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Full Length Research Article

PHYSICO-CHEMICAL ANALYSIS OF ARTIFICIALLY FORMULATED DIETS TO BE FED TO HONEY BEES DURING DEARTH PERIODS

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ABSTRACT

Six protein rich artificial diets were formulated & tested on *Apis mellifera* colonies during dearth periods. The main components of these diets were Defatted Soy Floor, Parched Gram, Brewer's Yeast, Skimmed Milk Powder, Protein Hydrolysate Powder, Spirulina, Natural Pollen, Sugar & Glucose Powder. Physico-chemical analysis of diet formulations was carried out to know the exact concentration of various nutrients. pH value and percent protein, carbohydrate, fat, ash, moisture & energy varied between 5.2-6.4, 10-25%, 57-70%, 2.47-6.48, 1.67-3.75%, 310-390% in Diet 1 to 6 respectively.

INTRODUCTION

Honey bees can survive on a pure carbohydrate diet for long periods but like most of animals, they also require proteins, fats (lipids), vitamins, minerals and water for normal growth and development (Loper and Berdel, 1980). All these nutrients must be present in the honeybee diet in an optimum ratio. These nutritional needs are satisfied by the collection of pollen, nectar / honey and water. Pollen which is collected by worker honey bees from a wide range of flowering plants normally satisfies the dietary requirements for protein, minerals, lipids and vitamins. Nectar collected by honey bees from either floral or extra floral nectarines of flower, is mainly a source of carbohydrates (White, 1963; Haydak, 1970). In terms of nutrition, protein is essential for the normal growth and development of honey bee tissues, muscles and glands such as hypo-pharyngeal glands (Moritz and Crailsheim, 1987; Schmidt *et al*., 1995) and it is well known that a pollen and protein-rich diet promotes development of larvae and ovarian and egg development (Woyke, 1976; Hays, 1984; Wheeler, 1996; Lin and Winston, 1998; Pernal and Currie, 2000; Hoover *et al*., 2006; Schafer *et al*., 2006; Dietemann *et al*., 2007). Bees require dietary lipids (fatty acids, sterols and phospholipids) in their diet as source of energy for synthesis of reserve fat and glycogen and as an essential structural component of many cell membranes (Lunden, 1954). Water is a vital element in the diet of honey bees. It serves some very important functions in the bee, including carrying dissolved food materials to all parts of the body, assisting in the removal of waste products, digesting and metabolizing food. Honey bees also require water to dilute thick honey and utilization of honey and crystallized sugar, to maintain optimum humidity within the hive to ensure egg hatch and to prevent larval dessication and to maintain proper temperature in hot weather (Herbert, 1992).

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MATERIALS AND METHODS

Six protein rich artificial diets were formulated & fed to honeybees during dearth periods (Table 1). Quantitative analysis of Proteins, Fats, Carbohydrates, pH, Ash and Moisture content of all the diet samples was carried out in Entomology Research Laboratory, School of Studies in Zoology, Jiwaji University Gwalior as per methods described below.

Table 1. Composition of diet formulations

Diet Code	DSF	PG	BY	SKM	PH	SP	P	S	G	H
Diet 1	16.7%	16.7%	16.7%	------	--------	--------	------	33.3%	16.7%	-------
Diet 2	20.7%	-------	20.7%	------	--------	8.3%	------	33.3%	16.7%	-------
Diet 3	16.7%	-------	16.7%	------	16.7%	--------	------	33.3%	16.7%	-------
Diet 4	16.7%	-------	16.7%	------	8.3%	--------	8.3%	33.3%	16.7%	-------
Diet 5	-------	-------	-------	------	--------	16.7%	------	-------	--------	83.3%
Diet 6	30%	-------	10%	10%	--------	--------	------	50%	--------	-------

DSF: Defatted Soyflour, PG: Parched Gram, BY: Brewer's Yeast, SKM: Skimmed Milk Powder,
PH: Protein Hydrolysate, SP: Spirulina, P: Pollen, S: Sugar, G: Glucose, H: Honey

