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ANALYSIS OF ESSENTIAL AMINO ACID CONTENTS IN FORAGER BEES OF *APIS MELLIFERA* L. FED ON ARTIFICIAL DIETS

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ABSTRACT

The present study attempts to investigate the effect of some artificial diets on essential amino acids of worker bees of *Apis mellifera* L. There were no great changes in different essential amino acids contents between control bees and those fed on artificial diets. The results obtained from treated groups were quite positive as the concentration level of different essential amino acids in treated forager bees were according to the requirements of the forager bees under natural conditions. Thus the tested diets may be served to honeybee colonies during "Dearth".

INTRODUCTION

The period when no food source (bee flora) is available for honeybees for their nutritional requirements, growth of colonies and development of broods is termed as dearth. *Apis mellifera* L. was introduced in India from west where the seasonal changes are well defined and the bees have well adapted biological cycle. But this biological cycle was disrupted when the 'Italian bees' were imported to India, especially to the northern plains of India. In temperate and subtemperate climates honeybees are confined to the hives for a few months during severe winters due to low temperature and non-floral availability. On the other hand in tropical and subtropical areas summers are very harsh to honeybees [1]. Generally, no floral source is available to bees from June to August, whereas some subsistence sources may be availed by bees during September and October. As a result yet instead of reproduction and egg laying, the bee colonies stop or greatly reduce 'Brood rearing' during harsh periods of Pollen and Nectar Dearth resulting in colonies getting weak just before flows and consequently effects the production of bee products and pollination activity.

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Food shortage causes quick dwindling and even perishing of bee colonies [2] [3] [4]. In this reference a beekeeper needs a judicious feeding. The raw materials to strengthen colony stores are provided by artificial diet (pollen and nectar substitute). A colony may be short of reserves because of poor flow or when the colonies are used for pollination for longer periods. Under these conditions artificial feeding to poor bee colonies becomes quite essential to keep up their proper growth. Stimulative feeding is a normal practice, which enhances brood rearing and colonies enter the season with good strength. It is seen that in *Apis mellifera* fat bodies are greatly developed which are able to sustain 'brood rearing' during the harsh period of pollen and nectar dearth, but still these reserve are insufficient enough for effective and efficient brood rearing process [5]. So pollen along with nectar holds a great significance as the 'Honey bee diet', both for its larvae and adults. Pollen and nectar jointly are a rich source of protein, fat and carbohydrates along with essential vitamins. Pollen and nectar dearth is a disastrous factor for bee colonies as the colony strength greatly dwindles. The reason for this fluctuation is that the old deprecate bees gradually dies with a constant rate but the new eggs are not reared significantly to maintain the colony strength with no further chances of propagation. Artificial feeding has therefore, to be provided to keep

brood rearing activity continuing and for maintaining colony strength as well as regular and continuous production of bee products. A number of research articles have described the successful rearing of insects on artificial diets but the success in rearing *Hymenopterans* has been quite limited [6]. The above-mentioned work is quite encouraging and has forced us to work out on artificial diets which could not only fulfill the basic requirements of the bees as the natural feed does but economically affordable also for Indian beekeepers during the conditions of pollen and nectar dearth.

MATERIAL AND METHODS

Bee Rearing in Laboratory, Description of Diet Formulations Used and Method of Analysing The Effects of Different Artificial Diets on Bee Colonies

The bees were reared in Zoology Deptt of Govt PG College, Bisalpur using standard Langstroth cages with wax sheet foundation frame under controlled conditions. The initial bee colonies were obtained from a nearby Apiary being run by Mr. Rajesh Gangwar (Expert of Apiculture). They were acclimatized for five days in the cages before experimental tests under laboratory conditions. The cages were observed everyday regularly. The temperature and relative humidity maintained were 25-30^{o}C (± 2 ^{o}C) and 60-65 R.H., respectively. Each experimental cage was started with five frames having about 200 bees per frame i.e. 1000 bees per cage and each cage was introduced with mated queen bee. The feeding of experimental bees was stopped 5 hours before the commencement of the experiments.

The experimental bee colonies were fed on different types of protein rich and nutritive pollen and nectar substitutes (artificial diets) as shown in Table -1. 50% sugar syrup ad libitum was used as control diet (Diet-1). Gram flour, black pulse, whole egg, soya flour and partly skimmed milk powder (Amul Spray manufactured by M/s. Kaira District Co. Op. Anand-388001, India, containing important vitamins and minerals) were used as diet-2, diet-3, diet-4, diet-5 and diet-6, respectively alongwith 50% sugar syrup in 1:1 ratio (table-1). Each diet (excluding control) was added broad-spectrum antibiotic, Gentamycin (M/s. Fulford India Limited, Hyderabad, India) and multivitamin and multimineral capsule (Becadexamin, M/s. GalaxoSmithkline Pharmaceuticals Limited, Banglore, India). The capsule was used to withstand the needs of vitamins and minerals. Each capsule contained following vitamins and minerals.

