Comparative Analysis of Protein content in Haemolymph of worker Honey Bees *Apis Mellifera* L. fed on laboratory formulated Diets

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ABSTRACT

During Dearth period it's necessary to own adequate supply of pollen substitutes to take care of bee colonies health. We compared three laboratory formulated diets (LFD): 1. Gram flour based, 2. Soya flour based diet, 3. Black pulse flour based diet, with bee collected pollen (as Natural feed) and Sugar syrup (used by beekeepers in most of parts of India during dearth period as bee feed) by measuring their effect on Haemolymph protein contents of young bees exclusively gulped up these diets, which may be a fast and cheap assay. The substitute diets included a non-soy-based, pollen substitute diet named **LFD-1** having Gram flour (*Cicer arietinum*), a Soya based diet, named **LFD-2** having Soya flour (*Glycine max*) and a Black pulse (*Phaseolus mungo*) based diet, named **LFD-3** having pulse flour. All the diets got within the sort of patty to groups of 100 European honey bees *Apis mellifera* L. in Langstroth cages, maintained and fed from emergence until 10 days old. Sucrose, within the variety of syrup (50% ad libitum) was used as a protein free control diet. The results so obtained showed that **LFD-1**, **LFD-2**, **LFD-3** and pollen increased protein content within the Haemolymph by factors of 2.50, 2.29, 1.69 and 1.76 respectively, over protein content analysed in bees fed only sucrose solution. The bees devoured **LFD-1** and **LFD-2** had their Haemolymph significantly enriched in protein as compared to group of bees fed on **LFD-3** and the controls and to content slightly above those fed on pollen. All four proteinaceous diets were significantly superior to sugar syrup alone.

Keywords: Pollen substitute, Laboratory Formulated diet (LFD), Honey bee, Protein, Haemolymph, Soybean, *Cicer arietinum*, *Phaseolus mungo*.

INTRODUCTION

Honeybee plays a vital role in environmental conservation. Their work efficacy for nectar and pollen collection, colony management including brood rearing, hive making, food packing, bee flights, preparation of bee products (honey, propolis, royal jelly etc.) etc., need lots of biochemical constituents **(Crailsheim, 1990; Herbert, 2000; Gupta and Kumar 2003, Kumar and Agrawal 2014, Kumar 2016).** Amongst

them, proteins contents play a major role in the life of honey bees **(Amdam and Omholt, 2002) (House, 1961; Cohen, 2003).** Unavailability of protein in bee diet results in decrease in all vital activities of honey bee like brood rearing, bee strength, bee products (honey, propolis, royal jelly etc.) and bee pollination.

Low Protein in bee diet also affects the ability of honey bees to resist diseases **(Matilla and Otis, 2006);** and supposed to one of the factor, responsible for "Colony Collapse Disorder" **(Cox-Foster et al., 2007; Kulincevic, and Rothenbuhler B (1973B).** During dearth period pollen is not always available and then a pollen substitute becomes necessary to ensure continued bee colony development, **(Standifer et al., 1980; Goodwin et al., 1994; Herbert, 2000).** A number of pollen substituted diets (i.e. those that contain pollen) are advocated and a few of them are in market. The collection of dietary material by the bees may not mean that it is enough for bee colonies. During dearth periods, honey bees often collect substances that have no nutritional value for the bees. In our experiment we have used a technique that rapidly tests to LFDs, consists of feeding newly emerged adult bees in small Langstroth cages for five days and then estimating the protein contents in their haemolymph **(Cremonez et al., 1998)**. Bee bread which is fermented pollen stored in brood comb, gives the highest protein levels with this diet evaluation technique **(Herbert and Shimanuki, 1978; Gilliam, 1997),** especially when made from fresh pollen **(Pernal and Currie, 2000).** Various alternative bee diets have been found to be nutritionally poor or unpalatable and most are not well tested **(Herbert, 2000)**. Although consumption rates of pollen substitutes may prove their palatability, only through nutritional tests can their worth be evaluated. The method of investigating the efficiency of a protein source by detecting the level of protein in the haemolymph of worker bees fed on pollen and pollen substitute diets provides a means to determine the actual benefit that the bees obtain from pollen substitutes or supplements **(Bitondi and Simoes, 1996; Cremonez et al., 1998; Szymas and Jedruszuk, 2003).** Thus, the objective of our study was to determine the efficiency of artificial diets (LFD) for feeding to honey bees during dearth period **((Pham-Delegue, *et al*.,2000**; **Gupta and Kumar., 2003)** as protein supplements for honey bees by measuring total protein in the Haemolymph of caged honey bees fed these diets.

