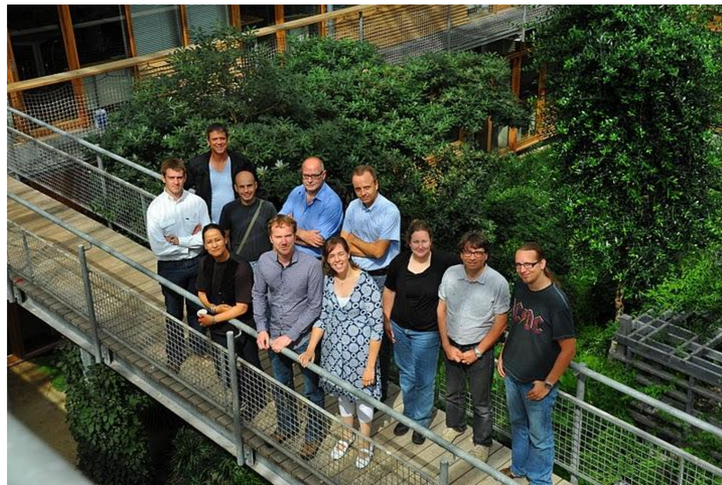
COLOSS Workshop

WG 3

Honey bee colony vitality

Wageningen 30 June – 1 July 2011



Meeting secretary: J. van der Steen (Wageningen UR, PRI bees)
Organization: J. van der Steen (Wageningen UR, PRI bees)

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Participants

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- Fabia Sgolastra (DISTA-Dipartimento di Scienze e Technologia Agroambientalli Bologna Italy)
- Pierre Giovenazzo (Université Laval Quebec Canada)
- Loic Flatrès-Grall (ITSAP-institue de l'abeille Avignon France)
- Coby van Dooremalen (WUR PRI bees Wageningen Netherlands)
- Bram Cornelissen (WUR PRI bees Wageningen Netherlands)
- Esther Stam (master student Wageningen University Netherlands)
- Jozef van der Steen (WUR PRI bees Wageningen Netherlands)
- Chiel Versluys (master student Wageningen University Netherlands)

Guests

- Chula Hok a Hin (lab technician PRI bees Wageningen Netherlands)
- Michiel Glorius (master student University Essex)

Location:
Wageningen University and Researchcentre (WUR)
Building “Lumen”, building nr. 100,
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Programme

Wednesday 29 June 2011

Arrival and welcome drink 20.00 h

Tuesday 30 June 2011

09.00 – 10.00 h. registration
10.00 – 10.15 h. opening workshop BY *Willem Jan de Kogel* Cluster manager Entomologie en virology PRI
10.15 – 11.00 h. plenary opening talk by *Frank van Langevelde*, Wageningen University and Researchcentre. Measuring vitality in host-parasite interaction. A butterfly - ant relation
11.00 – 11.30 h. *Pierre Giovenazzo*. Evaluation of the reproductive characteristics of honey bee queens produced during the beekeeping season.
11.30 – 12.00 h. *Bram Cornelissen*. What’s cookin’ in winter? Varroa and possible interactions with other pathogens.
12.00 – 13.30 h. lunch at Restaurant van de Toekomst on the campus
13.30 – 14.00 h. *Antonio Nanetti*. Considerations about factors affecting the colony vitality
14.00 – 14.30 h. *Fabio Sgolastra, Piotr Medrzycki*. The in-hive temperature measurements as a possible tool for colony condition estimation.
14.30 – 15.00 h. *Esther Stam*. Surviving honeybees in the field and at the laboratory
15.00 – 15.30 h. coffee- tea break
15.30 – 17.00 h excursion PRI bees, Study of impact of pollen income and imidacloprid on honey bee colony vitality and overwintering.
18.00 Social dinner

Friday 1 July 2011

09.00 – 09.30 h. *Chiel Versluys*. 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.
09.30 – 10.00 h. *Coby van Dooremalen*. Timing of varroa treatment and survival of honeybees.
10.00 – 10.30 h. *Jozef van der Steen*. Number of honeybees, number of sealed brood and mean colony hemolymph vitellogenin as parameters for colony vitality.
10.30 – 11.00 h. coffee- tea break
11.00 – 12.00 h. Discussions and evaluation about parameters to assess bee’s and colony vitality
12.00 – 13.30 h lunch at restaurant van de Toekomst on the campus
13.30 – 15.30 h. Continuation discussion and recommendations, collective making of the minutes and closure of the workshop.