Vitamins

1. Vitamin A (as concentrate oil form IP) - 5000 IU
2. Vitamin D_3 (calciferol IP)- 400 IU
3. Vitamin E (tocopheryl acetate IP)- 15 mg
4. Vitamin B_1 IP- 5 mg
1. Vitamin B_2 IP-5 mg
2. Nicotinamide-45 mg
3. D-Panthenol IP-5 mg
4. Vitamin B_6 IP-2 mg
5. Vitamin C IP-75 mg
6. Folic acid IP- 1000 µg
7. Vitamin B_{12} IP- 5 µg

Minerals

1. Dibasic calcium phosphate-70 mg
2. Copper sulphate- 0.1mg
3. Mangnese sulphate monohydrate 0.01 mg

Zinc monohydrate- 28.7 mg
Potassium iodide- 0.025 mg
Magnesium oxide- 0.15 mg

5.0% (w/w) of honey was essentially mixed to each diet to make the diets easily acceptable. The diets were provided to bee colonies through comb cells of frames. The observations were continuously made up to 10 days. The experiment was laid in a randomized block design, which consisted of six treatments replicated thrice including control. Three honeybee colonies reared in standard Langstroth cages having 8-frame capacity for each treatment were placed in test area at appropriate distance. The artificial diets as mentioned in table 1 were provided to the colonies thrice a week.

Estimation of Amino Acids

The amino acids contents were analyzed only in forager bees because most of the proteins in flying insects are due to presence of the flight muscles, of which 1/3 is mitochondrial protein [7]. Only foragers use their flight muscles for vital activities like foraging. However, the bees less than 20 days remain inside the hive and they perform a few vital role of wings for hive work. Samples of 30 forager bees were collected randomly from each of the four replicates of control and treated bee colonies (fed on artificial diet). The individuals were randomly selected for analysis from control and treated colonies. After the removal of gut contents, they were dried to constant weight under vacuum at 40 ^{0}C and their amino acid contents were determined. Duplicate analysis on foragers bees for nitrogen were performed by a micro-kjeldal method [8] and amino acids were analysed by a single-column buffer system after acid hydrolysis. The nitrogen analysis enabled the amino acid analyses to be expressed as gram per 16 gram of nitrogen. A Technicon amino acid auto analyzer (114-AAA, Technicon Instruments Limited, U.K.) was used to separate the amino acids with a modified buffer system in single columns [9] [10]. 9% cross-linking: average particle size 24µm was used as cationic resin [10]. The colour reagent was 2,4,6-trinitrobenzene sulphonic acid. The pumping rate was adjusted to 0.9 ml/min. Each chromatogram took about 10 hours to complete. The control and treated dry honeybees from different colonies were ground separately as finely as possible and individually placed in a two-necked 1-litre round bottom

flask. About 800 ml. of oxygen-free 6N HCl was added and boiled under a reflux condenser for 20 hours under a stream of nitrogen, cooled in a stream of nitrogen, and 20 ml of norleucine standard was added (norleucine standard: 0.2624 gram norleucine in 500 ml 0.1 N HCl). These analyzing samples were washed into a flask individually, made up to 1 litre and filtered (Whatman No. 54). About 25 ml of filtrate was collected and evaporated to dryness on a rotary evaporator at 40^0C; 2.5 ml N HCl was added to dissolve the residue, and it was made up to 25 ml. A portion was stored in a deep-freezer until analyzed. Amino acid analysis was performed on 1 ml of solution supplied on the top of the appropriate ion-exchange column. The effluent was pumped from the column through the heating coil to develop the colour, which was recorded as peaks on a logarithmic chart. The peaks were integrated by triangulation. The accuracy of the analytical technique was examined by analyzing 12 samples from standard mixture containing known amounts of each of the amino acids determined [10]. Norleucine was used as an internal standard. In no case the standard error of the mean colour factor for any amino acid was greater than ± 0.02. The test of significance was calculated by adopting Fisher's 't' test at $p<0.05$*, $p<0.01$** and $p< 0.001$***.

