

ACIDIC-BENZOIC FEED AND NOSEMA DISEASE

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S u m m a r y

It is not unusual that beekeepers add acetic or benzoic acid to the winter feed. It is effective for preventing formation of mould in feeders and may also have other effects. The chemical composition of the food may have an impact on the spore germination of the intracellular parasite *Nosema apis*, but there are contradictory results on the impact from acidified food on nosema infections. We have studied the effect from acidic and benzoic feed on nosema development in field studies and laboratory experiments. Bee colonies in the field were given winter feed with different concentrations of acetic acid (n=82) or benzoic acid (n= 41). Samples of adult bees from each colony were investigated for nosema disease in the fall at the time of feeding and in the following spring. Bees (n=225, acetic acid, n=120, benzoic acid) in the laboratory were individually fed the same solutions as in the field studies, but with addition of 10.000 *N. apis* spores per bee. Control bees (n=75/60) received sugar solution or acidified sugar solution only. Samples were taken 4, 8 and 12 or 14 days post infection and the amount of spores in the midgut was counted in a haemocytometer. In a second experiment, also with addition of 10.000 *N. apis* spores per bee but using only the highest concentration of acetic acid compared to non-acidified sugar solution, the rate of infected bees was investigated (n=210). No effect from altering the pH by addition of acetic acid, or from benzoic acid could be found, neither on the quantitative disease development of the parasite, nor on the infection rate of individual bees. The results from the field experiments support the laboratory results: addition of acetic or benzoic acid to the feed of honey bees has no influence on nosema prevalence or development.

Keywords: acetic acid, benzoic acid, winter feed, *Nosema apis*.

INTRODUCTION

The microsporidian parasite *Nosema apis* infects the epithelial cells of the ventricles of the honey bee (*Apis mellifera*) (Bailey 1972; Graaf 1991). *N. apis* has spread world-wide (Nixon 1982) but is not considered an important problem in tropical and sub-tropical climates (Wilson and Nunamaker 1983). In temperate climates, infections by *N. apis* must be considered a serious disease. *N. apis* has large negative effect on the production capacity of honey bee colonies in temperate climates (Farrar 1947, Fries

1984) and the survival of the colony during winter is affected by the disease (Farrar 1942, Fries 1988a). The problem with supersedure of infected queens adds to the economic damage caused by the parasite (Farrar 1947).

The addition of acetic acid into winter feed may have positive effects in preventing different diseases. Based on an experiment in Norway it was stated that acetic acid in the feed reduced the occurrence of chalk brood (Pedersen 1981), but the results have not been repeated. Laboratory experiments in Belgium suggested that acidified feed

decreased the development of *N. apis* in the midgut (Mottoul 1996), but field studies performed in France demonstrated no impact from acidified feed on nosema development (Vaillant 1989).

A laboratory study in Ukraine on the prophylactic effect of benzoic acid on the development of nosemosis in bees, suggests that benzoic feed have an impact on the disease symptoms, although no effect on the spore production in individual bees could be observed (Efimenko et al. 1998).

The chemical composition of the feed may have a general impact on the intestinal microflora and disease manifestation (Gilliam 1997), as well as a direct impact on the spore germination of *N. apis*. When the spore enters the midgut of the bee, it germinates under the influence of the gut juices. Many chemical stimuli cause germination in vitro (Laere 1977) and it is possible that changing the chemical environment (i.e. lower the pH) in vivo could have an impact on spore germination. On the other hand, the pH-value of the honey is very low, 3.2-4.5, averaging about 3.9 (Crane 1975).

The aim of this experiment was to study the effect from acidic and benzoic feed on the development of *N. apis* under laboratory conditions and in the field.

MATERIALS AND METHODS

Acetic acid

Field study

Eighty-two colonies in 4 different apiaries were randomly treated in three different ways in the fall 2002 at the time of winter feeding.

1. Sugar solution 2:3 w/v
2. Food consisting of sugar solution 2:3 w/v in addition of 2 ml conc. acetic acid/1000 ml (Acid 1)
3. Food consisting of sugar solution 2:3 w/v in addition of 4 ml conc. acetic acid/1000 ml (Acid 2)

In connection with the feeding, bee samples were taken to determine the occurrence of *N. apis*, and the pH-value in the food from a number of colonies in the different categories of treatment were measured. Colonies were also sampled and analyzed (60 bees per colony) for *N. apis* the following spring.

