Action FA0803

Agricultural institute of Slovenia

COLOSS Workshop "Honey bee nutrition”

Bled, Slovenia, 22-23 October 2012

Organized by Coloss Work Group 3

Speakers:
Katherine Aronstein, USDA/ARS, USA
Karl Crailsheim, Karl-Franzens-University Graz, Austria
Jozef Van der Steen, Wageningen UR, Netherlands

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Agenda

Sunday, 21 October 2012

Time	Activity
19.00 -	Welcome dinner

Monday, 22 October 2012

Time	Activity
09:00 – 09:20	Registration
09:20 – 09:30	Welcome and plenary session: organizational matters Aleš Gregorc
09:30 – 09:45	Welcome of the WG3 and achievements of the WG3 Karl Crailsheim
09:45 – 10:45	Plenary talk by Katherine Aronstein (Invited Speaker) & discussion
10:45 – 11:15	*Coffee break*
11:15 – 12:15	Plenary talk by Karl Crailsheim (Invited Speaker) & discussion
12:15 – 12:45	10-15 min talks by participants & discussion Anika Löwe Maja Ivana Smodiš Škerl
12:45 -14:15	*Lunch*
14:15 – 15:45	10-15 min talks by participants & discussion Robert Brodschneider Simone Tosi Janeš M *Csáki Tamás* Asger Søgaard Jørgensen
15:45 - 16:10	"Possible Common research topics" (submitted Ricola plan; Karl Crailsheim) & discussion
16:10 - 16:40	*Coffee break*
16:40 - 17:30	Discussion: determination of the future research topics
20:00 -	*Social dinner*

Tuesday, 23 October 2012

Time	Activity
09:00 - 10:00	Plenary talk by Jozef Van der Steen (Invited Speaker)
10:00 - 11:15	10-15 min talks by participants & discussion Fani Hatjina Piotr Medrzycki J. Ruiz Martínez Antoine Lecocq Geoff Williams
11:15 - 11:45	*Coffee break*
11:45 - 12:30	Discussion: experimental set up and their participants; Plenary discussions of current WG3
12:30 - 14:00	*Lunch*
14:00 - approx 19:00	Boat trip to Bled Island and/or Bled Castle and/or Apiculture Museum Radovljica (depend on weather)
End of workshop meeting	

Table of Contents

Table of Contents

How *Varroa* Parasitism Affects the Immunological and Nutritional Status of the Honey Bee.

Aronstein, K.A., Angela Douglas

USDA/ARS

Author for correspondence: Kate.Aronstein@ars.usda.gov

Bee health and colony productivity remains at the center of attention of many research groups world wide. Since it was first discovered in the US, *Varroa* infestation of managed bees has become one of the most urgent problems facing the beekeeping industry. However, the mechanisms by which honey bee health and nutritional status are impacted is not understood fully. The purpose of this study was to quantify the impact of experimental *Varroa* infestation on the immune response and nutritional status of honey bee colonies. The data are analyzed at the level of the individual insect and the colony level. The implications of the physiological relationship between immune function and nutritional condition are addressed to better understand how the nutritional status of individual bees shapes the overall health of the colony.

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For your remarks:

Feeding pollen and pollen substitute to caged honey bees.

Robert Brodschneider, Caroline Haidmayer, Karl Crailsheim

Department of Zoology, Karl-Franzens-University Graz, Universitätsplatz 2, 8010 Graz, Austria.

Author for correspondence: Robert.brodschneider@uni-graz.at

Adult honey bees need protein-rich food for their development. Bee-collected pollen or bee bread may contain honey bee pathogens, hence we tested the suitability of a pollen substitute based on non-hive products to nourish caged bees. In 2-7 replicates we compared the effect of bee collected pollen and the pollen substitute Feedbee® on the physiological development (hypopharyngeal glands and flight muscles) of 100 caged honey bees over a period of 5 or 12 consecutive days. Bees fed with a protein-free diet showed a retrogression in the size of the acini diameter of the hypopharyngeal glands compared to those of newly emerged bees ($p<0.05$, Fisher LSD). No differences in the size of the acini diameter or thorax weight between bees fed with pollen compared to bees fed with Feedbee® were found ($p>0.05$). We also tested the effect of artificial brood pheromone on the consumption of diets and physiological development. Pollen was less consumed on day 3 and 4 when brood pheromone was present ($p<0.05$, Mann-Whitney). The consumption of Feedbee® was lower on day 1 in cages with brood pheromone ($p<0.05$). The acini diameter of the hypopharyngeal glands of bees fed with any protein diet were smaller in the brood pheromone groups compared to the respective control groups without brood pheromone ($p<0.05$, Fisher LSD). Thorax weight was much more influenced by nutrition than by brood pheromone. Our results suggest an effect of brood pheromone on caged bees: pollen consumption and development of hypopharyngeal glands were reduced. With regard to the protein uptake, the development of the hypopharyngeal glands and the flight muscles, Feedbee® is an adequate nutrition for caged honey bees and shows no disadvantages compared to bee-collected pollen.