RESULT

Table 2 represents the essential amino acid analyses of forager honeybees fed on different artificial diets. An analysis of variance (ANOVA) of these observations indicated that there were no great changes in different essential amino acids contents between control bees and those fed on artificial diets. The results obtained from treated groups were quite positive as the concentration level of different essential amino acids in treated forager bees were according to the requirements of the forager bees (table 3.). The amino acids showing significant alterations over control forager bees were leucine (-10.38%*), threonine (+16.66%*) and valine (-11.66%*) for diet-2, histidine (+17.39%*), phenyl alanine (-15.15%*) and threonine (+27.77%**) for diet-3, histidine (+8.69%*), phenyl alanine (+15.15%*), threonine (+30.55%**) and valine (+13.33%*) for diet-4, arginine (+16.66%*), isoleucine (18.00%*), lysine (11.54%*), methionine (+11.54%*) and threonine (+11.11%*) for diet-5 and histidine (+13.04%*), lysine (+17.30), mathionine (+16.66%*) and threonine (+19.44%*) for diet six. From these observations, it becomes clear that all the artificial diets (diet-2 to diet-6) provided all the essential amino acids and thus good growth and development of experimental colonies took place. Therefore, all the diets may be used as nectar and pollen substitutes during the period of dearth. The foragers were morphogenetically observed quite perfect with normal wing expansion and flapping strength. These observations indicate the proper development of flight muscles in foragers, which are very essential for foraging activity. The total essential amino acid contents in forager bees fed on diet-4 and diet-6 were significantly higher ($P < 0.05$*) as compared to control group fed on plane sugar syrup.

Table 1. Combination of Control and Artificial Diets Fed to Experimental Groups of Forager *Apis mellifera* L.

Diet-1 (Control)	50% Sugar syrup
Diet-2	Gram flour + 50% sugar syrup in ratio 1: 1 by weight + Gentamycin (0.1ml/100g feed) as antibiotic + Multivitamin and multimineral capsule (1 capsule / Kg feed).
Diet-3	Ground Black pulse and sugar syrup in ratio 1: 1 + Gentamycin (0.1 ml/100g feed-Ranbaxy) as antibiotic + Multivitamin and multimineral capsule (1 capsule / Kg feed).
Diet-4	Whole Egg + 50% sugar syrup in ratio 1: 1 and + 1% Sodium bicarbonate (w/w as preservative) + Gentamycin (0.1 ml/100g feed) as antibiotic + Multivitamin capsule (1 capsule / Kg feed).
Diet-5	Partly skimmed milk powder (Amul spray) with 50% sugar syrup in ratio 1: 1 by weight + Gentamycin (0.1 ml/100g feed) as antibiotic + Multivitamin capsule (1 capsule / Kg feed).
Diet-6	Soya flour + 50% sugar syrup in ratio 1: 1+ Gentamycin (0.1 ml/100g feed) as antibiotic + Multivitamin capsule (1 capsule / Kg feed).

Table 2. Essential Amino Acid contents in Forager bees fed on artificial diets.

Sl.	Essential Amino Acids	Diet-1 (Control)	Diet-2	Diet-3	Diet-4	Diet-5	Diet-6
1	Arginine	4.2 ± 0.72 (---)	4.4 ± 0.34 (+4.76)	4.5 ± 0.39 (+7.14)	4.3 ± 0.41 (+2.38)	4.9 ± 0.58* (+16.66)	4.5 ± 0.71 (+7.14)
2	Histidine	2.3 ± 0.13 (---)	2.1 ± 0.09 (-8.69)	2.7 ± 0.19* (+17.39)	2.5 ± 0.15* (+8.69)	2.4 ± 0.23 (+ 4.34)	2.6 ± 0.31* (+13.04)
3	Isoleucine	5.0 ± 0.67 (---)	4.9 ± 0.84 (-2.00)	5.1 ± 0.79 (+2.00)	5.4 ± 0.61 (+8.00)	5.9 ± 0.46* (+18.00)	5.3 ± 0.44 (+6.00)
4	Leucine	7.7 ± 1.44 (---)	6.9 ± 0.51* (-10.38)	7.9 ± 0.36 (+2.59)	8.1 ± 0.52 (+5.19)	8.4 ± 0.75 (+9.00)	8.3 ± 0.98 (+7.79)