Summary

The objective of the workshop was to discuss parameters to assess the honey bee colony's vitality. Vitality is a broad, multi interpretable conception. Therefore the participants agreed to restrict to parameters to objectively description of the status of the honey bee colony (see Definitions).
The discussions about the presentations, and about the final version of these minutes were respectful, objective, collegial and fruitful.
These minutes contain an introduction of the objective, definitions, physiological, morphological, behavioural and socio physiological parameters and the abstracts of the presentations.

Introduction.

The honey bee colony must be considered as a super organism. The super organism's vitality is the result of many interactions and feedback mechanisms between individual honeybees .
The vitality of individual bees is affected by
- environment: pollen, pesticides, climate changes etc.
- pests and pathogens: (Varroa, *Nosema apis*, *N. ceranae*, viruses, etc.)
- genetics

Because of social interactions between the bees, the measurement of various biological parameters of individual bees can only give an **indication** of the impact on colony level.
Therefore the status of this super organism must be established on the colony level. One should be cautious to interpret the result of individuals to the colony.

Environment and genetics are conditioning factors of the colony status. They can be assessed by other types of study beyond the scope of this workshop.

Definition

How to define the honeybee colony vitality?
1. Honey bee colony / super organism vitality or fitness?
2. Health status or well being?
3. status?

The participants of the workshop decided to use the descriptions of the physiological status, morphological status and socio-physiological status to describe a honeybee colony / super organism.

Physiological parameters: hemolymph vitellogenin (total and fraction of total hemolymph protein), quantification of virus and others, pathogens, brood pattern, drone quality.

Morphological parameters: morphological characteristics

Behavioural parameters: number of stored bee bread cells, drones in the colony, brood pattern, hygienic behaviour, behaviour abnormalities, flight activity.

Socio-physiological parameters: odour, sound, temperature weight, # bees, # sealed brood, humidity, food consumption.

How to assess?

1. Haemolymph vitellogenin and total protein: pooled samples of in-hive 25 bees (see sampling summary J. van der Steen);
2. Quantification of pathogens (see BeeBook);
3. Brood counts / brood pattern (counting proportion of open cells in 10 x 10 sealed brood cells parallelogram (standard);
4. Drone quality: mobility, quantity and ratio dead / alive sperm cells (le Conte, Loic), viability eosine-nigrosine stain (vital stain);
5. Morphological characteristics: symptoms of diseases and developmental malformations;
6. Bee bread: photographing and counting cells, assessment of stores categories;
7. Drones in the colony: # drones, drone period;
8. Hygienic behaviour (pin-test, liquid nitrogen test, BeeBook);
9. Behavioural abnormalities: aggressiveness (BeeBook), flight and walking abilities.
10. Odour, no methods available yet
11. Sound, idem
12. Temperature / RH: sufficient numbers of sensors (Piotr, Fabio)
13. Weight: continuous weighting scale, periodic weighing with a platform scale
14. # bees: photographs and counting bees (Liebefeld method and modifications)
15. # open and / or sealed brood cells: idem
16. Food consumption during winter: see weight

Proposals

We made proposals to
- inform each other about studies. Protocols and parameters,
- disseminate method for vitellogenin analysis to interested collegeagues,
- perform a ring test vitellogenin analysis.