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For your remarks:

Trophallaxis and protein nutrition of honey bees

Karl Crailsheim

Department of Zoology, Karl-Franzens-University Graz, Universitätsplatz 2, 8010 Graz, Austria

Author for correspondence: karl.crailsheim@uni-graz.at

In honey bees, protein has a completely different fate compared to carbohydrates. By applying different sources of solid and liquid food at different locations in the colony we can investigate the uptake of diets by different aged workers. The location and the way in which diets are applied in the hive have impact on the age cohorts that feed on those diets. In particular when feeding protein diets, one must consider that many of the bees are also found feeding on a diet that generally do not consume other protein than processed jelly given by middle aged nurse bees. There are at least two temporal worker castes functioning as turntables for nutrients arriving in a colony. First, the food storer bees (aged 18-26 d), who receive feedings from returning foragers do also consume liquid sugar solutions within the hive. The second important group consists of 4-16 day old nurse bees. This group feeds on bee bread (stored pollen), has enzymes to digest protein and has developed hypopharyngeal glands. Nurse bees are feeding larvae and provide adult members of a colony with protein by trophallaxis. Artificial feeding results in an increase of incoming nutrients. Bees who are consuming the diet just for its sweetness are might also receive undemanded protein. Consequently, components of the artificial diets can be found in honey and brood. The physiological needs of honey bees and the impact of artificial provision will be discussed.

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For your remarks:

The impact of different nutrients on colony development and Nosema prevalence.

Fani Hatjina[1], Leonidas Charistos[1], Aikaterini Karatasou[2]

[1]Hellenic Institute of Apiculture - Nat. Agr. Org. 'DEMETER', N. Moudania, 63200, Greece
[2] Federation of Greek Beekeepers Associations, 4 Kouma Str, 412 22 Larisa, Greece

Author for correspondence: fhatjina@instmelissocomias.gr

In order to test the impact of different nutrients on colony development two types of experiments were designed. A) Three groups of colonies were overwintered with floral honey, honey-dew honey or sugar candy and the development of the colonies in terms of population and brood area was assessed before and after winter (November and April). Nosema prevalence was also recorded in November, January, March and April. B) Eight groups of colonies were fed with different supplements containing various vitamins during spring development. A ninth group was fed only sugar syrup and a tenth group was provided with extra queen pheromone. The enhancement of population development as well as of brood area was assessed before and after feeding stopped (after 8 weeks). The results showed: A) significant differences in overwintering between the three groups of different feeding type, with floral honey being the most suitable. B) Some differences also found in the development of the colonies in respect of the different nutrients fed. Nosema prevalence was also found different between the groups.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

The importance of water availability for honeybee colonies.

Hegić G, Janeš M, Filipi J, Prđun S, Dražić M, Bubalo D, Kezić N

Faculty of Agriculture, University of Zagreb

Author for correspondence: mjanes@agr.hr

Water plays an important role in honeybee diet. Bees must have continuous and adequate supply of water for normal development and survival. The lack of water may cause indigestion and irregular thermoregulation. Water can also be source of infection and poisoning. Beekeepers use various water supply containers that usually have many disadvantages (inadequate loading capacity, inconstant supply of water, unsatisfactory sanitary conditions). New model of hygienic water supply container has been constructed in order to study permanency, consumption and hygiene of water supply. The experiment took place from 26th of July to 29th of September in 2006 and it included six isolated apiaries of equal strength of colonies. During experiment, 83 colonies consumed 374.1 litters of water. The average water consumption was 5.19 ± 2.88 litters and the average daily water consumption was 0.12 ± 0.007 litters per colony.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

Do Bees Shun the Limelight?
A foray into the social behaviour of health-compromised honey bees.