Total Protein: Total protein content of diet formulations was determined with the help of spectrophotometer method proposed by Lowry *et al*., (1951) with minor modifications. To 4 ml of the sample in a test tube, 5.5 ml of the reagent mixture (50 ml of 2 % Sodium Carbonate in 0.1 N of NaOH+1 ml of 0.5 % CuSO4 solution in 1 % Sodium potassium tartarate solution) was added and kept undisturbed for 15 minutes. Then 0.5 ml of Folin-Ciocalteu reagent was added with vigorous shaking. The test tubes were then placed in dark for 30 minutes. The color (blue) intensity was measured at 660 nm in visible spectrophotometer (SYSTRONICS, INDIA) as optical density. The blank test tube included distilled water in place of protein (unknown) sample and in standard protein (known) test tube, 4 ml of BSA solution (5 mg/ml) was taken. The concentration of protein in unknown samples was calculated with the help of a standard graph of Optical Density versus Concentrations, plotted using five serial concentrations of BSA as samples.

Total Carbohydrate: Total carbohydrate content of diet formulations was determined by Dubois *et al*., (1956) method.

Total Fats: Total fat percentage was analyzed by Soxhlet extraction method (Stanley *et al*. 1974) with minor modifications. Five grams of sample was weighed on butter paper. The paper was folded in such a manner so as to prevent escape of the sample. Then this folded paper was placed in a thimble. Cotton was plugged on the top of thimble to help uniform soaking and extraction of the sample. Thimble was placed in Soxhlet apparatus and extracted in petroleum ether for about 16 hours. After extraction thimble was removed. Solvent and fat was transferred to a beaker. Beaker was then kept on water bath to evaporate ether. Finally it was dried in oven at 100^0 C for about an hour. Place beaker in desiccator, allow it to cool up to room temperature. Now weigh the beaker to obtain the final weight. Percentage of fat can be calculated by the formula given as under:

Fat Percentage: W_2-W_1/W x 100

Where, W: Weight of the sample, W_1: Weight of the beaker, W_2: Weight of beaker + fats after extraction.

Ash Content: Ash content of samples was determined using Muffle Furnace. Ten gram of the sample was weighed accurately in a pre-weighed silica disc. The sample was ignited in a Muffle Furnace at 620^0 C till white ash is obtained. The dish was pulled in a desiccator and allowed it to cool up to room temperature. Now weight of the dish was obtained and the final weight of ash was calculated by the formula given as under.

Ash Percentage: W_2-W_1/W x 100

Where, W: Mass in gm of the empty dish, W_1: Mass in gm of the dish with the material taken for the test, W_2: Mass in gm of the dish with ash

Moisture Content: Moisture content of diet samples was determined by using Hot Air Oven. Five gram of the sample was accurately weighed in a silica disc of known weight. It was dried in oven at 105 degree calcius for one hour. Then the dish was pulled in a desiccator and allowed it to cool up to room temperature. Now weight of the dish was recorded to obtain the final weight. Moisture percentage was calculated by following formula:

$$\text{Percent Moisture: } \frac{100\ (W1 - W)}{(W - W2)}$$

Where, W: Weight of dish with sample taken for test, W1: Weight of dish with Ash, W2: Weight of dish with dried sample

Total Energy: Total energy in the formulated diets was calculated with the help of bomb colorimeter. Five mg of sample was weighed accurately and pressed in to pellet form. It was then placed in capsule within the bomb made up of heavy steel material. Then the lid was closed. Pressure of about 300 pounds was maintained inside the bomb calorimeter. Then bomb was immersed in water. The sample was then ignited by means of electric fuse and reading reflects in form of kcal/100gm on the screen after three minutes.

pH value: The pH of formulated diets was analyzed by electronic pH meter method (Gonnet, 1977) using SYSTRONICS µ pH system 361 meter. To 5 mg of accurately weighed sample, 10 ml of distilled water was added and the pH was read directly from pH meter. The instrument was calibrated with standard buffer solutions of pH 7 and pH 4, prior to measuring the pH of samples. Three subsequent readings were taken and recorded.