Abstracts of the presentations

Evaluation of the reproductive characteristics of honey bee queens produced during the beekeeping season

Pierre Giovenazzo

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Young mated *Apis mellifera* honey bee queens were sampled from various Canadian suppliers from May 2008 to September 2008 at three week intervals. Each sample consisted of 20 young laying queens (2 weeks old) from each supplier. In May 2008, we also sampled queens imported from California and Hawaii (USA). Upon arrival, queens were euthanized, weighed and measured. The abdomen of each queen was dissected in order to remove the spermatheca and isolate the two ovaries. The total number of spermatozoa in each spermatheca was evaluated using a Cell-vu® hematocytomer. The two ovaries were paraffin-embedded and prepared for histological study. Microtome transverse sections (7 microns) of ovaries were stained with Masson Trichrome solution and mounted on standard microscope slides. All ovarioles in each ovary were counted using a photomicroscope (35X).

The queens from the different Canadian breeders and sampled from May to September (N=390) had the following measurements (average ± standard error) : body weight of 204,4 ± 24,1 mg; abdominal length of 10,4 ± 0,6 mm; abdominal width of 5,0 ± 0,3 mm; total number of ovarioles in both ovaries of 358,2 ± 67,5; total number of spermatozoa in spermatheca 8 734 908 ± 4 156 617. There are significant correlations ($P<0,05$) between the different morphometric variables checked (weight, abdominal length, abdominal width) and the different sampling dates, the total number of ovarioles and the total number of spermatozoa. Regression analysis indicates that queen reproductive qualities (ovariole and spermatozoa counts) show a significant positive linear trend with the sampling dates: lowest quality queens are produced at the beginning of the breeding season in May and the highest toward the end (August-September).

What's cooking in winter?

possible interactions of Varroa with other pathogens

Bram Cornelissen, Sabrina Schmid, Richard van Hoof, Coby van Dooremalen, Chula Hok A-Hin, Sjef van der Steen and Tjeerd Blacquière

One of the focal point for bee health is *Varroa destructor* and its control. Moreover its interaction with several viruses are known to relate to winter mortality. Besides these obvious interactions, others are possible as weakening of the immune response by *Varroa destructor* is likely to affect the susceptibility of honey bees to other pathogens. To test this hypotheses we followed colony development and survival and pathogen occurrence on a colony level.

From July 2009 until march 2010, 30 colonies were divided in two groups of which one (n=16) was treated against *Varroa destructor*, whilst the other group (n=14) wasn't, in order to manipulate varroa populations.
Colony development (bees and brood) was measured in July, September and November 2009 and March 2010. At similar intervals bee samples of approx. 100 workers were taken and stored at -80°C. 60 bees were individually checked for Varroa mites to establish infection rates. 30 varroa-free bees were then freeze-dried and pulverized using a bead-beater. RT-PCR single-plex was used for the detection of Deformed Wing Virus (DWV), Acute Bee Paralysis (ABPV), *Nosema apis* and *Nosema ceranae*.

During the course of the experiment, treatment effects were found on colony development and survival. Colonies treated and untreated were of equal size in early July 2009, consisting of 2283 bees (±760) and 2163 (±444) respectively. Treated colonies contained more bees than untreated colonies in November 2009 and in March 2010. No difference was observed in the amount of brood throughout the experiment. Nine colonies died between November to March, of which 8 were not treated. Survival rates for treated and untreated colonies were 94% and 43% respectively.
Infection rates of varroa in treated and untreated colonies differed in November and March. In November the infection rate in untreated colonies was 17.1%. In treated colonies 1.0% of the bees was infected. In March the infection rates were 12.3% and 2.2%.
ABPV was not detected, but DWV was. No difference could be established between the treated and untreated colonies for the collective data. Nonetheless in November in 70% of the untreated colonies DWV was detected compared to 25% of the treated colonies.
N. apis was found in three colonies, once in November and twice in March. *N. ceranae* was detected in all colonies in July 2009 and March 2010. In contrast *N. ceranae* was not found in all colonies in September and November. On these observation dates a higher infection rate was found for treated colonies (Sept: 67%, Nov: 75%) compared to untreated colonies (Sept:50%, Nov: 43%).
No correlation was found between pathogens, except for Varroa and DWV (R^2=0.50, $P<0.01$). No effect of pathogens, but varroa ($P<0.001$) was found on colony development. DWV (R^2= -0.55, $P<0.01$) and varroa (R^2= -0.74, $P<0.01$) also affected colony survival.