Antoine Lecocq

University of Copenhagen, Denmark

Author for correspondence: Antoine@life.ku.dk

One of the cornerstones of social behaviour is the interaction between two or more individuals of the same group. Honey bees form dense colonies of related workers in constant interaction with one another, for communication, hygienic or nutritional purposes among other things. However, while physical interactions are crucial to the running of a colony, they are also ideal for the transmission of pathogens and other harmful substances to the detriment of the colony as a whole. A number of collective immune defences have been identified in social insects including honey bees to reduce or limit the threat of parasites or harmful substances, such as division of labour, use of self-produced chemicals, waste management and cleaning of infected cells. Here, we investigated whether honey bee workers infected with *Nosema ceranae* have the ability to decrease their disease transmission potential by refusing to interact, and exchange food, with another healthy colony worker or whether healthy colony members can choose to interact with other healthy workers over health-compromised ones. We placed one bee in each of three chambers of a purpose made arena. A starved bee, healthy or infected with *Nosema ceranae*, had the option to beg for, through a mesh, and receive food from a healthy bee or a bee infected with *Nosema ceranae*. The behaviour of all the bees was recorded over one hour.

Preliminary results are still to be analysed and the experiment is ongoing. The results will include data on the time spent by the hungry begging bee on each side of the arena and its location relative to the separating mesh, the location of the donor bees within their own chambers. Finally we will

identify every instance of likely trophallaxis events and the *Nosema* spores count of all bees post-experiment.

Keywords: Trophallaxis, transmission, pathogen, social immunity, interaction

For your remarks:

Honey bee trophallaxis in an arena.

Anika Löwe, Karl Crailsheim

Department of Zoology, Karl-Franzens-University Graz, Universitätsplatz 2, 8010 Graz, Austria

Author for correspondence: anika.loewe@uni-graz.at

Trophallaxis (the dissemination of food from mouth to mouth) is a significant social interaction in the complex honey bee society. The amount and quality of food which is transferred can differ from the temporal castes. The castes in a honey bee colony are not rigid but change with age polytheism which might influence the trophallactic interactions. The aim of this study is to determine if different aged honey bees receive equal amounts of sugar solution provided from a nurse-aged bee (donor). In arena experiments, the trophallactic interactions between a donor (raised for 7-9 days in a honey bee colony) and one-day old bees as well with other nurse-aged bees from the same colony as the donor bee were investigated. For each experiment donor was caged respectively with one recipient (one-day old bee, nurse-aged bee) in an arena (plastic purpose observation container). In preliminary experiments we observed that newly emerged bees are capable to survive longer starving periods of more than six hours. Moreover the recipients only showed begging behavior when their honey stomach was emptied. For this reason all recipients were forced to regurgitate before the experiments started and had to starve additionally for three hours to assure that their honey stomach is empty. In turn, the donor bees were fed with 15µl of a 3 M glucose solution 10 minutes. before they were introduced into the arena. A single trial lasted maximum 15 minutes or until the first trophallactic interaction occurred. The following parameters were recorded: the time until the first trophallaxis took place and the duration of the trophallactic contact. Furthermore after the

following parameters were recorded: the time until the first trophallaxis took place and the duration of the trophallactic contact. Furthermore after the first observed trophallactic interaction the honey stomach of both the recipient and the donor was dissected and weighed to measure the amount of transferred sugar solution. In our arena experiments first median trophallactic contacts occurred within 66.6 sec. for one-day old bees (n= 31) and showed no significant difference to nurse-aged bees (Mann Whitney test, $p>0.05$). The median duration of one trophallactic contact lasted 7.78 sec for one-day old bees and also showed no significant difference (Mann Whitney test, $p>0.05$). But the median amount of 9.02mg sugar solution a nursed-aged bee received is significant higher compared to that a one-day old bee gained (Mann Whitney test, $p<0.05$). We hope that these arena trials will allow us to study protein dissemination in the different temporal castes in further experiments.

For your remarks:

Nutritional status and other parameters influence results of toxicological tests on bees.

