The effect of three ways of sugar feeding on the intake of pollen and the development of nukes and its effect on the vitellogenin content of the worker bees of *Apis mellifera* L.

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Abstract

Currently beekeeping is under pressure of declining numbers of honey bee colonies, a problem because of their important role in pollination and the resulting socio-economic aspects. It has been shown that the parasite *Varroa destructor* plays a major role in the loss of colonies. *V. destructor* individuals use the bees' hemolymph as their food. The hemolymph and vitellogenin consumption by the *Varroa* mite has an impact on the amount of protein available for the developing bee and whole colony. Where *V. Destructor* can use up to 25% of the nutritional reserves available in a bee pupa. In this research we looked for a relation in supplemental sugar feeding and pollen intake on colony level.

Two types of sugar were provided to colonies, i.e. sugar syrup and sugar paste (group A *ad libitum*), where sugar syrup was provided either in big amounts (group C) or dispersed (group B). In this research the amount of pollen under different feeding strategies were examined, important because differences in pollen intake may result in different vitality, brood rearing, and overwintering abilities of bee colonies.

After seven weeks of feeding, brood size, amount of bees and pollen intake results where analyzed. The results show that pollen intake did not differ significantly among the three feeding groups ($P=0.142$). But a significant difference ($P=0.007$) in brood size among the feeding groups was found. Colonies provided with sugar syrup at once did have less brood compared to sugar syrup provided dispersed over time. Feeding with sugar paste showed no difference with both sugar syrup provided once and sugar syrup provided dispersed. The amount of bees did not differ significantly ($P=0.399$). Vitellogenin titers were higher ($P<0.001$) between bees fed with sugar syrup provided at once and bees of the other two groups. Thus, the results show no significant differences toward the pollen intake and different supplemental sugar sources. But it is shown that a difference in brood rearing can be caused by the way sugar syrup is provided.

Winter bees will develop when pollen supply is low (parameter), causing a smaller brood nest with production of winter bees. Those winter bees have a higher vitellogenin level. But the development of winter bees is possibly induced by more parameter which might be nectar/carbohydrate availability as maybe shown by this research.

1. Introduction

At this moment beekeeping is under pressure of declining numbers of honey bee colonies. This is a problem because of honey bees' role in pollination and the resulting socio-economic aspects. It has been shown that the parasite *Varroa destructor* plays a major role in the loss of colonies (Neumann and Carreck, 2010). *Varroa destructor* infestation leads to weaker colonies to which different direct and indirect factors add and which finally results in collapse of colonies and eventually a declining number of colonies. Factors which might play a role are insecticides, food availability, bad bee keeping practice, virus- and bacterial diseases and pests (Neumann and Carreck, 2010).

V. destructor individuals use the bees' hemolymph as their food. The consumption can amount 25% of the nutritional reserves available in a bee pupa (Garedew et al., 2004). This large consumption in the pupa has an impact on the amount of protein available for the developing bee. The amount of protein available is essential for the development of brood and for the general colony vitality and development (Cremonez et al., 1998). The decline in protein present in pupae demonstrates the harmful effect of *V. destructor*. The combination of *V. destructor* and availability of protein seems determinative for the growth of larvae and young bees. The combination of *V. destructor* and available protein is finally determinative for the colony vitality and growth.

Current practise among beekeepers in the Netherlands is to make new colonies prior to the period the bee colony prepares to swarm. Often one or more colonies are made by splitting from one colony which needs to be supplied with extra sugar. Supplying extra sugar has the aim to boost colony growth and make the colony strong enough for the winter (Free and Spencerbooth, 1961). Besides the boosting of new colonies, colonies older than one year are supplied with sugar syrup when nectar supply is low or when the honey is harvested from the hive. A solution of table sugar and water of 33% sugar or 50% sugar is normal practice. Where 33% is often used to boost a colony, 50% is used for winter preparation. Next to this, there are other sugar supplements available like sugar paste which is more like a dough.

From the perspective of nutrients, a bee colony needs besides sugar as a carbohydrate source also pollen which serve as a source of protein, lipids, vitamins and minerals. Those nutrients, protein, lipids, vitamins and minerals are of major importance for the development of the larvae, young bees, and therefore also for the whole colony development. Keller et al., (2005) have shown that a the amount of pollen in the diet has a positive correlation with the spring colony growth and might influence the overwintering capacities of the colony. It is known that supplemental feeding does have a boosting effect on the colony (Pesante et al, 1992) as well that pollen patties do have an effect on colony development and honey yields (DeGrandi-Hoffman et al., 2008). But also in the natural way, the amount of brood in a colony is influenced by the amount of pollen available (Fewell and Winston et al., 1992, Keller et al., 2005). In the colony a certain equilibrium of pollen is maintained (Fewell and Winston et al., 1992). More brood will result in more pollen foraging, but a low availability of pollen will result in less brood rearing. Excluding colonies from pollen will finally result in a total stop of brood rearing (Keller et al., 2005). In smaller colonies pollen equilibrium might be different, smaller colonies might gain more profit by foraging more on pollen to maximize brood rearing.

Population growth is a major measure for the strength of the honeybee colony and the survival chance in winter. Knowing that pollen intake is affecting the brood production, the pollen flow is determinative for the colony vitality (Fewell and Winston et al., 1992).

Important for boosting the colony is the amount of food (Pesante et al., 1992) and the way it is provided. When there is an abundance of sugar syrup provided it will be stored in the cells and made ready for winter storage. When this syrup is sealed the bees will not use it until it is essentially needed for survival. Besides the population growth also vitellogenin is a parameter for winter survival. Vitellogenin is a glyco-lipoprotein regulating the juvenile hormone (JH). Carbohydrate and proteins availability in the field and maybe artificially provided leads to changes in the vitellogenin level. A shortage of carbohydrates and protein leads to higher JH levels causing sensory changes resulting in foraging behaviour (Amdam et. Al., 2006). The transition to winter bees seems to be induced by lower pollen availability. Due to the lower pollen availability the amount of brood in the colony is reduced (Mattila and Otis 2007) causing a lower production rate of larvae food. The lower production of larvae food causes a higher protein availability in the bees itself resulting in higher vitellogenin levels and winter bees (Mattilla and Otis 2007, 2004).

When sugar syrup is provided, the bees will try to store it as fast as possible. Such behaviour can have an effect on the collection of pollen to boost a strong and vital colony (Avni et al., 2009, Cremonez et al., 1998). To avoid the behaviour of immediate storage of the sugar syrup, sugar can be fed as a sugar paste which is only limitedly stored by the bees and which can therefore constantly be used as an energy source by the bees whenever needed. The bees will use sugar paste as energy source for normal tasks, besides that less time to forage for nectar might be needed, resulting in more time for pollen foraging. By keeping the pollen-flow high the bees will become - and stay - more vital. The sugar source provided in the research can be used directly or stored and consumed during winter. The sugar (mainly sucrose see feeding 3.2) will be broken down by enzymes (invertase) in the honey stomach after which it can be stored in the combs or used in metabolism by the bee itself. The stored not jet converted sucrose will even be further hydrolyzed by invertase (sucrase, sacchrase) during comb storage to glucose and fructose. Ripening of honey is done in the hive, and not during flight (Crane E., 1979).

In this research we compared feeding of sugar syrup and sugar paste and analysed the resulting brood rearing and pollen flow. When pollen intake is high in colonies constantly provided with sugar paste and lower in sugar syrup fed colonies this might result in different vitality, brood rearing, and overwintering ability of the bee colonies.

In this research the amount of honey produced is not taken into account and only the development of the bee colonies is examined.

2. Research question

I. Does feeding of *Apis mellifera* L. colonies with sugar paste or sugar syrup result in a changed (higher or lower) intake of pollen?

    a. Is the effect of sugar paste and sugar syrup different?

II. Does feeding of *Apis mellifera* L. colonies with sugar paste or sugar syrup result in a changed (higher or lower) brood and colony development?

III. Is there a difference in bee colony vitellogenin titer among bees from the different colonies and the colony groups and their diet?

3. Methods

The research was performed on 36 small starting bee colonies (nukes) of *Apis mellifera* L. (see 3.1 preparing colonies). The 36 colonies were divided into three groups (A, B and C) of 12 colonies, taking into account the origin of the queens (see 3.1 preparing colonies).