MATERIALS AND METHODS

The experimental bees were reared in Zoology Department of Govt PG College, Bisalpur, Pilibhit using standard Langstroth cages with wax sheet foundation frame under controlled conditions. The initial bee colonies were obtained from a local Apiary being run by Mr. Rajesh Gangwar of Village Mahaba, Bisalpur (Expert of Apiculture). The temperature and relative humidity maintained were 25-30oC (± 2 oC) and 60-65 R.H., respectively. Each experimental cage was started with 200 newly emerged bees per cage. The experimental setup was created in June 2017 and continued till August 2017.

Formulation of Lab. Diets

All the three pollen substitutes were formulated as per detail

LFD-1 having Gram flour (*Cicer arietinum*) + 50% syrup in ratio 1:1 by weight + Gentamycin (0.1ml/100g feed, Brand Genticyn) as antibiotic + Multivitamin and multimineral capsule (1 capsule / Kg feed, Brand Revital),

LFD-2 having Soya flour (*Glycine max*) + 50% syrup in ratio 1:1 by weight + Gentamycin (0.1 ml/100g feed, Brand Genticyn) as antibiotic + Multivitamin capsule (1 capsule / Kg feed, Brand Revital)

LFD-3 having Black pulse flour (*Phaseolus mungo*) + 50% syrup in ratio 1:1 by weight + Gentamycin (0.1 ml/100g feed, Brand Genticyn) as antibiotic + Multivitamin capsule (1 capsule / Kg feed).

Preparation of Diets

To formulate different diets in laboratory, the grams, soyabean and black pulse were obtained from local market of Bisalpur and converted into flour as per requirement. All diets had a similar final consistency and were prepared by mixing one part (by weight) of the proteinaceous powder with two parts of commercial grade finely granulated sucrose, which was ground with a spice grinder to powder consistency. Enough water was added to obtain a paste.

Bee feeding

The bees were randomly fed one of three lab formulated proteinaceous diets: **LFD-1**(Gram flour based diet), **LFD-2** (Soyabean flour based diet), **LFD-3** (Black Pulse Flour based diet) and fourth one as natural pollen (purchased from Shanti Kunj Krishi Ghar, Hapur, India). Pollen was stored at about -4ºC in a refrigerator for up to a week, until it was incorporated into the diets. All diets were administered as patties and the bees were allowed to feed *ad libitum* **(Cremonez et al., 1998).** Water was also supplied in glass tubes capped with cotton balls. Approximately 24g ±0.5g of each diet was placed in a hoarding cage, in a shallow plastic container and the diets were replaced every day. A protein free control diet was provided by feeding a fifth group of bees with 50% (by weight) sucrose syrup. A trial consisted of five cages, one for each diet and three trials were made, sequentially. Consumption rates were not recorded.

Biochemical Analysis

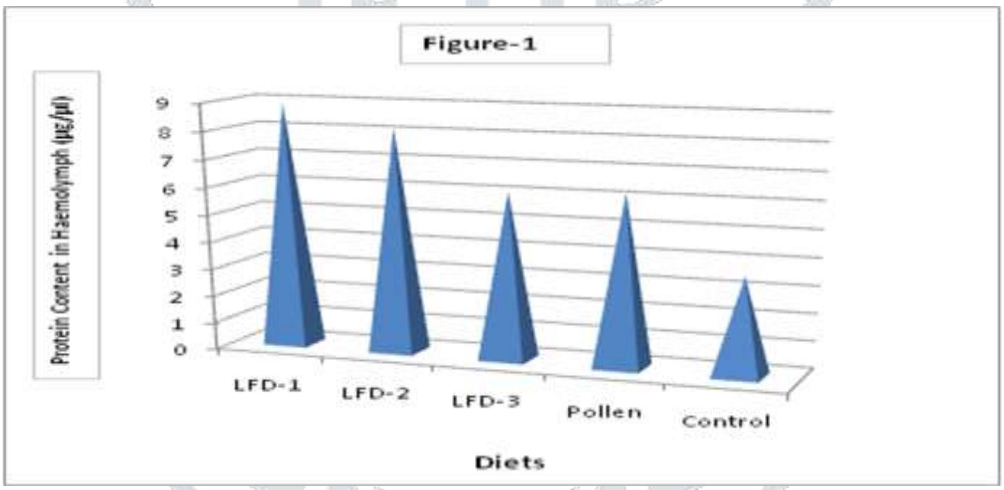
After the newly emerged bees had been fed for five days, five to six bees were removed from each cage and haemolymph was collected from a small incision at the level of the 3rd dorsal tergite, into microcapillary tubes previously washed in a 0.1% (wt:vol) phenylthiourea solution in water. The protein concentration was determined spectrophotometrically (using Systronics 166, India) for each bee by **Cremonez et al., 1998** method. The mean haemolymph protein concentrations of the bees fed on the various diets were compared by analysis of variance (ANOVA) and comparisons between the diets were made by Fisher's 't' test **(Chandel, 1993).**