Piotr Medrzycki, Simone Tosi

Agricultural Research Council, Research Unit for Apiculture and Sericulture; Via di Saliceto 80, 40128 Bologna, Italy

Author for correspondence: piotr.medrzycki@entecra.it

The official guidelines for risk assessment of pesticides on honeybees are based on specific protocols. They contain procedures which must be respected in order to make the results valid for the registration process. Nevertheless, in many cases the application of the same official guidelines by different research teams leads to quite different results of toxicological tests. This worrying phenomenon makes the tests not reliable and the reason of a similar variability may be searched in some test parameters which play an important role as cofactors in bee response to the stressors, but are indicated in the guidelines with insufficient precision. In fact, for some of the test parameters, often a broad range of values are acceptable, while some others are not considered at all.

The present contribution is aimed to discuss some of the test parameters which may significantly influence the results of toxicity tests.

For example, the EPPO guidelines allow to run laboratory toxicity tests at the temperature of 25 +/- 2°C. In our studies we have noticed that the LD_{50} value may vary significantly within this temperature range.

Also the nutritional status of the bees used for tests is described in the guidelines in a generic way. In our studies we have observed that the nutritive quality of pollen consumed by the bees after emergence may influence their susceptibility to pesticides.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

Another parameter influencing results of toxicological tests is bee age. Forager bees seem to be less resistant to the stressors than younger ones. For this reason the bee collection method and, as a consequence, bee age are important parameters to be taken into account in the guidelines.

One important deficiency of the official laboratory test protocols is related to the diet consumption, which is quite different according to the bee's task. Since the exposure to toxic substances is related to the food uptake (which is limited in the lab tests), this point should be also taken into account. Two methods to increase diet uptake in lab tests are reported: making foragers fly and forcing them to transfer test food to other bees through trophallaxis.

The current guidelines allow to the subject interested in pesticide registration, to run toxicity tests with such a set of test parameters (temperature, nutritional status, bee age, sanitary conditions etc) which produce lower negative effects.

It is concluded that an international initiative aimed to study synergistic effects and/or influence of test parameters on pesticide toxicity to bees is needed.

For your remarks:

An adequate feeding can decrease the loss of colonies in Spain.

J. Ruiz Martínez, M. Gutiérrez Tirado.

APOIDEA SL
Font del Riego, 38. 14009-Córdoba (Spain)

Author for correspondence: info@apoidea.es

Multiple factors of Colony losses in Spain, as technical management, environment and health, are analised relating them to the nutrition of the colonies. It presents an essay on the influence of sugary foods (commercial and artesanal) in developing colonies of *Apis mellifera*, reaching conclusiones the following:

1. - Commercial sugar food produced a greater increase in the number of population and breeding, which enabled more swarms at an early stage, thereby offsetting its higher cost.
2. - Licensing of these preparations is a guarantee of food security and comfort and ease in handling contribute to significant time savings.
3. - In adverse environmental conditions such as drought or lack of pollen the development of colonies may be limited despite of sugary food supply.
4. – So, it is essential to further investigate a balanced diet with protein supplements.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

Impact of different sugar syrups on individual caged honeybee performance.

Maja Ivana Smodiš Škerl, Mitja Nakrst, Veronika Kmecl, Aleš Gregorc

Agricultural Institute of Slovenia
Hacquetova ulica 17, 1000 Ljubljana

Author for correspondence: maja.smodis.skerl@kis.si

In beekeeping practice, during the lack of nectar and for winter feeding the consume sugar (sucrose) and several other sugar syrups are used. In our experiment, we conducted feeding with different syrups in laboratory conditions assessing performance of caged workers in terms of survival, load of *Nosema* spp. spores and size of hypopharingeal (food processing) glands. We also analysed haemolymph proteins and sugars (glucose, fructose and trehalose). We found that 90 % of workers survived 10 days in all four tested diet groups. The longest survival was found in workers fed by 'sugar syrup' and 'TruSweet', where approx. 10 % of bees remain alive in cages 39 days. Mortality rate was higher in cages where workers received saccharose-fructose-glucose “Apiinvert” or fructose-glucose-saccharose “Ambrosia” syrup. The lower *Nosema* spp. spore load, under 10^6 per bee, was detected in workers received 'TruSweet', 'Apiinvert' and 'Ambrosia' and the highest spore load, varied from 1.4×10^6 to 48×10^6 per bee, was found in workers received only 'sugar syrup'. We found no differences in the acini size of hypopharingeal glands in workers of all feeding groups. Total protein content varied among the feeding groups, and significant differences in 8 days old bees ($P < 0.01$) was found. We also found significant differences of haemolymph fructose titre between feeding groups ($P < 0.0001$). Different sugars syrups in a bee diet have an important influence on workers longevity, *Nosema* spp. spore load in the intestine and protein and sugar titre in the haemolymph. Further tests are in progress to establish potential effects of sugar syrups on colony level performance with special attention on overwintering.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

Pollen availability for honeybees in an agricultural landscape and mitigation of pollen scarcity by planting of bee forage.