Laboratory experiment I

Adult bees were individually fed (10 μ l per bee, 30 bees per treatment) with the same sugar solutions as in the field study, but with the addition of 10000 spores of *N. apis* per 10 μ l in the following combinations (Table 1) As shown, the spores were distributed either in acidified food or in sugar solution, followed by feeding either with sugar solution or acidified food.

Table 1.

Combination of treatment (group number), 30 bees per treatment, experiment I.

Initial treatment	Additional treatment		
	Sugar solution	Acid 1	Acid 2
Sugar solution + spores	I	II	III
Acid 1 + spores	IV	V	VI
Acid 2 + spores	VII	VIII	IX
Sugar solution only	X	XI	XII

Sugar solution (sugar: water 3:2, pH 7.91)

Acid 1 (sugar solution + 0.2% acetic acid, pH 3.6)

Acid 2 (sugar solution + 0.4% acetic acid, pH 3.2)

Table 2.

Combination of treatments and number of bees per treatment, experiment II.

Treatment	Number of cages/Bees per cage	Total number of bees
Sugar solution	1/15	15
Acid 2	1/15	15
Sugar solution + spores	6/15	90
Acid 2 + spores	6/15	90

The bees were incubated at + 30°C in 50% RH, with constant access to food. Five bees per treatment were examined 4, 8 and 12 days after treatment, and the number of spores in the midgut was counted in a haemocytometer. Twelve days after treatment, the rest of the bees were killed and examined for nosema.

Laboratory experiment II

To refine eventual impact from acidified food on *N. apis*, yet another separate experiment were conducted. In this experiment bees were fed spores initially in sugar solution, followed by additional feeding of sugar solution, or spores in acidified food followed by additional feeding with acidified food only. Two groups of bees were only fed sugar solution and acidified food respectively. These bees were used as control groups. (Table 2).

The bees were individually fed (10 µl/bee) with sugar solution or acidified

food with addition of 10000 spores of *N. apis* per 10 µl. All of the bees were killed and examined for nosema 14 days after treatment.

Benzoic acid

Field study

Forty-one colonies in 4 different apiaries were treated in the fall 2004 at the time of winter feeding.

A control group (n=20) were fed sugar solution (2:3 w/v) only, and the other group (n=21) were fed sugar solution 2:3 w/v in addition of 30 g benzoic acid/100 l. In connection with the feeding, bee samples were taken to determine the occurrence of *N. apis*. Colonies were also sampled and analyzed (60 bees per colony) for *N. apis* the following spring.

Laboratory experiment

Adult bees were individually fed (10 µl per bee, 30 bees per treatment) sugar solution with the addition of 10000 spores of *N. apis* per 10 µl in the combinations

Table 3.

Combination of treatment (group number), 30 bees per treatment.

Initial treatment	Additional treatment	
	Sugar solution	Benzoic acid
Spores in sugar	I	II
Spores in sugar/benzoic acid	III	IV
Sugar solution only	V	
Benzoic acid only		VI

Sugar solution 1:1 (sugar: water) pH 7.9

Acid (sugar solution + 0.03% benzoic acid) pH 3.3

tabulated in Table 3. As shown in Table 3, the spores were distributed either in benzoic feed or in sugar solution, followed by feeding either with sugar solution or sugar solution with benzoic acid. Two groups of bees were only fed sugar solution and benzoic feed respectively. These bees were used as control groups (Table 3).

The bees were incubated at + 30°C in 50% RH, with constant access to food. Five bees per treatment were examined 4, 8 and 14 days after treatment, and the number of spores in the midgut was counted in a haemocytometer. Fourteen days after treatment, the rest of the bees were killed and examined for nosema.

RESULTS

Acetic acid

Laboratory experiment I

No impact on the quantitative development of *N. apis* from acidified food could be traced in any of the treatments compared to controls in this experiment (Figure 1).