RESULTS AND DISCUSSION

Quantitative analysis of Proteins, Fats, Carbohydrates, pH, Ash and Moisture content of the formulated diets was carried out in Entomology Research Laboratory, School of Studies in Zoology, Jiwaji University; Gwalior. Results obtained are presented in Table 2. The highest percentage of protein was calculated to be (24.21±0.40 percent) in diet 4 (both pollen and protein hydrolysate powder were added as protein supplement). The percentage of protein in diet 3 in which protein hydrolysate was added to balance the protein was

Table 2. Quantitative analysis of diet formulations

Sample Type	*Parameters Analyzed*						
	Energy (kcal/100gm)	*Protein (%age)*	*Carbohydrate (%age)*	*Fat (%age)*	*Ash (%age)*	*Moisture (%age)*	*pH*
Diet 1	310.5±2.17*	16.70±0.40	57.41±1.63	5.66±0.28	3.75±0.21	15.60±1.08	5.4
Diet 2	389.1±1.04	21.68±0.27	61.18±1.72	4.37±0.30	2.93±0.17	11.21±0.59	5.6
Diet 3	323.4±2.20	22.12±0.19	60.15±1.42	4.30±0.14	2.70±0.27	10.92±0.77	6.4
Diet 4	353.5±2.00	24.21±0.22	57.15±1.17	5.63±0.33	2.83±0.36	10.41±0.40	6.1
Diet 5	447.4±4.37	10.90±0.26	69.20±1.79	2.47±0.27	1.67±0.17	18.30±0.49	8.7
Diet 6	352.3±1.07	16.60±0.69	62.84±1.14	6.48±0.38	4.21±0.28	12.20±1.07	5.2

* Values shown in table are Mean±SE

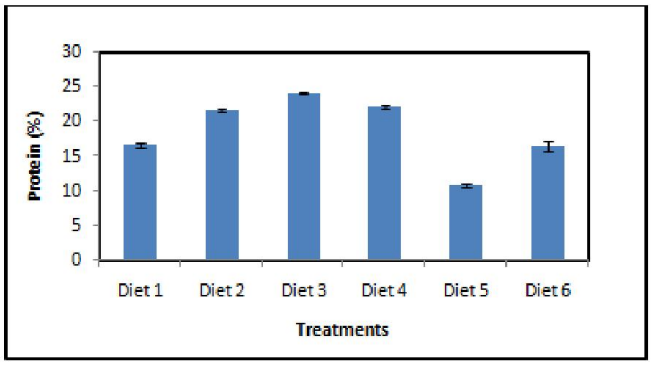

Figure 1. Total Protein in Diet Formulations

22.12±0.19 percent. The percentage of protein in Diet 2 (blue green algae-Spirulina was added as pollen substitute) was calculated to be (21.68±0.27 percent). The percentage of protein in diet 1 and diet 6 in which no natural pollen was added, was analyzed to be (16.70±0.40) and (16.60±0.69 percent) respectively. Least amount of protein (10.90±0.26 percent) was calculated in diet 5 in which Spirulina alone, was used in combination with honey (Figure 1). The percentage of carbohydrate varied from 57 to 70% (Table 2). Although carbohydrates do not play any role in the brood or egg development but is used as a source of energy by adult bees but it has been reported that feeding bee colonies with carbohydrate rich diet enhance food and honey stores of the bee colonies. The highest percentage of total carbohydrates (69.20±1.79) was analyzed in diet 5 in which 5.0 parts of honey was added as sweetener followed by (62.84±1.14) and (61.18±1.72) in diet 6 and 2 respectively. Diet 4 and Diet 1 were analyzed to have minimum carbohydrate percentage (57.15±1.17) and (57.41±1.63) respectively (Figure 2).