These result support the hypothesis that varroa and known secundary interactors (DWV) play a key role in colony development and survival. This can not be said about ABPV and *N. apis* which were not detected or detected sporadically. As a fairly novel pathogen in honey bees (*Apis mellifera*) *Nosema ceranae* is considered to be a possible candidate explaining winter mortality. We could not find any clue in that direction as it was found in all colonies, survivors and non-survivors. We saw a difference in the infection with *N. ceranae* between treated and untreated colonies, where more treated colonies were infected than untreated colonies. Based on these preliminary results we are unable to explain this. We hypothesize that ecological conditions in colonies treated for varroa are more optimal for *N. ceranae* than in colonies that are not treated and that *N. ceranae* does not affect winter survival in NW-Europe.

Considerations about factors affecting the colony vitality

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Abstract

Honey bees and apiculture have to deal with a large variety of challenges.

Most exigent seems to be the relationship between bees and various kinds of possible contaminating chemicals, coming from different sources. For instance, the interaction with agriculture may be at the same time beneficial due to the increased availability of food resources, but also a detrimental source of undesirable contaminants. Highly toxic pesticides like neonicotinoids represent a well-known case of that.

Unfortunately, honey bees get chemically contaminated also because of the treatments that the beekeeper must administer to control pests and diseases, namely varroa mites. Most of those contaminations are unavoidable and occur at regular basis in the time.

In most cases, the effects of the chemical contaminations are described in terms of individual mortality and/or of variations in the adult population and in the brood area of a colony. However, a quantitative approach does not account for the superorganismic nature of a bee colony, where the quality of the interactions between large numbers of individuals is very important.

In this context a clear definition of what "sublethal effects" mean in a honey bee colony is extremely necessary, as well as more adequate parameters that can be measured at colony level or in single individuals.

Another urgent question is posed by the impact of climatic changes. Colony development, symbiotic interactions with plants, bee-pathogen relationships, efficacy of treatments etc. are severely affected by the environmental changes that are taking place presently.

Therefore, definitions for vitality and well-being of a colony urge to a better understanding of the ongoing situation and to put into practice the needed countermeasures to protect the bees.

The in-hive temperature measurement as a possible tool for colony condition estimation

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The colony strength, measured as the quantity of adult bees, brood and stores, is one of the most important parameters in the study of the effects of pesticides, pathogens and other stressors on honeybee colonies. The method of "sixths" is widely used to estimate this parameter. It consists in the ideational division of each comb side in six parts and counting of the total number of sixths covered by adult bees, brood cells and stores. This method is characterized by some faults: low objectivity, stressfulness for the colony and impossibility to collect data in a continuous way. An indirect estimation of the colony strength could be given by the measure of the in-hive temperature. In the present study we equipped four beehives with three temperature data loggers each: one in the central position of the nest (in the brood area) and the other two in the lateral positions. Another data logger was placed at the centre of the super during the honey production period. The measurements were done at regular intervals (hourly) from May 2009 to September 2010. We found that in the central position the daily temperature oscillation was strongly affected by the brood extension. In fact, as well-known, the nest temperature is one of the most precisely controlled parameters in a honeybee colony. Adult workers keep the brood temperature within narrow limits between 32 °C and 36 °C with a mean of 34.5 °C and several studied showed that even small deviations (1-2 °C) from the optimal level affect many traits of emerged bees, including learning abilities, outdoor activities, task specialization, longevity, pesticide and parasite susceptibility (Tautz et al., 2003; Groh et al., 2004; McMullan and Brown, 2005; Jones et al., 2005; Becher et al., 2009; Medrzycki et al., 2010). These individual effects can affect the whole colony with a "snowballing positive feedback loops" (Oliver, 2010).