There was no significant difference in the proportion of infected bees between the different treatments (*Chi-square*, $P > 0.05$). The treatment of the groups is specified in Table 1. In the group where bees were initially fed spores in sugar solution only (group I, II and III), 86% of the bees were infected with *N. apis*. In the groups fed spores in Acid 1 (group IV, V and VI) and Acid 2 (VII, VIII and IX) 92-93% were infected. Bees examined 4 days post

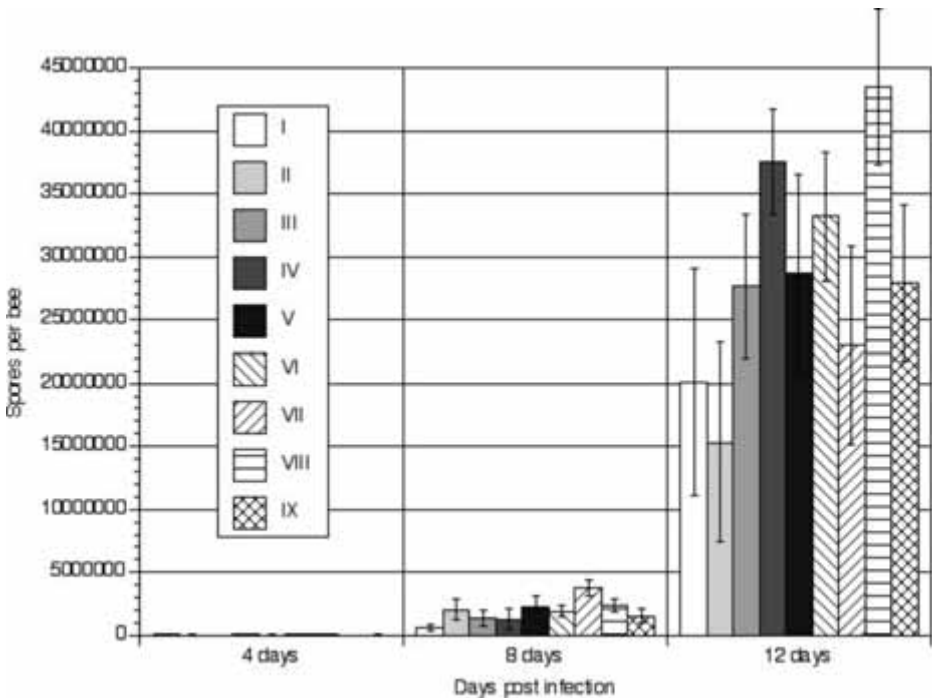


Figure 1. Acetic acid results. Average number of spores in the ventriculus of five bees examined for *N. apis*, 4, 8 and 12 days post infection. Group I-III initially fed with spores in sugar solution only, group IV-VI fed spores in sugar solution + 0.2% acetic acid, group VII-IX fed spores in sugar solution + 0.4% acetic acid. Group numbers corresponds to Table 1. Error bars represent standard error of means.

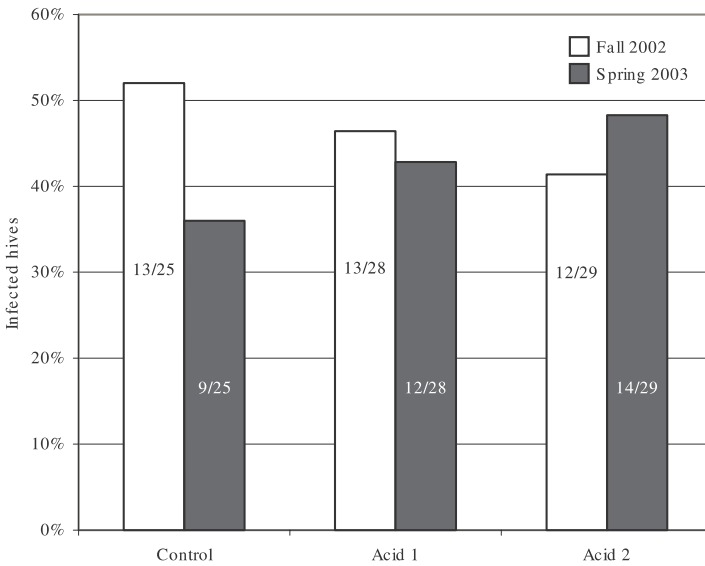


Figure 2. Proportion of infected bee colonies in the fall and spring respectively in the control group fed sugar solution and the two groups fed different concentrations of acidified food (combined). Numbers in bars correspond to number of infected hives over total number of hives.