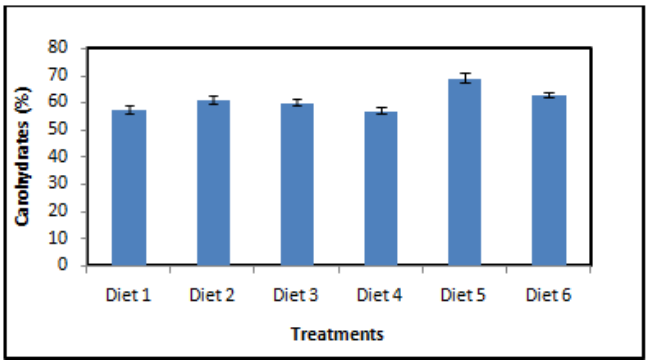

Figure 2. Total Carbohydrates in Diet Formulations

Bees require dietary lipids (fatty acids, sterols and phospholipids) in their diet as source of energy and as an essential structural component of many cell membranes (Lunden, 1954). Percentage of fat was found to be very less among all the diet. The maximum percentage of fats was calculated (6.48±0.38 percent) in diet 6 in which dried skimmed milk was added and minimum percentage (2.47±0.27 percent) was analyzed in diet 5. Diet 1, 2, 3 and 4 was found to have (5.66±0.28 percent), (4.37±0.30 percent), (4.30±0.14 percent) and (5.63±0.33 percent) respectively (Figure 3). The percentage of ash was calculated to be (3.75±0.21 percent), (2.93±0.17 percent), (2.70±0.27 percent), (2.83±0.36 percent) and (1.67±0.17 percent) and (4.2±0.28 percent) percent in the diet 1 to 6 respectively. However the ash content does not affect bee colony parameters (Figure 4). Moisture content was calculated at 105^{0}C. Total moisture content was calculated to be (15.60±1.08 percent), (11.21±0.59 percent), (10.92±0.77 percent), (10.41±0.40 percent), (18.30±0.49 percent) and (12.20±1.07 percent) in diet 1 to 6 respectively (Figure 5). It was observed that pH of all the samples varied in between 5.2 to 8.7. The maximum pH (8.7) was analyzed for diet 5 followed by (6.4), (6.19, (5.6), (5.4) for diet 3, 4, 2 and 1 respectively. The minimum pH (5.2) was analyzed for the diet 6 (Figure 6). Total energy in the formulated samples was analyzed and calculated with the help of bomb colorimeter. The maximum amount of energy (447.4 kcal/100gm) was