- Group A was provided with sugar paste, available throughout the test.
- Group B sugar was provided syrup 50% (see 3.2 feeding) on a basis of two times a week, corresponding to the energy provided by the sugar paste.
- Group C was supplied with sugar syrup 50% (see 3.2 feeding) at once (4,6L) corresponding to the food use of group A (sugar paste). When the sugar paste was almost finished a new amount was applied this did take six weeks (applied in week five) in total sugar in once was applied two times during the test.

All the colonies started with an amount of food in the combs, which was already collected before by the bees. Colonies were checked for availability of food, to prevent possible starvation. The pollen weight, closed brood area and vitellogenin titer of individual bees were the variables in this research (see pollen intake, development of the bee colony and vitellogenin) and these were assessed at the end of the experiment. The hives were located on the apiary of Wageningen University and Research Centre, the Netherlands. All colonies had the same forage availability and similar weather conditions. The hives for each of the three groups A, B and C (factors) were positioned together to minimise interactions of bees between the groups (robbing and displacement to wrong hives). The hive entrances where located towards the South-East.

3.1 Preparing colonies

The colonies were provided by and in collaboration with a commercial beekeeper company (Inbuzz) using *Apis mellifera* L. Making of the colonies was done by the simplified method described by Calis et al. (2008). Starting point is a full grown colony in a ten frame hive. From this hive the queen is removed, leaving the colony queen-less. The colony will build emergency cells (queen cells), which will be full grown after 14 days. Then the colony will be split into smaller colonies of about 2500 – 3000 bees. Every colony will get one queen cell/young queen and after approximately one week the queen will perform a mating flight.

To provide all groups with a fair and secure start all newly made colonies did get additional food in the form of sugar paste for the first three weeks and were treated against *Varroa destructor* with oxalic acid spraying.

After the mating flight colonies with queens with good egg production and brood rearing were used for the research and placed on location.

The newly made bee colonies were divided in the three groups according to their origin. This means that from one main colony minimally three queens were obtained. Those three queens and their hive will be divided among the three groups. This will guarantee a certain level of genetic comparability between the groups. Table 3.1.1 shows an

Table 3.1.1 Example of queen division, up to 11 queens per group.

Group A Sugar paste	Group B Sugar syrup 33% weekly	Group C Sugar syrup at once
2 Queens from hive 1 1 Queen from hive 2 Up to 12 queens in total	2 Queens from hive 1 1 Queen from hive 2 Up to 12 queens in total	2 Queens from hive 1 1 Queen from hive 2 Up to 12 queens in total

example of two hives as source of queens for the three groups.

3.2 Feeding

The food was provided *ad libitum* for group A with sugar paste, provided in packages of 2.5 kg, consisting of 90% powder sugar and 10% water (Fondabee, consisting of sucrose (83%), higher sugars (12%) maltose (2.75) and dextrose (2.25%). Group B and C were provided with sugar syrup BEE FIT® HM 76% diluted to 50% sugar (table 3.2.1) (consisting of maltose (37%), glucose (26.5%), fructose (24.5%) and higher sugars (12%). The sugar sources like glucose, fructose, saccharose (sucrose), maltose, trehalose, melezitose; and the unsweet: arabinose, xylose, galactose, cellobiose, raffinose, mannitol, sorbitol.can be utilized well by bees (Haydak M.H., 1970) where those sugars are also mainly found in nectar in different quantities or only sacharose is present or absent (Crane E., 1979).

The amount provided to group B and C was adjusted to the amount of sugar paste used in group A. During the experiment the colonies fluctuated in adsorption rate and therefore also the amount of sugar provided fluctuated. Group B was provided twice a week during the whole experiment with syrup 50%. Syrup was provided in glass jars with holes in the cover of approximately Ø 1-2mm, and placed upside down on the frames (figure 3.2.1). Group C did also get sugar syrup according to the amount used by group A. As a starting point the sugar paste consumption was estimated to be 2.5 kg in two weeks (experience of Johan Calis). In practice during the experiment the sugar paste usage fluctuated (see discussion).

Table 3.2.1 Food division

Calculation	Amount food provided	Group
Sugarpaste (90% sugar = 2.25kg)	2.5 kg of sugarpaste provided at once	A
Sugar syrup 2.25kg sugar and 2.25kg water	4.5 kg provided in approximately 5 weeks 0.440 kg 2 times a week.	B
Sugar syrup 2.25kg sugar and 2.25kg water	4.5 kg of syrup provided at once	C

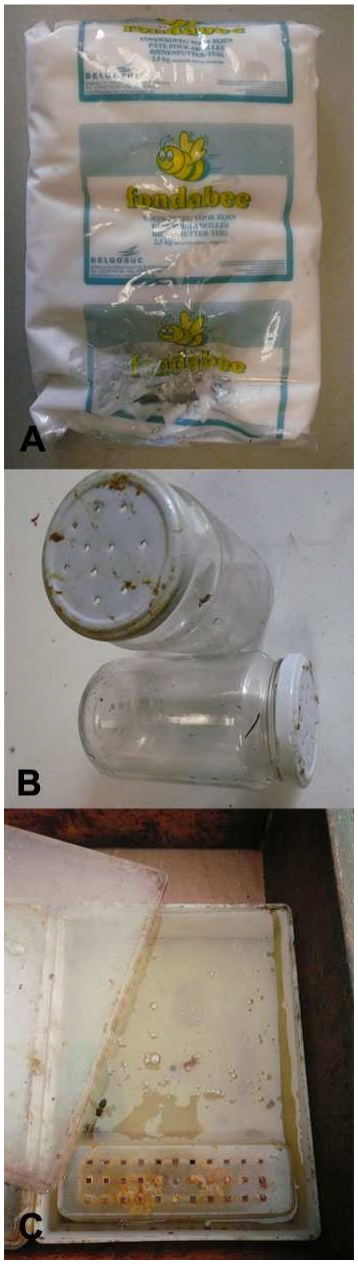

Figure 3.2.1 showing the three types of feed provide to the colonies. A. Sugar paste, B. Sugar syrup provided with intervals, C. Sugar syrup applied at once.

3.3 Pollen intake

To measure the pollen flow we used a pollen trap (home made, figure 3.3.1 shown without top plate). This trap has the dimensions of a medium honey super and all forager bees are forced to enter through a plastic (Nicod) strip with holes (figure 3.3.1 A and figure 3.3.1 B). Those holes (Ø 5mm figure 3.3.2) cause pollen to fall off and the pollen are collected in a drawer after falling through a grid (figure 3.3.1 B). The grid prevents the bee to have access to the fallen pollen Figure 3.3.1 E. The pollen trap is positioned on top of the brood chamber providing easy access needed for turning on (access through the Nicod strip) and off (open access, strip removed). All pollen traps were 'on' during one day per week from the start of the experiment in July until the half of September simultaneously (Figure 3.4.1). Every time the trap was used for one day during daylight, starting at 14:00 until the next day 14:00. The pollen harvested per hive were collected in tubes and weighed directly. After weighing the pollen were stored in a refrigerator and stored for possible further research or follow up research.

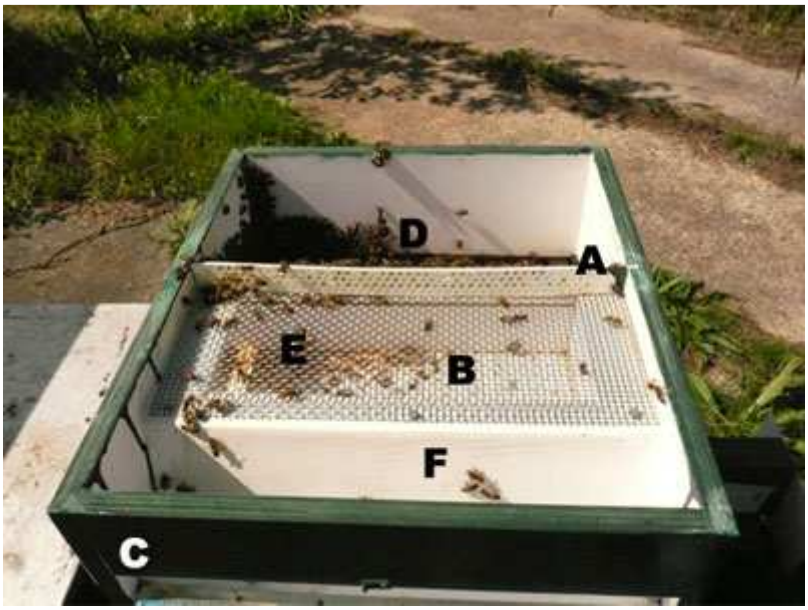

Figure 3.3.1 Open pollen trap shown from above, where A. shows the plastic strip with holes where bees go trough and pollen are falling off due to the small size of the holes, B. the grid where fallen pollen and bees stay separated, C. the entrance to the pollen trap, D. the entrance and actual hive, E. the pollen under the grid stored in a tray, F. slope for easy access of bees to the trap.

