6. Review of this fact sheet

The fact sheet will be reviewed to assess whether it is still up-to-date after a reasonable period of time and adapted as necessary.

Fact sheet in cooperation with:

Bundesinstitut für Risikobewertung, Abteilung Sicherheit in der Nahrungskette *(Federal Institute for Risk Assessment, Department 'Safety in the Food Chain')*, Max-Dohrn-Str. 8-10, D-10589 Berlin

Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES) - Institut für Bienenkunde Celle *(Office for Consumer Protection and Food Safety of the Land of Lower Saxony (LAVES) – Institute for Apiculture in Celle)*, Herzogin-Eleonore-Allee 5, D-29221 Celle

Verein der Zuckerindustrie e. V. *(Association of the German sugar industry)*, Friedrichstraße 69, D-10117 Berlin

Further reading

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Consumer Protection and Food Safety of the Land of Lower Saxony (LAVES) – Institute for Apiculture, Celle).

7) Ros-Polski, V., Popović, V., Koutchma, T. (2016): Effect of ultraviolet-C light treatment on Hydroxymethylfurfural (5-HMF) content in high fructose corn syrup (HFCS) and model syrups. Journal of Food Engineering 179, 78-87.

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Sources of illustrations

i. Figure I: Zirbes, L., Nguyen, B.K., Graaf, D.C., De Meulenaer, B., Reybrock, W., Haubruge, E., Saegerman, C. (2013): Hydroxymethylfurfural: A possible emergent cause of honey bee mortality? J. Agric. Food Chem. 61, 11865.

ii. Figure II: Copyright Fotolia, Photographer: Aubord Dulac.

Legal foundations cited

Food and Feed Code (*Lebensmittel- und Futtermittelgesetzbuch*) in the version published on 3 June 2013 (Federal Law Gazette p. 1426), last amended by Article 1 of the Act of 30 June 2017 (Federal Law Gazette I p. 2147)

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (OJ L 31 of 1 February 2002, p. 1), last amended by Regulation (EU) No 2017/745

Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene (OJ L 35 of 8 February 2005, p. 1), last amended by Regulation (EU) No 2015/1905

Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC (OJ L 229 of 1 September 2009, p. 1, L 192 of 22 July 2011, p. 71), last amended by Regulation (EU) No 2017/2279

Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials (OJ L 29 of 30 January 2013, p. 1) as amended by Regulation (EU) No 2017/1017

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