In the lateral comb position and in the super the temperature fluctuated from 25 to 35 °C and it depended on bee presence and external conditions. In the fall, with the interruption of brood rearing, we found a rapid temperature decreased inside the hive; similar results could be observed in case of swarming, queen death or induced interruption of brood rearing. Thus, the in-hive temperature can be useful not only in order to follow the development parameters of the colony but also to evidence particular events during the good season.

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Timing of anti-Varroa treatment and survival of winter bees

Coby van Dooremalen, Tjeerd Blacquiere, Jozef van der Steen, Bram Cornelissen, Lonne Geritsen, Frank van Langevelde.

Colony Collapse Disorder has multifactorial causes such as habitat degradation, invasive species, pesticides, and agricultural and beekeeping practices. In this study, we focused on the effect of *Varroa destructor* on the vitality of the bee colony. Timing of anti-varroa treatment can possibly affect the transition to winter bees. The use of the chemicals or the presence of mites during the development of the winter bee pupae possibly reduces the lifespan of the bees, causing the bees to die before spring has been reached.

Assigned 12 hives to 4 groups, differing in timing of anti-Varroa treatment: July, August, September, or not treated at all. Anti-Varroa treatment consisted of three weeks of formic acid evaporation in the hives. All groups were additionally treated in December using oxalic acid trickling. Mite fall was measured to check the Varroa infestation and the affectivity of the treatments. Lifespan was measured, marking 100 newborn bees (cohort) per hive every two weeks and counting the number of survivors every two weeks during July and April the next year. The cumulative survival from these curves was used as a measure for lifespan. Brood was also counted every two weeks. Winter survival was determined by counting the number of frames occupied with bees in April the next year.

Mite fall showed that indeed more mites died during and after anti-Varroa treatment in July, August, or September than after treatment. However, treatment in December caused the largest reduction in mite fall. Cumulative survival showed that the lifespan increased during the fall (August-November), showing the transition to winter bees, and showed that the bees of the hives treated in July had the longest lifespan of all groups. Bees of the hives treated in August and September had a longer lifespan than bees in hives that were not treated. The amount of brood indeed decreased with the increase in lifespan during the season. In April the next year, the hives that were not treated with formic acid during the experiment, showed much lower probabilities for the frames in the hives to be occupied (1 out of 10 frames) with bees compared to the hives that were treated during July, August, or September (6 to 7 frames out of 10 frames).

Although beekeepers often do not like to treat their hives against *V. destructor* during July, the period that they are most probably collecting honey, treating the bees before their transition to winter bees does increase their lifespan and thus increases their chances to survive winter. Treating hives against Varroa in August or September results in lower lifespan of the winter bees than treating them in July, but a longer lifespan than not treating them at all. Differences between the numbers of frames with bees in April the next year were in this study to small to be significant between anti-Varroa treatment in July, August or September. However, it would be interesting to follow these hives for multiple years. The differences in winter survival could potentially lead to larger differences in winter survival over multiple years.

Effects of pollen availability and Varroa destructor infestation on *Apis mellifera*

Esther Stam, esther.stam@wur.nl

Abstract:

For almost forty years the European honeybee *Apis mellifera* experiences strong parasitic pressure from the mite *Varroa destructor*, which until then only was present in Asian and African honeybee species. Much is known now about the lifestyle of both *A. mellifera* and varroa mites, in wild and increasingly in *in vitro* reared honeybees. Control methods have been proposed in order to lower the number of varroa mites. An additional cause of honeybee colony loss is the homogenization of pollen availability in the field. Moreover, being a very important pollinator, protection of *A. mellifera* will ultimately lead to the saving of agricultural crops, which are a very important food source for society. The aim of this research was therefore, to investigate the effect of both pollen availability and varroa infestation and their interaction on honeybee health, measured in protein amount and content of the body and body weight. For protein amount results indicated that there is no significant difference between varroa infested and non-infested honeybees (ANOVA, df=2, P=0.075), but is significant for the pollen availability (ANOVA, df=1, P=0.012). The protein amount in the abdomen is significantly more reduced than in the head (ANOVA, df=2, P=0.016) with respect to varroa infestation. There is a significant difference in body weight between varroa infested and non-infested honeybees (ANOVA, df=2, P=0.000). Results on the protein amount of honeybees with different varroa infestation and pollen availability make clear that pollen availability has a greater effect on the protein amount than does varroa infestation. However, all other results are comparable and in line with other research, so pollen availability and varroa infestation remain to be considered as important factors in protecting *A. mellifera*.