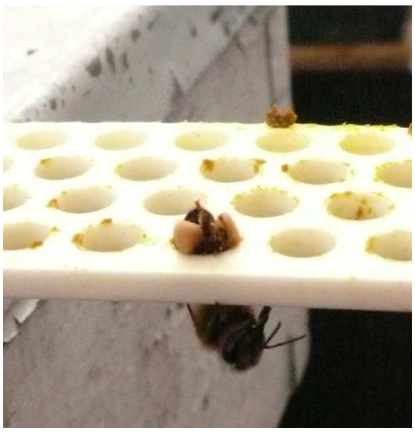

Figure 3.3.2 Bee pressing herself through a hole of the pollen trap, after which the pollen will fall off.

3.4 Colony development and control

The development of the colonies was measured by estimation on three occasions. Once at the start (date: 19-07-2010), once in the middle (date: 31-08-2010) of the project and once at the end (date: 20-09-2010). Estimations where made of the number of frames occupied by bees and the surface area of sealed brood. The estimations of brood area were done using the unit dm^2, because one comb side is $7dm^2$ this is a practical unit. On one dm^2 of occupied comb there are approximately 125 bees. By multiplying the counted surface in dm^2 with 125 the number of bees on one side of the comb could be calculated (Cornelissen et al., 2009). The results of the estimations where used to estimate the colony size in order to measure growth. Simultaneously with the colony size estimation several other factors were checked:

Figure 3.4.1 Row of hives with pollen traps on top and super for feeding multiple diets.

- The presence of brood. This will be checked for all phases: eggs, larvae, closed brood. If there is no brood present there might be a problem with the queen, food, etc.
- Food availability, if a lack of food was present food could be provided earlier than the scheme, or extra.

3.5 Vitellogenin

To gain insight in the average vitellogenin level of the bees in a colony hemolymph was taken of 25 bees from every colony. The sampled bees were collected from an outer comb. An outer comb is used because of the low risk of sampling the queen, and is allowed since the age cohorts of bees were shown to be homogeneously distributed over the frames in the colony (Steen 2009, unpublished). The bees were kept in a plastic container (figure 3.5.1) with access to sugar paste provided in a small container preventing sticking. Hemolymph was sampled within 2 hours after removal of the bees from the hive. The sampled bees were anesthetized with CO_2 and then fixed with needles onto a Styrofoam strip (figure 3.5.2). To extract their hemolymph a capillary (2µl) is placed in the abdomen between the second and third segment. In this way, the hemolymph can be extracted (Amdam et al., 2006). Per bee two µl of hemolymph was extracted (figure 3.5.2), pooled and kept cold after which it was stored. The hemolymph was stored and vitellogenin was measured according the 'Protocol eiwit electrophoresis met phast system'. Thereafter different processing phases are applied, starting with preparing a standard (B-galactosidase Sigma G8511-1vl 500ug) for quantifying the vitellogenin in the last phase. Then a serial dilution is made, and the samples are made ready for SDS-electrophoresis. Then electrophoresis is applied using one ul sample solution. After which the program is started, and the protein are transported through the gel. Thereafter the gel is colored with Blue R (standard tablet phastgel) and support liquids. Finally the gels are photographed and analyzed. This is done by using the earlier applied standard (B-galactosidase) and software (Phoretix 1D advanced version 5.20) and.

Figure 3.5.1 showing anesthetized bees within the sample container, just before sampling the hemolymph for vitellogenin analysis. Within the yellow box in white the sugar past is shown, preventing the bees from a deficiency of carbohydrates.

Figure 3.5.2 showing the bees on Styrofoam strip, the upper hand is sampling hemolymph by using a 2µl glass capillary.

3.6 Statistical analysis

Number of replicate colonies needed.

In order to get results that are with 95% certainty not caused by chance a minimal group size is needed. In this experiment three variables will be measured. According to estimations derived from earlier research, the vitellogenin titer (highest value 55 µg vitellogenin per µL, lowest value 5 µg vitellogenin per µL) and sealed brood cell numbers (highest 10.000 cells and lowest 8.000 cells) the variance number could be used. For sealed brood and vitellogenin titer the variance was respectively 62.500 cells and 156,25 µg vit. per µL. As difference Δ we used for vitellogenin titer 12,5 µg vit. per µL and for sealed brood 250,0 cells. Using a probability level of α=0.05 and a power of β=0.9 a number of 11 colonies per treatment is needed to get reliable results. To compute the numbers of colonies needed Genstat statistical software version 13.2 (VSN International Ltd) was used.

Data analysis

The research consisted of three observation periods within seven weeks. The data was analyzed on significance differences against the factors of group A, B and C.

Statistical analysis was done by using one way analysis of variance (ANOVA). Except from the pollen intake due to the non normal distributed residuals (heteroscedasticity) (figure 3.6.1). Here a general linear mixed model of variance was used (Restricted Maximum Likelihood Methods). The significance was analyzed by using an F-test.

From seven weeks, the pollen intake was measured. Visually the first three weeks and last four weeks differed (figure 4.1.1). Linear regression was used to prove a possible relation between the weather influences (parameters: temperature, sun, wind, precipitation) and the observed difference between the first three weeks and last four weeks. Also a multiple linear regression was used to include all parameters at once.

When the F-test shows a significant effect then pair wise comparison was done using the Tukey test (p=0.05). Tukey test was used because it includes a correction for the number of treatment means to be compared, in other words it corrects for the increased chance that a type I error is made (experiment-wise error rate).

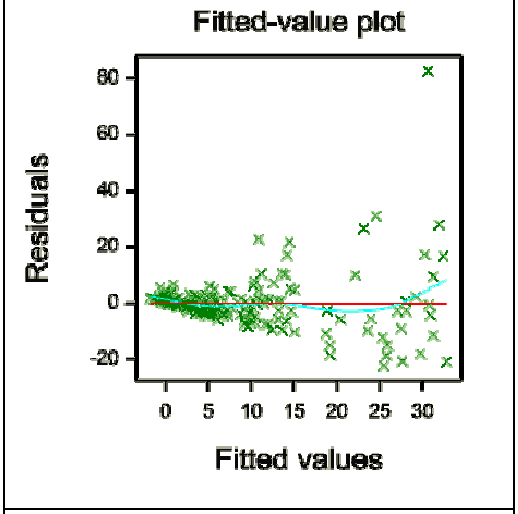

Figure 3.6.1 Fitted-value plot showing skewness of the non normal distributed residuals of the pollen intake results.

For all results the residuals were evaluated on normal distribution using the skewness values, fitted value plot and normal plot of the residuals. Only the residuals from the pollen intake were not normally distributed and showed a relation with the fitted values. On the non-normally distributed residuals logarithm transformation was applied to gain independence and a log-normal distribution.

Tukey studentized test was used to compare the different food groups (A, B, C) among each other and the pollen intake per week.

The analyses were done using Genstat version 13.2 from VSN international Ltd.

4. Results

4.1 Pollen intake

The results of the pollen intake showed that the measurements were non-normally distributed, the residuals showed heteroscedasticity (figure 3.6.1).

The pollen intake did not differ significantly among the three feeding groups which was measured among seven weeks (P=0.142) (figure 4.1.2). A significant difference (P<0.001) was seen between the averages of the pollen intake during the first three weeks and the and last 4 weeks. Linear regression was used to show possible relations with weather influences but no relations where found comparing weather influences (temperature, sun, wind, precipitation) with the pollen intake.