Asger Søgaard Jørgensen , Flemming Vejsnæs, Rolf Theuerkauf

Danish Beekeepers Association, Fulbyvej 15. DK 4180 Soeroe

Author for correspondence: asj@biavl.dk

Denmark has the world's most intensive agricultural landscape. More than 60 % of Denmark is arable land of which 92 % is under crop rotation.
The main crops are Winter Wheat, Maize, Sugar Beets and Potatoes. For the bees we have about 170.000 hectares with Winter Rape Seeds. Some areas with organic grown pastures for organic milk production and on the Eastern Islands we have 4.000 hectares with production of White Clover seeds.
There are some orchards with fruit production apples, strawberries, raspberries.
Towns, roads etc. are 20 %, 12 % forest and the rest is heather, dunes, meadows
In the agricultural landscape we have abundant nectar and pollen some periods of the season, but other periods the landscape is a desert for honeybees and other beneficial insects.
Observations bee-yards with electronic scales and temperature registrations have been set up.
From the observation bee yards we can see that some parts of the season there is an abundant nectar flow, but regularly we find in the agricultural landscape a dearth period during June with lack of flow. The flow stops normally mid July.
There is a lack of nectar yielding bee forage in parts of the season. What I want to investigate is if the same counts for the pollen supply.
The project has several activities involved.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

The beekeepers apply pollen traps on one hive in the observation bee yards monthly to build a calendar of the most important pollen plants in the area.
By means of Google maps, field data and direct surveys the main pollen plants around the bee yards.
The data are compared the data collected with similar data from 60 years ago.
In cooperation with the Hunters association, the Ornithological Society and the Danish Nature Conservation association we produce information and inputs to politician, administrators, hunters, farmers and beekeepers on how to improve the conditions not only for honeybees but for wildlife, birds and insects in the agricultural landscape.
The project is planned for a three years period.

For your remarks:

Are we preparing summer or winter colonies for winter?

Jozef Van der Steen, Chula HokaHin, Bram Cornellissen, Coby van Dooremalen

Bees@WUR, Plant research International Wageningen UR

Author for correspondence: sjef.vandersteen@wur.nl

The vitality of a honeybee colony depends on several factors among which pollen supply , parasites, beekeeper practice and pesticides. We described the vitality of a honey bee colony with the parameters total hemolymph protein, mean hemolymph vitellogenin concentration, number of bees and number of sealed brood cells. As the colony has compensation mechanisms to cope with low protein income and since vitellogenin is age and task dependent, only an overall reduction of vitellogenin is relevant to be measured. Our studies demonstrated that low diversity of pollen, resulted in postponed transition to winter colonies in September. This may have serious consequences for the success of the Varroa control in July/August and the composition and size of the winter population at the start of the winter period in October.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

Treatment efficiency in higher need of protein sources.

Csáki Tamás[1], Máthás Sciller[2], Békési László[2]

[1]Szent István University, Institute for Wildlife Conservation, 2101 Gödöllő, Páter K. u. 1. Institute for Small Animal Research & Co-ordination Centre For Gene Conservation.

[2]Institute for Bee-breeding, 2101 Gödöllő, Isaszegi u. 100.

Author for correspondence: csaki.tamas@gmail.com

In the overwrought production in beekeeping needs sufficient nutrition for the bees. Focusing on protein natural pollen sources different pollens have different nutritional value to honey bees. The crude protein (CP) content of natural pollens and their levels of amino acids are depending on specific situations to determine them as a proper proportion. For instance colonies dealing with nosemosis may need more CP with different proportion of amino acids. Using pollen substitutes with good palatability, good digestibility and proper balance still may not be enough to fight back nosemosis. In our work we intend to combine various treatments and diets against the two types of nosemosis.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

Does the pollen available to the colony influence the health of the bees?