5	Lysine	5.2 ± 0.81 (---)	5.4 ± 0.58 (+3.85)	5.7 ± 0.16 (+9.61)	5.5 ± 0.77 (+5.76)	5.8 ± 0.65* (+11.54)	6.1 ± 0.47* (+17.30)
6	Methionine	1.8 ± 0.07 (---)	1.5 ± 0.29 (-16.66)	1.7 ± 0.43 (-5.56)	1.9 ± 0.04 (+5.56)	2.1 ± 0.37* (+16.66)	2.1 ± 0.12* (+16.66)
7	Phenylalanine	3.3 ± 0.68 (---)	3.5 ± 0.44 (+6.06)	2.8 ± 0.71* (-15.15)	3.8 ± 0.23* (+15.15)	3.1 ± 0.45 (-6.06)	3.7 ± 0.43 (+12.12)
8	Threonine	3.6 ± 0.28 (---)	4.2 ± 0.97* (+16.66)	4.6 ± 0.30** (+27.77)	4.7 ± 0.19** (+30.55)	4.0 ± 0.21* (+11.11)	4.3 ± 0.79* (+19.44)
9	Valine	6.0 ± 0.98 (---)	5.3 ± 0.91* (-11.66)	6.2 ± 0.43 (+3.33)	6.8 ± 0.84* (+13.33)	5.5 ± 0.18 (-8.33)	6.5 ± 0.87 (+8.33)
Total		39.1 ± 3.93 (---)	38.2 ± 5.44 (-2.30)	41.2 ± 1.61 (+5.37)	43.0 ± 3.09 (+9.97)*	42.1 ± 2.11 (+7.67)	43.4 ± 6.21 (+10.99)*

Each value is the mean of four replicates.
Values are expressed as mean ± S. E.
Significance at *P < 0.05, ** P < 0.01.
Values in parentheses indicate percent increase / decrease over control.

Table 3. Comparison of the Essential Amino Acid contents (g/16gN) in various pollen supplements, pollen, broods, worker bees and foragers.
[1, 7] [11]; [2] [12]; [3] [13]; [4] [14]; [5][15]; [6, 8 & 9] [16]

Sl. No.	Essential Amino Acids	Royal Jelly[1]	Soyabean Flour[2]	Casein[3]	Whole Eggs[4]	Pollens[5]	Brood[6]	Honeybee Requirments[7]	Foragers (Field Conditions)[8]	Foragers (Lab Conditions)[9]
1	Arginine	5.1	7.7	3.4	6.2	5.3	3.0	4.5	4.2	4.2
2	Histidine	2.2	2.3	2.7	2.4	2.5	1.5	1.0	2.3	2.3
3	Isoleucine	5.3	5.3	5.7	5.8	5.1	4.0	4.0	4.7	5.0
4	Leucine	7.7	8.0	8.7	9.0	7.1	4.5	3.2	7.5	7.7
5	Lysine	6.7	6.6	6.9	7.5	6.4	3.0	3.4	4.6	5.2
6	Methionine	1.9	1.4	2.8	3.3	1.9	1.5	3.0	1.3	1.8
7	Phenylalanine	4.1	5.1	4.8	4.8	4.1	2.5	2.2	3.2	3.3
8	Threonine	4.0	3.9	3.9	4.7	4.1	3.0	3.0	3.6	3.6
9	Valine	6.7	5.3	6.6	6.8	5.8	4.0	5.8	5.9	6.0
	Total	43.7	45.6	45.5	50.5	42.3	27.0	30.1	37.3	39.1

DISCUSSION AND CONCLUSION

Amino acids are one of the most important constituents of all living bodies. These are the fundamental units of all kinds of proteins found in living beings. The concentration and kinds of α-amino acids present in an animal body reflect on the basic requirement of the protein contents of that animal in the form of its diet [17]. The understanding about the essential amino acid requirements, their types and their level in honey bees with respect to the effect of the artificial diets on their level may help in developing an ideal diet for bees required at the time of dearth and to sustain the bee colonies and regular honey production [16,18]. When the amino acid contents of adult honeybee were compared with those of various pollen substitute and pollen (Table 3), the amounts of only lysine and phenylalanine were found to be much different among essential amino acids, indicating that these pollens are almost ideal food substances for worker honeybees and brood development of *Apis mellifera* L [19-21]. The results obtained in present study support their use as an ideal diet for honey bees. Thus, soybean flour, casein (skimmed milk), whole eggs, gram flour and black pulse should be almost perfect protein and minerals sources for worker honeybees and larvae development. However, in casein the amount of lysine as compared to arginine is high, and this may seriously reduce the availability of the arginine, as these amino acids compete for absorption at the same active site [22].

Therefore, lots of care is needed in any study of protein metabolism when casein is taken as a major source of amino acids The results obtained from this study are quite useful in reference to know the dietary need of the bees and the variations in diet required to maintain the proper growth of bee colonies at the time of nectar and pollen dearth.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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