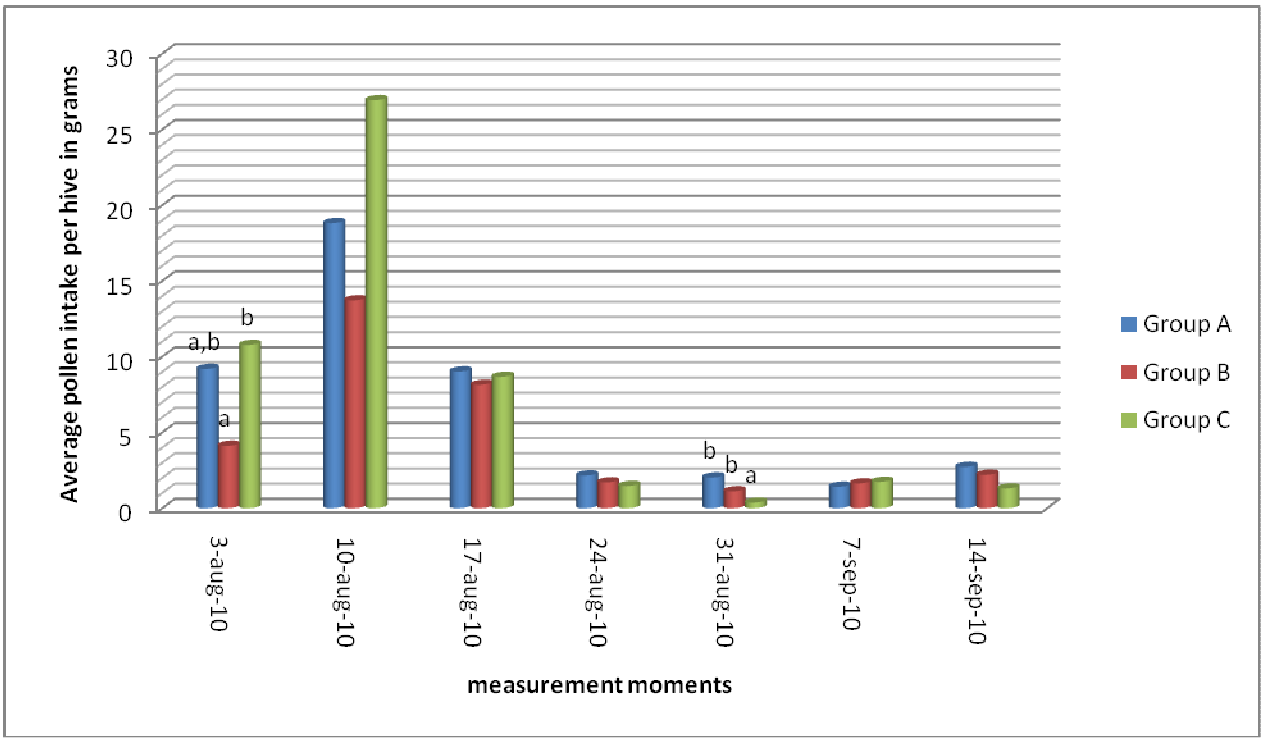

Figure 4.1.2 showing the pollen intake in grams (y-axis) per measurement weeks 1-7 (x-axis), where weeks 1 (3-aug-10) and 5 (31-aug-10) showed significant differences. For s.e. values see table 4.1.1.

Differences among the food treatment groups were seen on week 1 and week 5. Group C was foraged two times during the test, once one week before week one and in week five. The measurements close to those dates show significant differences in pollen intake. Although not fully corresponding for the first week feeding could be the factor causing this difference.

Table 4.1.1. Mean and s.e. from every group and the seven measurement groups.

	3-aug-10		10-aug-10		17-aug-10		24-aug-10		31-aug-10		7-sep-10		14-sep-10	
Group	Mean	s.e.*	Mean	s.e.*	Mean	s.e.*	Mean	s.e.*	Mean	s.e.*	Mean	s.e.*	Mean	s.e.*
A	9.191 [a,b]	3.238	18.815	6.630	9.023	3.179	2.154	0.759	2.026 [b]	0.714	1.415	0.498	2.720	0.959
B	4.116 [a]	1.530	13.717	4.607	8.142	2.735	1.704	0.572	1.116 [b]	0.375	1.635	0.549	2.199	0.739
C	10.774 [b]	3.459	26.932	8.648	8.671	2.784	1.469	0.516	0.366 [a]	0.118	1.741	0.583	1.299	0.417

* standard errors are only approximates since the model is not linear

4.2 Feeding and colony development

The results show that there is a significant difference (P=0.007) (figure 4.2.1) in brood size among the feeding groups at the end of the research period (20-sep-2010).

Colonies provided with sugar syrup at once did have less brood compared to sugar syrup provided dispersed over time. Feeding with sugar paste showed no difference with both sugar syrup provided once and sugar syrup provided dispersed (table 4.2.1).

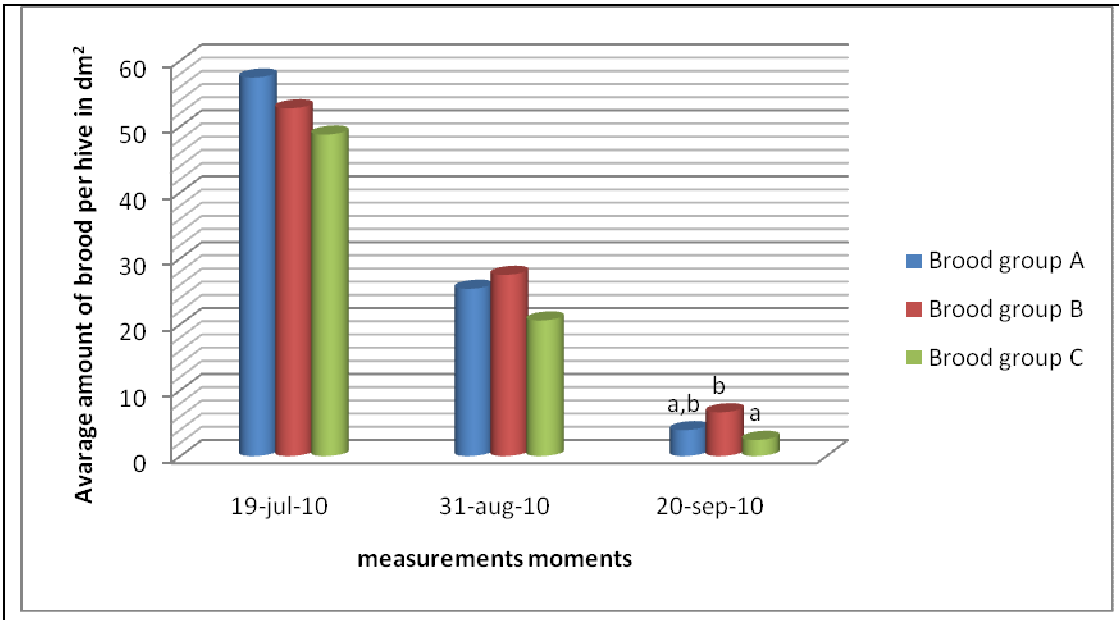

Figure 4.2.1 showing the mean amount of closed brood in the groups where blue is group A, red is group B and green is group C. The x-as shows the three measurements moments, where the y-as shows the amount of closed brood in dm^2. Mean and s.e. values in parenthesis, 19-jun-10 A: 27.78 (5.718) B: 25.45 (5.085) C: 26.23 (4.761), 31-aug-10 A 25.39 (14.76), B 27.5 (8.576), C 20.59 (5.305), 20-sep-10 A 3.944 (2.920), B 6.65 (2.148), C 2.455 (3.174). The amount of colonies was A: n= 9, B: n= 10, C: n=11.

The amount of bees (figure 4.2.2) did not differ significantly (P=0.399) among the last sample (20-sep-2010). Also in the earlier bee amount samples no significant difference was found (annex 3 table A12.5, A12.6 and A12.7).