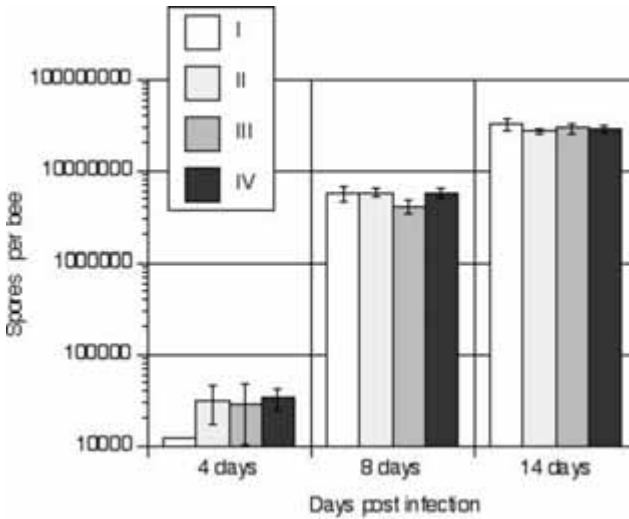


Figure 3. Benzoic acid results. Average number of spores in the ventriculus of five bees examined for *N. apis*, 4, 8 and 14 days post infection. Group I and II initially fed with spores in sugar solution only, group III and IV fed spores in sugar solution + 0.03% benzoic acid. Group numbers corresponds to Table 3. Error bars represent standard error of means.

infection were not part of the comparison calculations (Kruskall-Wallis) because not all infected bees have traceable amounts of spores already 4 days post infection (Fries 1988b).

Laboratory experiment II

In this experiment, the highest concentration of acetic acid was compared to non-acidified sugar solution. In the group fed sugar solution + spores, 25 of 90

bees were infected while in the group fed spores in acidified food, 30 of 85 bees were infected. Data demonstrate no reduction in the proportion of infected bees when spores are fed in acidified food and bees continued to be fed acidified food post infection (*Chi-square*, $P>0.05$). The treatment of the two groups is specified in Table 2.

Field experiment

The proportion of infected hives in the fall of 2002 is shown in Figure 2. Neither in the fall nor in the spring is the difference between treatments significantly different from the controls (*Chi-square*, $P>0.05$). There was an unexpected reduction of spores per bee from the fall of 2002 to the following spring in all colonies including the controls, but there was no significant difference between the reductions of spore levels between any of the groups.

Benzoic acid

Laboratory experiment

No impact on the quantitative development of *N. apis* from benzoic feed could be traced in any of the treatments compared to controls in the experiment (Figure 3).

Field experiment

There was an increase in the proportion of infected hives from the fall of 2004 to the spring 2005 in both the treated and the control group, but the over all infection rates was very low in both groups. In the control group ($n=20$), the amount of infected hives increased from 1 to 6, whereas in the group fed benzoic winter feed ($n=21$), the number increased from zero to 3 infected hives. Thus, the difference in infection rate is not significant between the groups (*Chi-square*, $P>0.05$).

DISCUSSION

The laboratory results on acetic acid demonstrate that the infectivity or quantitative development of *N. apis* in honey bees is not influenced by the acidity of the food when spores are consumed. This conclusion is valid irrespective of whether the spores are consumed in acid solution and then bees are fed normal sugar solution, if spores are fed in sugar solution and bees then given acid solution, or if spores are fed in acid solution and bees then fed acid solution.

The results from the field experiment with acetic acid supports the conclusions made from the laboratory experiments. From Figure 2 it can be seen that the trend (non-significant) is contrary to the hypothesis that acidification of food will lower the incidence of nosema disease. Thus, the field results support the conclusions of the laboratory experiments.