RESULTS

The protein content in the haemolymph (µg/µl) of the caged honey bees varied significantly among the diet groups ($P < 0.01$, ANOVA, Table 1 Figure 1). **LFD-1** gave the highest protein levels, but it was not significantly superior to **LFD-2**. However, **LFD-1** performed significantly better than pollen, **LFD-3** and sucrose, while **Dearth Feed-2** was significantly superior to **LFD-3** and sucrose, but not to pollen. All of the proteinaceous diets were superior to sucrose syrup in their ability to elevate the protein content of bee haemolymph.

Table 1 and figure -1 representing data of Crude protein percentage of the protein sources and mean protein content (µg/µl haemolymph) in haemolymph of individual five days old *Apis mellifera* L. workers, fed on LFD and pollen from day 0 (when the newly-emerged bees were placed in the cages). *Protein contents having same letter are not significantly different from each other (Fisher's 't'test, a = 0.05.).

Diet	% Crude protein content	Protein titer* in haemolymph	%age increase in protein Content over Control	Standard deviation of the protein content	N (number of bees tested)
LFD-1	33.6	8.89^{ab}	2.49	3.99	18
LFD-2	26.7	8.15^{abd}	2.28	3.45	16
LFD-3	22.5	6.09^{dc}	1.71	2.67	15
Pollen	19.9	6.24^{bc}	1.75	2.19	17
Sugar Syrup (Control)	0	3.57^{d}	-	1.62	17



ISCUSSION

The results so obtained clearly show that **LFD-1** and **LFD-2** have performed better than pollen (Table 1 and Figure-1), giving of 2.49 and 2.28 times more protein in the bee haemolymph, respectively, than the Sugar Syrup (control), while the increase obtained with pollen was 1.75. This may seem a surprising result, but although pollen is the natural protein source for honey bees, they normally consume it after it has been fermented, in the form of "bee bread" **(Herbert *et al.*, 1985)**. Bee bread is superior to bees collected pollen when haemolymph protein values of bees, fed on these materials are compared **(Cremonez *et al.*, 1998).** Though pollen is rich in protein **(Roulston and Cane, 2000),** it is apparently not completely available until it has been processed by the bacteria in bee bread **(Herbert and Shimanuki, 1978; Gilliam, 1997).** No information is available with us on

the plant origins of the pollen we used; this factor affects the nutritional value of pollen **(Barbier, 1970; Baker and Baker, 1983; Pernal and Currie, 2000;)**. The **LFD-3** flour gave nearly the same protein content as did the bee-collected pollen (1.71 times the protein found in sugar syrup fed bees), indicating that this LFD, used as pollen substitute has some value for honey bees. All four protein sources originally contained at least 20 % crude protein, before being mixed with sugar and water. The black pulse flour, we tested contained about 20% protein, similar to the levels found in pollen, but can vary from 8 % to 40 % crude protein, depending on the fraction of seeds included when it is produced **(Perriera *et al.*, 2006)**. **Amdam and Omholt (2002)** reported much higher amounts of vitellogenin in haemolymph what we found in the present study. Their results may reflect the temperate adaptations of the race of honey bees they studied, the time of year of their studies, and the requirements for storage proteins in northern temperate zone honey bees as they prepare physiologically for winter **Mardan and Kevan (2002).** Our study was also of the temperate adapted European honey bees *Apis mellifera* L. but in tropical region of North India. Queenless groups of worker honey bees in Langstroth cages might moreover be expected to divert protein ingested into ovarian development.

Statistically, **LFD-1** and **LFD-2** have been found superior to fresh bees over collected pollen and therefore are confirmed to be adequate alternatives for bee feeding, especially during dearth period. The laboratory trials analyzing haemolymph protein in small groups of caged bees found successful and demonstrated that laboratory formulated diets (LFD), used as pollen substitutes can be superior to, or as good as, bee collected pollen, with the added advantages of lower cost without risk of spreading bee diseases. This protein substitute analysis method also provides a means of determining whether easily available protein materials such as black pulse flour may be good sources for the development of efficient and inexpensive bee diets.

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