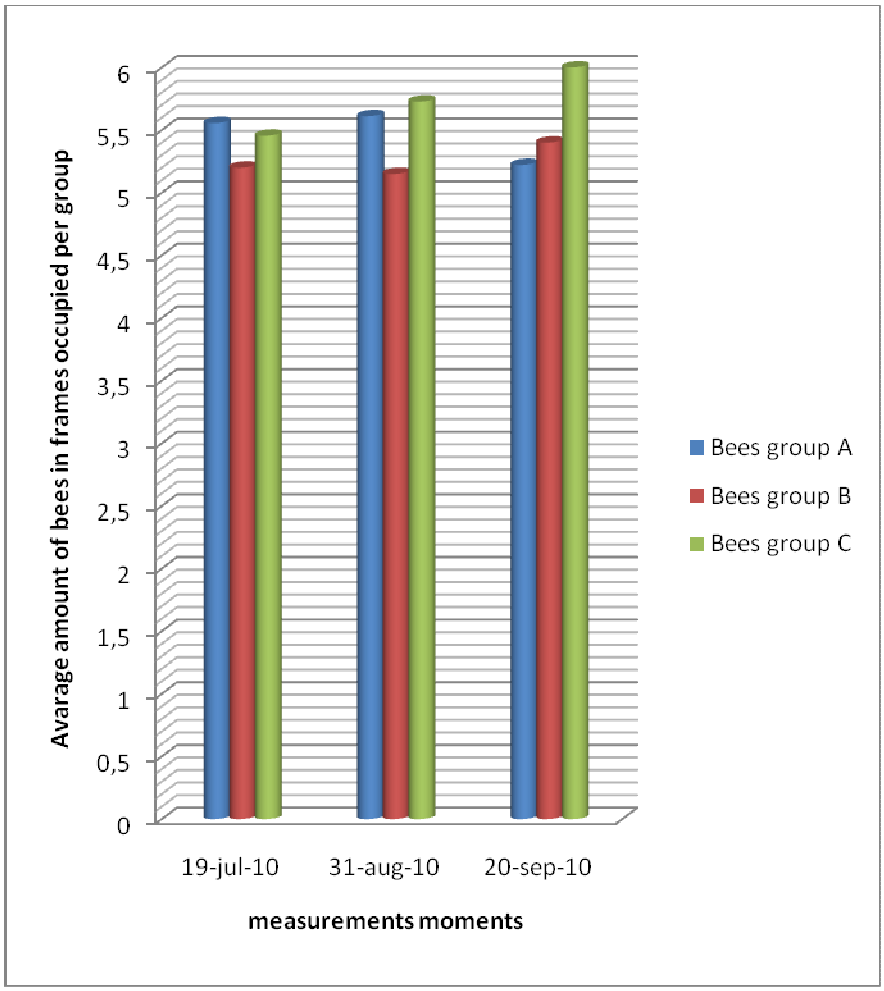

Figure 4.2.2 showing the mean amount of bees in the groups where blue is group A, red is group B and green is group C. The x-axis shows the three measurements moments where the y-as shows the amount of bees in frames occupied a half frame is approximately 875 bees. Mean and s.e. values in parenthesis, 19-jul-10 A 5.556 (1.236), B 5.2 (1.687), C 5.455 (1.293), 31-aug-10 A 5.611 (1.833), B 5.15 (1.156), C 5.727 (1.330), 20-sep-10 A 5.222 (1.770), B 5.4 (1.308), C 6 (0.894).

4.3 Vitellogenin titer among the 3 groups

Analysis of the vitellogenin concentration in the hemolymph shows that there is a significant difference (P<0.001; annex 13 table A13.1) in vitellogenin titer among the different feeding groups (table 4.3.1).

Group C where sugar syrup is provided at once has a higher vitellogenin titer compared to the two other groups (figure 4.3.1). Vitellogenin was only measured at the end of the experiment where hemolymph was taken from 15 to 17 September.

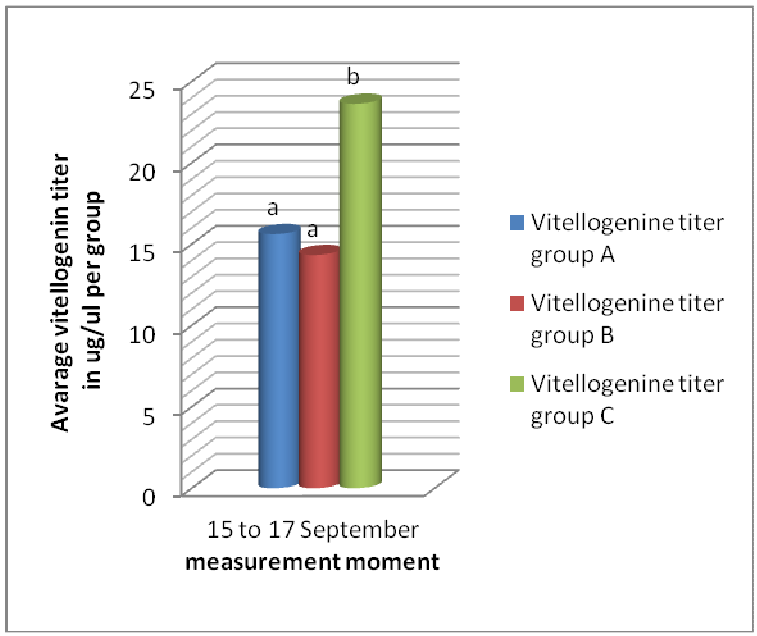

Figure 4.3.1 Showing the group mean of vitellogenin titer in (ug/ul (y-as). Where blue is group A, red group B and green group C (y-as). Mean and s.e. values in parenthesis and number A 15.617a (1.874) N=18, B 14.335a (1.695) N=22, C 23.627b (1.559) N=26.

5. Discussion

5.1 Pollen intake and food

The measurements of this research show no significant results toward the pollen intake in treatments with different supplemental sugar sources. Multiple causes might take their part, feed was accessible in different methods. Where sugar paste and sugar in once were applied on top of the pollen trap (Figure A15.1 and 5.1.1.). The sugar syrup dispersed provided was directly placed on the colony. The difference where caused by the cavity between colony and food (sugar paste and sugar in once) and had especially a negative influence on sugar paste (figure 5.1.1.). Where the sugar paste stayed more solid due to the colder temperature and less humid air. The way in which food was provided could be changed in the further (see changed improved pollen trap annex 15). For example, the problem with supplying sugar paste was that it was provided far further from the colony itself (figure 5.1.1.). It could be provided on the same way as the glass jars but this would change the access of bees to the colony. The sugar paste package would seal at least half of the colony access, which could be beneficial or harmful. For sugar paste the easiest way of absorbing is when it is slightly heated by the colony as well as becoming humid trough the colony. By placing the sugar paste above the pollen trap the heat and humidity were often not optimal, the sugar paste even tended to dry out. In some cases also wasps (*Vespula vulgaris*) tended to rob the sugar source due to the relatively unprotected position of the food, especially with sugar paste and sugar provided at once. This was often the case in small colonies or with cold weather. The normal position of the food is directly above the storey with the brood. The way of presenting the food might have an influence on absorption speed and cause different fluctuations among the groups. This was also seen by the fact that the bees did take almost five weeks instead of the predicted two weeks to empty a package of 2,5 kg sugar paste. In the presented new pollen trap model this feeding problem is mainly solved.

Figure 5.1.1. The hive as used in the research with every part explained.

The pollen intake was measured on seven moments and appeared to be lognormally distributed.

In this test the amount of sugar paste used by the colonies of group A was the measure for providing sugar syrup to the other two groups of hives. The reason for choosing sugar dye is that sugar dye is normally *ad libitum* provided when used as a feed. Another way which might be more fair could be presenting a standard amount of sugar for every hive. This makes a comparison more reliable and guarantees that every hive receives exactly the same amount of sugar. Of course a provision method as closely as possible to *ad libitum* provision of sugar dye should be the target. Providing a fixed amount is also easier because in the last two weeks the speed of sugar paste use increased. Six of the 12 hives finished the sugar paste in less than two weeks, compared to five weeks of the other hives. Technically providing a fixed amount might not change the results but can make the test more easy.

5.2 Weather

There is visually and statistically (P<0.001)(annex 11, table A11.2) a difference in average pollen intake between the first three weeks and the last four weeks (figure 4.1.1). This difference could not be explained by the weather. Within the first three weeks the temperature was higher (16.6℃ vs 13.9℃). Wind speed was lower (maximal hour aver age speed 6.25m/s vs 8.0 m/s), as well as precipitation (3.2mm vs 5.3mm) and sun hours (3.76 and 4.87 average hours sun per day). Only sun was opposite from the bees preferable weather conditions. As shown the weather was different but it did not differ significantly (p=0.05), and a clear difference between period 1 (first three weeks) and period 2 (last four weeks) was not seen.

Even so, the weather may have had its influence, the summer was with 17,7˚C more than one degree warmer than the long term average (16,6˚C). This might be positive towards foraging for pollen, but also resulted in a shortage of water available for plants. Within the month August the weather was very instable with heavy rains. In August the long term normal precipitation is 62mm, during the test it was 170 mm(KNMI , 2010). This rain may have caused a very fluctuating pollen yield. During this month it could be bad weather on Monday and Tuesday, when the pollen trap was closed for 24 hours, resulting in no or little foraging activity. Such days may not have been representative and may have influenced the observed pollen intake by the colonies. This could possibly have been avoided by trapping pollen more often or during prolonged periods.