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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Supervision by dr. Tjeerd Blacquiere and prof. dr. Marcel Dicke

Minor thesis 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 were analysed. 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.

Figure 1 showing bee going through the actual pollen trap, pollen trap entrance and pollen from pollen collection

Worker bees, sealed brood, bee bread and vitellogenin as parameters for colony vitality.

Jozef. van der Steen
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The vitality of an individual honey bee depends on several factors e.g. the age related physiological condition, stress factors like diseases and parasites and quantity and quality of the protein feed. The honey bee colony is a super organism with trophallaxis and feed- back mechanisms to maintain the vitality of the colony. Therefore the vitality of the colony must be established on colony level. This raises the questions what matrix should be used and what is a representative sample of a colony. Bees from the flight entrance do not represent the colony as older forager bees are over represented. In case in-hive bees are sampled, knowledge of the age distribution is important. On all brood frames in summer, the age distribution is about 25% 1 week bees, 25% 2 week bees, 25% 3 week bees and 25% 4 + 5 week bees. On storage frames the older bees are overrepresented.
Parameters to describe the colony to assess the (differences in) vitality can be (not complete) hemolymph protein (vitellogenin, HSP, immune related proteins), number of worker bees. Cells sealed brood, cells bee bread, food gland development, fat body protein etc.
In our studies we used hemolymph protein / vitellogenin, number of worker bees, number of sealed brood cells as parameters in vitality studies. Vitellogenin is the main storage protein, essential to synthesize larval feed and regulation of the immune system. In individual hemolymph vitellogenin titres correlate strongly with levels of total hemolymph protein. The same goes for representative colony samples. A pooled sample of the hemolymph of 25 bees appears to be representative for the colony to assess the total hemolymph protein and vitellogenin. For other hemolymph parameters like carbon hydrates and immune related proteins this may be different.
The number of bees is an obvious parameter. The parameter "number of sealed brood cells" is chosen instead of "number of brood cells: eggs, larvae, pupae" because the number of eggs and larvae are affected by normal mortality and cannibalism.
Hemolymph protein, vitellogenin, number of bees and number of sealed brood cells are related to each other via feed-back systems. However external factors like Varroa, pollen diversity affect / disrupt the feed-back mechanisms, affecting the colony vitality. E.g. feeding bee bread results in more hemolymph protein a vitellogenin than feeding just sugar. Bees, being parasitized in the pupal phase by Varroa synthesize less vitellogenin than bees that have not been parasitized in the pupal phase.
In our studies it is demonstrated that the number of bees and the fraction vitellogenin is positive related in September; the more bees, the more vitellogenin. The demonstrated negative impact of Varroa on the synthesis of vitellogenin in individual bees can also be demonstrated on colony level, demonstrated it is an over-all negative effect of Varroa on colony vitality. Pollen diversity and pollen quantity have a positive effect on colony vitellogenin.
The combination of the parameters is needed to describe the colony's vitality. The combination of the parameters, determined in a 2010 study show that in a pollen rich and poor environment, in September the bees respectively stop breeding and don't,

consequently having a mean high and low fraction of hemolymph vitellogenin (0.45 and 0.33). This raises the question: do colonies in the pollen poor environment keep on breeding because of the low vitellogenin fraction which does not reach the "winter population' level of vitellogenin or are other factors involved? In general is it possible that, because of Varroa or environmental factors, vitellogenin cannot reach a certain level in September, the colony will keep on breeding and will not turn into a real winter colony?