It is interesting to note that the proportion of infected hives actually has decreased from the fall to the spring in this experiment with acetic acid (Figure 2), and that there has been a reduction in the number of spores per bee during the same time. This is contrary to what can be expected (Bailey and Ball 1991) and also contrary to our field experiment with benzoic acid 2005/6, but the trend is similar in all groups and remains unexplained.

The results from the laboratory experiments with benzoic acid demonstrate that the course of infection is not affected by addition of benzoic acid to the feed, at least not with the concentration applied. The quantitative development of *N. apis* is similar in all groups irrespective of treatment. In the field experiment, the proportion of infected hives increased during winter in both the treated and untreated group, but is generally to low to effectively compare treatments.

It has been suggested that the intestinal microflora of the adult honey bee may be affected by additional compounds in the feed, influencing the disease manifestation and survival of adult bees and that addition of benzoic acid can prolong the life-span of both nosema infected and uninfected bees (Efimenko et al. 1998). Our results demonstrate that neither infectivity, nor the quantitative spore production, is influenced by acidifying food by using acetic acid, or from addition of benzoic acid to the feed. Why addition of benzoic acid to the feed would increase the life-span of adult bees infected by *N. apis* (Efimenko et al. 1998) remains unexplained and these results need to be repeated since it is well documented that the response in life-span to nosema infection is highly variable and difficult to predict (Malone and Giacon 1996).

CONCLUSIONS

Addition of acetic or benzoic acid to the feed of honey bees has no influence on nosema prevalence or development. Neither the infectivity nor the quantitative development of *N. apis* in honey bees is influenced by the acidification of the feed by using acetic or benzoic acid in low concentrations.

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POKARM Z DODATKIEM KWASÓW OCTOWEGO I BENZOESOWEGO A ROZWÓJ NOSEMOZY

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S t r e s z c z e n i e

Nie jest niecodziennym dodawanie przez pszczelarzy kwasu octowego lub benzooesowego do karmy zimowej. Zapobiega to skutecznie tworzeniu się pleśni w podkarmiaczkach, a także może mieć inne działanie. Skład chemiczny pokarmu może wpływać na kiełkowanie zarodników wewnątrzkomórkowego pasożyta *Nosema apis*, ale dane na temat wpływu, jaki ma zakwaszony pokarm na rozwój nosemozy są sprzeczne. Badaliśmy wpływ kwasów octowego i benzooesowego na rozwój nosemozy w badaniach terenowych i doświadczeniach laboratoryjnych. Rodzinom pszczelim w terenie podawano pokarm zimowy o różnej koncentracji kwasu octowego (n=82) lub benzooesowego (n=41). Próbkę pszczoł z każdej rodziny badano pod względem występowania nosemozy w jesieni w okresie podkarmiania i następnego wiosny. Pszczołom w laboratorium (n=225, kwas octowy, n=82, kwas benzooesowy) podawano indywidualnie takie same rozcieńczenia, jak w badaniach terenowych, ale z dodatkiem 10,000 zarodników *N. apis* na pszczołę. Pszczoły kontrolne n=75/60) otrzymywały tylko roztwór cukru lub zakwaszony roztwór cukru. Próbkę pobierano 4, 8, i 12 lub 14 dni po zakażeniu a liczbę zarodników w jelicie środkowym liczono w hemocytometrze. W drugim doświadczeniu, gdzie także dodawano 10,000 zarodników *N. apis* na 1 pszczołę ale przy zastosowaniu tylko najwyższego stężenia kwasu octowego w porównaniu do nie zakwaszonego roztworu cukru badano udział zakażonych pszczoł (n=210). Nie stwierdzono wpływu zmiany pH na skutek dodania kwasu octowego lub benzooesowego ani pod względem ilościowego rozwoju choroby powodowanej przez pasożyta ani na stopień zakażenia pszczoł. Wyniki z badań terenowych potwierdzają wyniki laboratoryjne: dodatek kwasu octowego lub benzooesowego do karmy nie ma wpływu na występowanie nosemozy i jej rozwój.

Słowa kluczowe: kwas octowy, kwas benzooesowy, pokarm zimowy, *Nosema apis*.