5.3 Brood and food

The results show that colonies provided with a continuous supply of sugar syrup raised more brood than the colonies which were provided with sugar syrup at once (P=0.007)(figure 4.2.1, annex 12). The reason may be that there is always a certain amount of sugar available for nursing. Pesante et al. (1992) showed already that indeed a higher weight could be obtained by supplemental feeding with sugar syrup. But in our research the number of bees did not differ between the groups (figure 4.2.2).

5.4 Vitellogenin

Vitellogenin titers in September show a significant difference (P<0.001) (figure 4.3.1, table 4.3.1, annex 13 table A13.1) between group C where sugar syrup is provided and the other two groups. Among group C the vitellogenin titer is higher (annex 13 table A13.1). There might be several reasons for this difference.

The higher level of vitellogenin in Group C might be explained by the possibility that the colonies had a better start with a large(r) amount of sugar in stock, which might cause a more stable start for the bees. The results of the first two weeks show a higher intake of pollen in group C after which the order changed in the next weeks (figure 4.1.1 annex 14 figure A14.1). This difference in pollen intake (higher intake) was only significantly different for the first week (annex 11 table A11.3) and between group C (higher) and B (lower). Group A showed no significantly different pollen intake than groups B and C. A negative significant difference was found in week 5 (annex 11 table A11.4) where a lower pollen intake was seen among group C compared to group A and B. This boost of sugar feeding might be a way to enhance pollen intake if on the long term.

It would be interesting to know if vitellogenin levels are different among the hives with different corresponding pollen intake, there might even be a genetically variety origin to.

Another reason why the vitellogenin titer in group C was found higher could be that more sugar is stored when provided at once. Unfortunately the amount of stored sugar was not measured. In this case the way of providing sugar might have an effect on the bees vitellogenin titer. The possible bigger amount of sugar available might result in a higher vitellogenin titer because fewer bees become foragers. Amdam and Omholt (2003) stat that a shortage of carbohydrates causes a fast reduction in vitellogenin titer which is causing the hive bee to become a forager. This process of transition starts by the internal repressor where under the control of the allatoregulatory central nervous system juvenile hormone (JH) is secreted. The JH represses the vitellogenin production and causes the bee to become a forager by a positive feedback loop to the allatoregulatory central nervous system pathway. Also less brood was found compared to the continuously with sugar syrup provided group B. This strengthens the idea that winter bees where formed. Winter bees are characterized by their high vitellogenin titers. Although it could be possible that a relation was seen between the amount of brood in September (20-sep-2010) and vitellogenin titer, this was not the case.

The bees of group C sugar at once had a significant higher vitellogenin level, this higher vitellogenin level might give them a better overwintering ability, although this higher level can be caused by a earlier start of producing winter bees with their typically high vitellogenin levels (Mattila et al., 2001). In this research the highest value found was 40.94µg/µl and the lowest value 5.82µg/µl. In order to get a more precise view on the actual development of the vitellogenin a regular sample moment of every two weeks towards winter could give more insight. This might elaborate the knowledge for countries where bees hibernate. An option could be to include also other proteins like Lipophorin, Pulvative storage protein, Thorax protein and the Putative jelly protein (Otis et al., 2004).

In short, bees in colonies provided with sugar paste and sugar syrup result in different vitality (vitellogenin titer), brood rearing and might have a different overwintering ability due to the vitellogenin level.

In conclusion colonies provided with sugar paste and sugar syrup result in different vitellogenin titters. It is shown that winter bees are formed when pollen amount is reduced including other unknown parameters. One of those parameters might be the availability of nectar and other parameters (Mattila and Otis 2007) in this test artificial food. Where the colonies with sugar syrup dispersed (group B) and sugar paste (group A) showed lower vitellogenin and the sugar syrup at once showed higher vitellogenin titters. This might show the earlier start of winter bee production as we can assume that pollen intake did not differ (P=0.142). Earlier production of winter bees might cause a lower vitellogenin level in winter and finally have influence on immune system and a lower buffer towards parasitism of mites.

5.5 Pollen trap experiences

Pollen traps were used for one day a week, from the afternoon until the next day afternoon. During the research this method showed some disadvantages. For example the bees had difficulties to get used to the roster within the trap (figure 3.3.2). This causes bees to fly out again and try another hive causing interactions of bees between hives, although the pollen traps where installed at all hives at the same time which should prevent major interactions.

Another problem arising with the pollen trap is the fluctuation in size of pollen, which might cause different amounts of pollen for the colony. For example one colony can go for a certain plant which results in smaller pollen nuggets and a other colony can go for a plant causing big nuggets. In the hive with big nuggets (figure 5.6.1) is a higher amount measured, but by better investigation they might have gathered the same amount (weight). Calibration could be a solution to this, but the weather and season didn't give enough pollen intake to do this.

The currently used pollen trap itself has also several negative characteristics: the entrance of the trap is to high (big) causing robbing (figure 5.5.1). The way from the entrance to the brood nest has obstacles which might hinder the colony with defense against intruders. Also there is a cavity which might result in less optimal temperature regulation. In annex 15 other improvement points are discussed and a improved model pollen trap is presented.

Figure 5.5.1 showing a bee that's removed out of the hive due to robbing.

5.6 General discussion

During the process of the research new views developed and mistakes were made and corrected. One of the problems was robbing among the colonies. Robbing occurred in the end on the season when the available pollen and nectar is lower (figure 5.5.1). On that moment bees get the tendency to rob honey and pollen from smaller or less protected colonies. Due to the big entrance of the pollen trap this behaviour might be even enhanced (see improvements for pollen trap annex 15). This problem was solved by making a small entrance in a duck tape strip with on one side plastic preventing bees to stick to the tape. This measure reduced robbing and therefore showed that the current pollen trap entrances are too big to prevent robbing.

Within this research queens were obtained by emergency cells (queen cells), at the end of the test several queens were not present or did not perform well. With an approach in which there is less pressure on producing queen cells might give better results (oral information Inbuzz / Calis). Producing queens in big and strong colonies may already help. In our research only hives with ten frames were used for queen raising, different than planned due to a misunderstanding.

In the proposal it was planned that checking and estimation of the size of the colonies was done every 2 weeks. Within the actual research it is only done three times; namely at the start (19-jul-2010), in the middle (31-aug-2010) and at the end (20-sep-2010) of the research period. On those occasions also all colony parameters were measured. Less checks were needed during the research due to the fact that during feeding already a good impression of the food situation was gained. The advantage was a minimal disturbance.

Figure 5.6.1 showing pollen harvested with the pollen trap.

The whole research could give more reliable answers if done from the start of the bee season, for example from May onwards. If repeated it would be possible to improve the results by placing mixed groups on different locations, minimizing the chance that differences are attributed to food where location might play a role. This can be especially important for the amount of brood and bees.

In this research the produced honey yields by the colonies are excluded from observation. Furthermore, it was assumed that the pollen availability is similar for the colonies and that the location is rewarding enough with forage for the whole group. In this research it is also assumed that sugar sources have the same composition or that slight differences will have no significant influence. Number of bees and brood size were estimated by observation in the field by man, this may have influenced the results slightly.

Furthermore we assume that all observations which are tested are independent, and means are normal distributed (as mentioned earlier in 3.6) and there is a equal variation among observations (homoscedasticity). If not normal distributed we assume that is was normal distributed after transformation.

6. Conclusions

The results of this research provide no evidence that feeding colonies with different sugar sources has an influence on the pollen intake. But it is shown that a difference in brood rearing can be caused by the way sugar syrup is provided, although the number of bees was the same among the groups. We can conclude that the manner in which food is provided has an effect on the amount of brood at the end of the season. In September the vitellogenin concentration of the hemolymph was higher when colonies had been supplied with a big lot of sugar feed at once, as compared with continuous feeding with sugar solution and sugar paste.

7. Future research

During this research different new questions arose, for instance: is there a direct link between pollen intake and vitellogenin titer in hemolymph? This relation was already shown by Cremonez et al., (1998) where the quantity and usability were correlated with vitellogenin levels. This idea shows the interaction, since winter bees will be made when pollen supply is low, causing a smaller brood nest with production of winter bees and higher protein levels are developing (Mattila and Otis 2007). But more parameters should be out there which might be nectar/carbohydrate availability as maybe shown by this research, but this needs further research. It might be good to research the start hibernation on different moments early (end August), middle (end September) and finally the end of October. The moment of hibernation could be controlled by nectar and pollen availability. To control if the colony is really ready for hibernating the vitellogenin level can be checked. The result is that hibernation is directed in different hibernation periods. Where a possible long and short hibernation periods might give different survival results in spring.