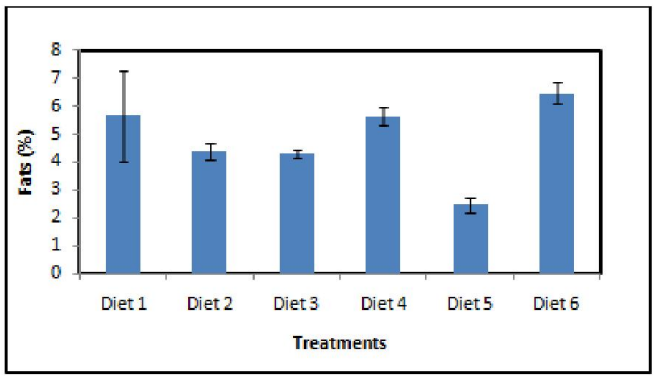

Figure 3. Total Fats in Diet Formulations

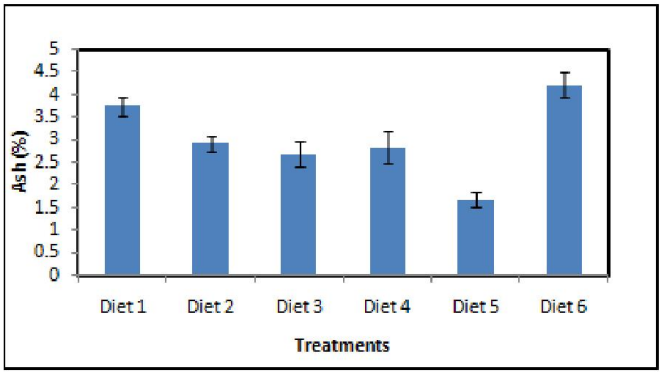

Figure 4. Total Ash in Diet Formulations

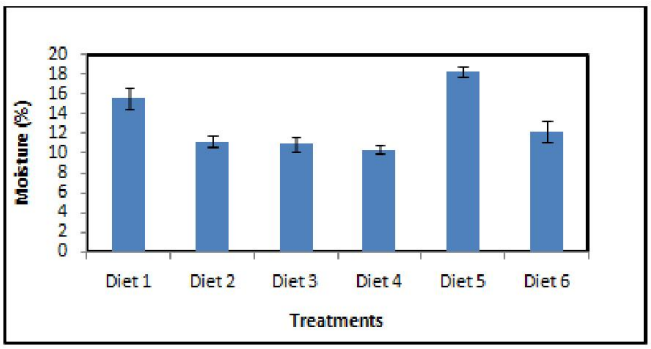

Figure 5. Total Moisture in Diet Formulations

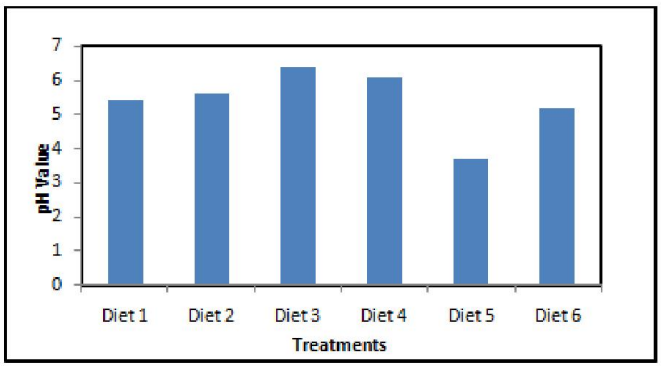

Figure 6. pH values in Diet Formulations

calculated in diet 5 whereas, all other diets were found to have almost similar amount of energy i.e. (389.1±1.04 kcal/100gm), (353.5±2.00 kcal/100gm), (352.3±1.07 kcal/100gm0, (323.4±2.20 kcal/100gm) in diet 2, 4, 6 and 3 respectively. Total Energy was calculated to be minimum (310.5±2.17 kcal/100gm) in diet 1 (Figure 7).

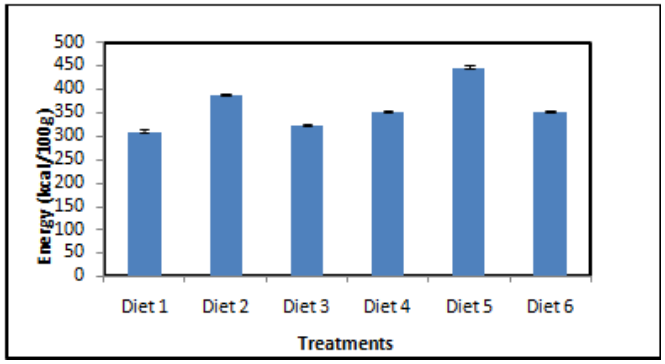

Figure 7. Total Energy in Diet Formulations

Diet no. 3 & 4 were having optimum level of protein & other nutrients required for the growth and development of honeybees. Results obtained during the study are concurrent with the findings of Standifer *et al*., 1960; Stranger and Laidlaw, 1974; Alexandru *et al*., 1977; Standifer *et al*., 1977; Peng *et al*., 1984; Shimanuki and Herbert, 1986; Abbas *et al*., 1995; Sabir *et al*., 2000; Saffari *et al*., 2004; Lakra, 2006; DeGrandi-Hoffman, 2010; Sihag and Gupta, 2011 who reported that honey bees require optimum level of protein (20-25%) & carbohydrates for their growth & development. Results obtained in case of pH are in line with that of Herbert and Shimanuki (1983a) who reported that the pollen substitutes with pH of 6.6, 5.5 and 4.7 was consumed more than that having pH less than 4.7 and above 8.0. It can be concluded at the end of study that diet no. 3 proved to be best formulation in terms of favorable biochemical (high protein and low fat contents) composition. Honeybees should be fed with balanced protein rich artificial diets during dearth periods.

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