Simone Tosi [1,2], Piotr Medrzycki[2], Diego Bergamini[1], Cecilia Costa[2]

[1]University of Bologna - Department of Agroenvironmental Sciences and Technologies, Via Fanin, 44 - 40127, Bologna – Italy

[2] Agricultural Research Council – Research Unit for Apiculture and Sericulture (CRA-API), Via di Saliceto, 80 - 40128, Bologna – Italy

Author for correspondence: s.tosi@unibo.it

It is commonly agreed that the phenomenon named CCD (Colony Collapse Disorder), related to the recent honey bee colony losses, is multi-factorial. One of the factors, suspected of playing an important role in these losses, is the nutritional status of the colonies.
Honey bees need to eat pollen to ensure the proper development and growth. Indeed, pollen is the main source of proteins for honey bees. Forager bees tend to collect pollens from different plant species and this behaviour helps to completely satisfy the nutritional requirements of the colony through a balanced and diverse diet. In fact, the nutritive value of the pollen can vary widely according to its botanical origin. Nevertheless, commercial colonies are often placed in agricultural landscapes, where there are few pollen-producing plant species available for the bees. For this reason, forager bees can collect pollen from only the few different plants species available, according to the flowering time.
The aim of this work was to investigate if the quality of the pollen available to a colony can influence the health of the bees.
Newly emerged bees were fed *ad libitum* with water, honey and fresh pollen. The pollen was collected in apiaries situated either in natural (NAT) or agricultural (AGR) ecosystems. The collected pollen was provided to two

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

separate groups of newly emerged bees. After 2 weeks of incubation, LD_{50} of fipronil and thiamethoxam in relation to the pollen diet was assessed.
The results showed that bees fed with AGR pollen, compared to those fed with NAT pollen, were characterized by: 1) higher mortality during the 2 weeks of incubation and 2) lower resistance to the intoxication by the aa.ii.
To conclude, the survival of the bees and their susceptibility to pesticides are influenced by the pollen nourishment. Thus, intensive agricultural ecosystems may have negative impact on honey bee colonies through both the widespread presence of pesticides and the low nutritional quality of the pollen available.

For your remarks:

The COLOSS *BEEBOOK*: honey bee nutrition standardization not so standard

Geoff Williams[1], Gina Tanner[1], Peter Neumann[1], Robert Brodschneider[2]

[1]Swiss Bee Research Centre, Research Station Agroscope Liebefeld-Posieux ALP-Haras, Switzerland

[2]Department of Zoology, Karl-Franzens-University, Graz, Austria

Author for correspondence: geoffrey.williams@alp.admin.ch

The primary purpose of the COLOSS *BEEBOOK* is to recommend standards for honey bee research methods. In cases where such standards could not be provided *ad hoc*, discussion on method selection, as well as future research directions for developing standards, are provided. In the chapter 'Maintaining adult worker honey bees under laboratory conditions', nutritional considerations are paramount. Whereas the importance of providing adults with carbohydrates is well known, the need for other macro-nutrients and vitamins is less clear. Additionally, much debate exists over sterilization techniques when providing bee products; ultimately, food must be nutritious yet free of contaminants that may confound results. Here we present recommendations and discussions from the COLOSS *BEEBOOK* on choosing and providing worker honey bees maintained under general laboratory conditions with carbohydrates, proteins, vitamins, and minerals.

Workshop "Honey bee nutrition", 22. - 23. 10. 2012, Bled, Slovenia

For your remarks:

PARTICIPANTS

ARONSTEIN Katherine Research Molecular Biologist USDA/ARS, 2413 E Hwy, 78596 Weslaco, TX, USA Kate.Aronstein @ars.usda.gov Cell phone: 956-373-8546	BEVK Danilo Doctor National institute of biology, Večna pot 111, 1000 Ljubljana, Slovenia danilo.bevk@gmail.com Tel: 0038659232783 Cell phone: 0038641960139
BRODSCHNEIDER Robert Doctor Department of Zoology, Karl-Franzens-University Graz, Universitätsplatz 2, 8010 Graz, Austria Robert.brodschneider@uni-graz.at Tel: 00433163805602 Cell phone: 00436508629954	BUBALO Dragan Prof.dr.sc. Faculty of Agriculture University of Zagreb, Svetošimunska cesta 25, 10 000 Zagreb, Croatia dbubalo@agr.hr Tel: +385 1 239 3977 Cell phone: +385 99 31 790 31
CRAILSHEIM Karl Doctor Karl-Franzens-University Graz, Department of Zoology, Universitätsplatz 2, 8010 Graz, Austria Karl.crailsheim@uni-graz.at Tel: 0043 316 380 5616 or 5597 Cell phone: 0045 6648463117	CSÁKI Tamás Mr., PhD student Szent István University, Institute for Wildlife Conservation HUNGARY-2103 Gödöllő, Páter K. u. 1. csaki.tamas@gmail.com Tel: +3628522086 Cell phone: 0036303326848
FILIPI Janja Doc. dr. Polytechnics Marko Marulic in Knin, Kralja Petra Kresimira IV 30, HR-22300, Knin, Croatia jfilipi@veleknin.hr Tel: 00385 22 668 126 Cell phone: 00385 91 3881 117	GREGORC Aleš Doctor Agricultural Institute of Slovenia Hacquetova 17, 1000 Ljubljana, Slovenia ales.gregorc@kis.si Tel: 00386 1 28 05 150