Furthermore it might be interesting to know what the influence is of different food types before winter and supplied for several months (>3, long term). on the parameters as used in this research (vitellogenin, colony development - bees and brood, and pollen intake). As well the results on the spring survival.

Vitellogenin gives bees the possibility to survive for several months on carbohydrates only (Brodschneider and Crailsheim, 2010). Therefore the vitellogenin concentration might act as a parameter to predict the survival chances of bee colonies in relation to the months towards spring. However, if a too low vitellogenin concentration is found in autumn there seems no remedy available at that time to recover, since pollen supplements did not have an effect on the total protein content of the bee. Not on the winter bees in autumn nor in the new nursed bees in spring (Mattila et. al., 2007). It might be still interesting to use vitellogenin as a parameter to predict colony survival rate, in order to prevent colony losses by applying different management strategies. This could be for example moving of the hives to locations were also in winter pollen are available as well new innovative ways of improving vitellogenin and protein content of the bees like hormone inhibition treatment.

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9. References

Amdam GV, Norberg K, Page RE, Erber J, Scheiner R (2006) Downregulation of vitellogenin gene activity increases the gustatory responsiveness of honey bee workers (Apis mellifera). Behavioural Brain Research 169: 201-205.

Amdam GV, Omholt SW (2003) The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. Journal of Theoretical Biology 223: 451-464.

Avni D, Dag A, Shafir S (2009) The effect of surface area of pollen patties fed to honey bee (Apis mellifera) colonies on their consumption, brood production and honey yields. Journal of Apicultural Research 48: 23-28.

Brodschneider R, Crailsheim K (2010) Nutrition and health in honey bees. Apidologie 41: 278-294.

Cornelissen B, Schmid S, Henning J, Steen J, Blacquière. 2009. Estimating colony size using digital photography. Unpublished.

Crane E (1979). Honey a comprehensive survey. Heinemann: 83, 103, 105, 186.

Cremonez TM, De Jong D, Bitondi MMG (1998) Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees (Hymenoptera : Apidae). Journal of Economic Entomology 91: 1284-1289.

DeGrandi-Hoffman G, Wardell G, Ahumada-Segura F, Rinderer T, Danka R, et al. (2008) Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. Journal of Apicultural Research 47: 265-270.

Fewell JH, Winston ML (1992) Colony state and regulation of pollen foraging in the honey-bee, apis-mellifera L. Behavioral Ecology and Sociobiology 30: 387-393.

Free JB, Spencerbooth Y (1961) Effect of feeding sugar syrup to honey-bee colonies. Journal of Agricultural Science 57: 147-151.

Garedew A, Schmolz E, Lamprecht I (2004) The energy and nutritional demand of the parasitic life of the mite Varroa destructor. Apidologie 35: 419-430.

Keller I, Fluri P, Imdorf A (2005) Pollen nutrition and colony development in honey bees: part I. Bee World 86: 3-10.

Mattila HR, Harris JL, Otis GW (2001) Timing of production of winter bees in honey bee (Apis mellifera) colonies. Insectes Sociaux 48: 88-93.

Mattila HR, Otis GW (2007) Dwindling pollen resources trigger the trasition to broodless populations of long-lived honeybees each autumn. Ecological Entomology 32: 196-505.

Mattila HR, Otis GW (2007) Manipulating pollen supply in honey bee colonies during the fall does not affect the performance of winter bees. The Canadian Entomologist 139: 554-563.

Neumann P, Carreck NL (2010) Honey bee colony losses. Journal of Apicultural Research 49: 1-6.

Pesante DG, Rinderer TE, Collins AM, Boykin DL, Buco SM (1992) Honey production in venezuela - effects of feeding sugar syrup on colony weight gains by africanized and european colonies. Apidologie 23: 545-552.

Websites

KNMI Koninklijk Nederlands Meteriologisch Instituut, Pers bericht zomer was warm zonnig en nat
http://www.knmi.nl/cms/content/85694/zomer_was_warm_zonnig_en_nat
Visited on 16-10-2010

10. Annex: Explanatory word list

Nukes	Core colonies / Kern volkjes
Group A	Group which is provided with sugar paste
Group B	Group which is provided with syrup in jars and 2 times a week
Group C	Group which is provided wit syrup in amounts corresponding to the uptake of sugar paste of Group A
Sugar paste	Mass of white dough, consisting of powder sugar whit 10% water (Fondabee). In this research sugar paste is applied *ad libitum* to group A.
Sugar syrup	Provided to groups B and C diluted of a 76% sugar concentration to 50% provided in once or dispersed.
Super	Name for a honey chamber in a bee hive, in Dutch hives there is place for 10 small hives.
Allatoregulatory	Part of the bees central nervous system which regulates secretion of juvenile hormone which represses the vitellogenin production.
Vitellogenin	Glycolipoprotein, with properties of sugar, fat and protein, it acts a a food storage protein and is important for successful hibernating.
Hibernating	The rest period bees taken in countries with cold winters

11. Annex: Statistical results pollen intake

Table A11.1 REML variance components analysis pollen intake logarithmic distributed

Fixed term	Wald statistic	d.f.	F statistic	d.d.f.	p
Gr (Groups)	3.95	2	1.97	180.1	0.142
Time	255.81	6	42.64	173.2	<0.001
Gr.Time	25.04	12	2.09	173.2	0.020

Table A11.2 REML variance components analysis pollen intake logarithmic distributed with division in time

Fixed term	Wald statistic	d.f.	F statistic	d.f.	p
Gr (Groups)	3.95	2	1.97	180.1	0.142
Time2 (first three weeks and last four weeks)	227.05	1	227.05	173.3	<0.001
Time2.Time (Time = 7 weken)	28.91	5	5.78	173.2	<0.001
Gr.Time2	11.09	2	5.55	173.2	0.005
Gr.Time2.Time	13.80	10	1.38	173.2	0.193

Significant differences pair wise comparison

Table A11.3 Pairwise comparisons of means; p= 0.05 (STUDENT) week 1 of pollen intake in grams

Groups	Mean
A	9.191[ab]
B	4.116[a]
C	10.774[b]

Table A11.4 Pairwise comparisons of means; p= 0.05 (STUDENT) week 5 of pollen intake in grams

Groups	Mean
A	2.026[b]
B	1.116[b]
C	0.366[a]

Non significant differences pair wise comparison

Table A11.5 Pairwise comparisons of means; p= 0.05 (STUDENT) week 2 of pollen intake in grams

Groups	Mean
A	18.81
B	13.72
C	26.93

Table A11.6 Pairwise comparisons of means; p= 0.05 (STUDENT) week 3 of pollen intake in grams

Groups	Mean
A	9.023
B	8.142
C	8.671

Table A11.7 Pairwise comparisons of means; p= 0.05 (STUDENT) week 4 of pollen intake in grams

Groups	Mean
A	2.154
B	1.704
C	1.469

Table A11.8 Pairwise comparisons of means; p= 0.05 (STUDENT) week 6 of pollen intake in grams

Groups	Mean
A	1.415
B	1.635
C	1.741

Table A11.9 Pairwise comparisons of means; p= 0.05 (STUDENT) week 7 of pollen intake in grams

Groups	Mean
A	2.720
B	2.199
C	1.299

12. Annex: Statistical results: number of closed brood cells and number of bees; colony development

Table A12.1 ANOVA results of the closed brood cells at start of the research 19-jul-2010

Source of variation	d.f.	s.s.	m.s.	F	p
Gr (Groups)	2	26.40	13.20	0.49	0.615
Residual	27	720.96	26.70		
Total	29	747.37			

Table A12.2 ANOVA results of the closed brood cells sample in the middle of the research 30-aug-2010

Source of variation	d.f.	s.s.	m.s.	F	p
Gr (Groups)	2	264.37	132.18	1.33	0.282
Residual	27	2685.80	99.47		
Total	29	2950.17			

Table A12.3 ANOVA results of the closed brood cells at the end of the research 20-sep-2010

Source of variation	d.f.	s.s.	m.s.	F	p
Gr (Groups)	2	93.826	46.913	6.02	0.007
Residual	27	210.474	7.795		
Total	29	304.300			

Table A12.4 Pair wise comparison of closed brood sample results with mean, number and s.e. values for 20-sep-2010

Gr (Groups)	A (sugar paste)	B (sugar syrup spread provided)	C (sugar syrup provided at once)
mean	3.944^{ab}	6.650^{b}	2.455^{a}
rep. (N)	9	10	11
s.e.	0.931	0.883	0.842

Table A12.5 ANOVA results of the amount of bees in frames at start of the research 19-jul-2010

Source of variation	d.f.	s.s.	m.s.	F	F pr.
Gr (Groups)	2	0.651	0.325	0.16	0.852
Residual	27	54.549	2.020		
Total	29				

Table A12.6 ANOVA results of the amount of bees in frames at the middle of the research 30-aug-2010

Source of variation	d.f.	s.s.	m.s.	F	F pr.
Gr (Groups)	2	1.904	0.952	0.45	0.640
Residual	27	56.596	2.096		
Total	29				

Table A12.7 ANOVA results of the amount of bees in frames at the end of the research 20-sep-2010

Source of variation	d.f.	s.s.	m.s.	F	F pr.
Gr (Groups)	2	3.411	1.706	0.95	0.399
Residual	27	48.456	1.795		
Total	29	51.867			

13. Annex: Statistical results vitellogenin titer from 15 to 17 September 2010

Table A13.1 ANOVA results of the vitellogenin titer in hemolymp of bees sampled from 15 sep to 17 sep 2010

Source of variation	d.f.	s.s.	m.s.	F	p
Gr (Groups)	2	1213.20	606.60	9.59	<.001
Residual	77	3983.53	63.23		
Total	79	5196.73			

Table A13.2 Vitelloganine results with mean, number and s.e. values in (ug/ul) end of research 15 sep to 17 sep 2010

Gr (Groups)	A (sugar paste)	B (sugar syrup spread provided)	C (sugar syrup provided at once)
mean	15.617[a]	14.335[a]	23.627[b]
rep. (N)	18	22	26
s.e.	1.874	1.695	1.559

14. Annex: Important dates

Table A14.1 dates of the pollen measurements

Sample	Date
Week 1	3-aug-10
Week 2	10-aug-10
Week 3	17-aug-10
Week 4	24-aug-10
Week 5	31-aug-10
Week 6	7-sep-10
Week 7	14-sep-10

Table A14.2 dates of the colony size and brood size measurements

Date
19-jul-10
31-aug-10
20-sep-10

Table A14.3 dates that sugar in once is applied.

Date
20 –jul-10
31-aug-10

15. Annex: Statistical results vitellogenin titer from 15 to 17 September 2010

The sugar paste and sugar in once were applied on top of the pollen trap (A15.1). The cavity between colony and food had especially a negative influence by using sugar paste. The sugar stayed and become more solid due to the colder temperature and less humid air.

The cavity inside the pollen trap has also other negative points as it gives the bees the opportunity to build comb in the trap although not seen during this research (figure A15.2, A15.3). When the colony has build in the cavity there is a change to damage the queen as well useless disturbance of the hive, because often also brood is reared there. A point which could be improved is locating the entrance a little bit higher on the front side of the trap, making the way to the hive shorter, and creating more space for the cavity where food can be provided.

Improved pollen trap

Recommended improvements for the pollen trap.
The mentioned problems can be solved by some modifications. The first change is making the entrance smaller and make the space to the actual colony smaller (figure A15.1 B and E). By making the distance to the actual hive smaller there is also a cavity where feed can be placed (figure A15.1 A). This allows the beekeeper to easily feed sugar paste or multiple jars with sugar syrup and prevents comb building in the pollen trap (figure A15.1). The last improvement is made to locate the entrance higher causing a shorter walk for the bees and more space for feeding. This is better because the situation is already different from normal practice, and every energy saving is welcome.

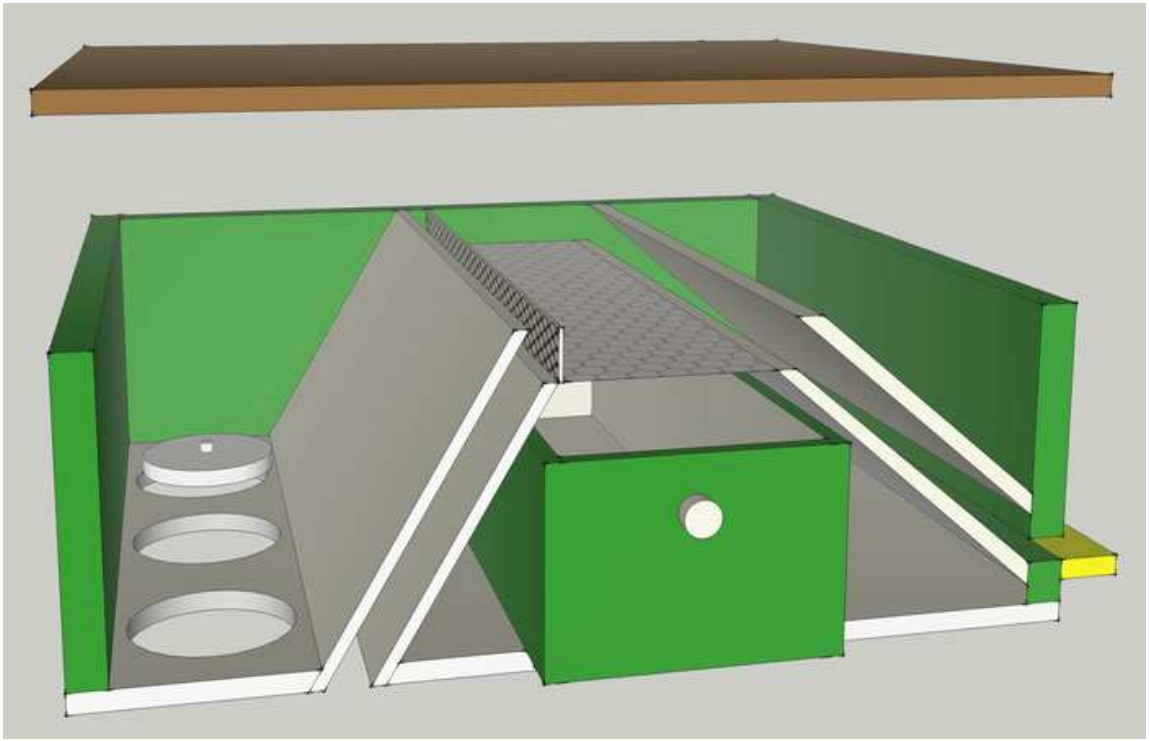

Figure A15.1 The improved pollen trap where a better solution is presented for feeding, pollen intake, temperature and comb building.. A feeding hole with cap, B walkway to hive, C grid where bees and pollen

become separated, D pollen fall trough bees can't enter, E walkway to grid, F pollen trap entrance, G tray were pollen is collected, H inner cover.

Figure A15.2 shows the top plate of the pollen trap, where the colony has build comb on the place the cavity is located.

Figure A15.3 pollentrap where the cavity is build out with comb (A).

16. Annex: Original planning time track

	1 26-apr	2 3-mei	3 10-mei	4 17-mei	5 24-mei	6 31-mei	7 7-jun	8 14-jun	9 21-jun	10 28-jun	11 5-jul	12 12-jul	13 19-jul	14 26-jul	15 2-aug	16 9-aug	17 16-aug	18 23-aug	19 30-aug	20 6-sep	21 13-sep	22 20-sep	23 27-sep	24 4-okt	25 11- okt	26 18- okt	27 25- okt
Making research proposal																											
ENT-51306 Frontiers in Medical and Veterinary Biology																											
Making colonys																											
Place of colonys on location																											
Pollen trap is added																											
Start new feeding week																											
Weekly control colonies																1					2						
Making photos for development control																											
Development analysis (photos)																											
Pollen flow ones a week and analysis																											
Vitellogenin sample																											
Supplemental winter feeding if needed on the end of research																											
Analyse vitellogenin																											
Analyze of data Start with report																											
Finish analyses Finish report																											
Presenting report																											
ENT-30806 Fundamental and Applied Aspects of the Biology of Insects																											

Minor thesis	24 ects	1 = end observation period one
Entomology course full time	6 ects	2 = end observation period two
Entomology course spread	6 ects	
Spare days ± 14 days		Total of 36 ects