PARTICIPANTS

HATJINA Fani Doctor Hellenic Institute of Apiculture, Nat. Agr. Org. 'DEMETER', 63 200 N. Moudania, Greece fhatjina@instmelissocomias.gr Tel: +30 23730 91297 Cell phone: +30 6947431299	JANEŠ Mateja Mag. ing. agr. Faculty of Agriculture University of Zagreb, Svetošimunska cesta 25, 10 000 Zagreb, Croatia mjanes@agr.hr Tel: +385 1 239 3793 Cell phone: +385 98 9345 241
JØRGENSEN Asger Soegaard Beekeeping consultant Danish Beekeepers Association, Fulbyvej 15, DK 4180 Soroe, Denmark asj@gefion1 Tel: + 45 5786 5470 Cell phone: + 45 2446 4991	KEZIC Nikola Prof. dr. Faculty of Agriculture, Svetosimunska 25, HR-10000 Zagreb, Croatia nkezic@agr.hr Tel: 00385 1 2393793 Cell phone: 00385 98 316 469
LECOCQ Antoine Mr University of Copenhagen, Thorvaldsensvej 40, 3 sal, 2000 Frederiksberg, Denmark Antoine@life.ku.dk Tel: +4581503509	LöWE Anika Dipl. Biol. Institutu for Zoology, Universitätsplatz 2, 8010 Graz, Austria Anika.loewe@uni-graz.at Tel: 0043 316 380 5602 Cell phone: 00436767801853
MAYR Josef Doctor AGES GmbH - Austrian Agency for Health and Food Safety, Spargelfeldstraße191, A-1220 Wien, Austria josef.mayr@ages.at Tel: 0043-50-555 -33130 Cell phone: 0043-699-10339900	MEDRZYCKI Piotr Doctor CRA-API, Via di Saliceto 80, 40128 Bologna, Italy piotr.medrzycki@entecra.it Tel: +39 051 353103 int.20 Cell phone: +39 333 6349921

PARTICIPANTS

NAKRST Mitja Ing. Agricultural Institute of Slovenia Hacquetova 17, 1000 Ljubljana, Slovenia mitja.nakrst@kis.si Tel: 00386 1 28 05 241	PRQUN Saša mag.ing. Agronomski fakultet, Svetošimunska 25, 10 000 Zagreb, Croatia sprdjun@agr.hr Tel: +385 1 239 3793 Cell phone: +385 98 1992 326
SMODIŠ ŠKERL Maja Ivana Doctor Agricultural Institute of Slovenia Hacquetova 17, 1000 Ljubljana, Slovenia maja.smodis.skerl@kis.si Tel: 00386 1 28 05 241	RUIZ MARTÍNEZ José Antonio APOIDEA SL, Font del Riego 38, 14009 Córdoba, Spain info@apoidea.es Tel: 0034 957 420633 Cell phone: 658 031 243
TOSI Simone Doctor CRA-API, Via di Saliceto 80, 40128 Bologna, Italy s.tosi@unibo.it Tel: +39 051 353103 Cell phone: +39 349 0610340	VAN DER STEEN Jozef MSc bees@wu,r PR, Wageningen UR P.O.Box 16, 6700 AA Wageningen, Netherlands sjef.vandersteen@wur.nl
WILLIAMS Geoff Doctor Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern, Switzerland geoffrey.williams@alp.admin.ch Tel: +41 79 437 93 40 Cell phone: +41 79 437 93 40	

For your remarks:

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