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Studies on the impact of fortified diet on selected tissue substrate mobilization in *Apis cerana indica*

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Abstract
The colour of honey is characteristics of its floral source due to minerals and other minor components. Honey is classified into seven colour categories water white, extra white, white, extra light, amber, light amber and dark amber. Food fortification or enrichment is a process of adding extra nutrients to food. Fortification is the practice of deliberately increasing the content of essential quality of the food supply with minimal health risk. In this study four groups of honey bees *[Apis cerana indica [(F.)]* were fed individually with carrot extract, tocopherol, egg albumin and a mixture of the three feed materials respectively while one group was allowed normal pasturage. The cages were kept in one kilometer distance to prevent cross feeding. The feeding experiment was continued for about three months and the total protein, free amino acid and free fatty acid content of the larval, pupal and adult tissues were analyzed. Protein and free amino acid increased in the bee groups fed with albumin and mixed feed. Free amino acid and free fatty acid increased in the bee groups fed with tocopherol and mixed feed. The food commodities used for fortification were supplied along with dilute honey and it was presumed that mobilization of substrates indicated the role of the various agents of fortification in the honey bee colony.

Keywords: protein, free amino acid, free fatty acid, fortification, tocopherol, egg albumin

Introduction
Honey the natural sweet liquid produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants which the honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. It is an important food for human beings. The colour of honey is largely a reflection of plants from which the nectar was sourced and also reported that honey colour is an important consideration as it determines the value of the product and the products final destination.

The normal diet of *Apis cerana indica* (F) consists of nectar and pollen collected by worker bees and stored in the combs of the super chamber. The worker bees feed upon any available source of sweetened liquor in the vicinity of the hive. This behaviour of worker honeybees is exploited by providing artificial diet to honey bees, fortifying the diets with tocopherol, egg albumin, carrot extract separately and also with a mixture of the three food products. Artificial fortification of the diet of honeybees has already been tried [8]. The worker bees feeding on the fortified artificial diets fill their crops with the provided diet and regurgitate the contents into the food combs. The developing larvae are fed by the worker bees that draw feed materials from the fortified nutrients stored in the hive. The effect of fortification is expected to be observed in the developing larvae in which consequent mobilization of important tissue substrates is expected. Total proteins, free amino acids and free fatty acids are important substrates expected to be modified through the enhanced provisioning of proteins, complex carbohydrates and tocopherol which is fat soluble. The mobilization of these substrates may be followed to find out the effect of the food materials used for fortifying the diet. Artificial fortification is beneficial to the different castes of honey bees in a bee hive as well as to their developing life stages. It is presumed that the quality of honey will improve depending on the type of materials used for fortification. [1]. An artificial diet containing added tocopherol will lead to the inclusion of tocopherol in the honey produced, which may be considered as enriched honey. The substrates available in larger quantities will result in increased bodyweight of larva, better growth and disease resistance, higher reproductive potential and fitness. The effect of egg albumin, carrot extract and tocopherol on the total protein, free amino acids and free fatty acids in the body tissues of the developing larvae has been analyzed in this study.

Materials and methods
Five separate sets of *Apis cerana indica* colonies were used in this study.

Table 1

Group I	-	**Control.**
Group II	-	Carrot
Group III	-	Tocopherol (vitamin E) albumin
Group IV	-	Egg albumin
Group V	-	Mixture of Carrot extract, tocopherol and egg albumin.

Biochemical Studies
The whole body tissues of larva (4 day old), pupa (5 day old) and adult worker bees (1 day old) of all the five groups were used for the biochemical estimations. Protein concentration of whole body tissue was determined by the method [6]. Free amino acids concentration of body tissues was determined following the method of [7] using leucine as the standard free amino acid. Free fatty acid of body tissue was determined by following the method [3].

Results and Discussion
Protein are essential nutrients all the living organisms. They are one of the building blocks of body tissues and can also

serve as a fuel, proteins provide as much energy density as carbohydrates - 4 kcal per gram in contract, lipids provide - 9 kcal per gram. The protein level in the body tissue of *Apis cerana indica* increased with fortification. In all stages of *Apis cerana indica* the colony fortified with mixed food recorded increased protein content in the body tissues compared to the individual food items. In the control larva the total tissue protein content is 24±1.459 mg/g. In the larvae fortified with the mixed food, the protein content is 27.1±1.552 mg/g. In the larvae fed with albumin the total tissue protein content is 17.1±1.552 mg/g. Mixed food and albumin significantly increased the protein content in the body tissues. In control pupae, the total protein content is 36.8±0.994 mg/g which increased in the body tissues of pupae fed with mixed food and albumin. In mixed food treatment the total protein content in the body tissue of pupae increased by 12.82 percent compared to the control. The tissue protein of worker bees also increased significantly when fed with mixed food and albumin.

Table 2: Tissue protein content (in mg/g) in the life stages *of Apis cerana Indica* raised on different enriched feed types

Life stage	Control	carrot extract	Tocopherol	Albumen	Mixed food
Larva	24+1.459	25± l.092 (1.25)	25±1.324 (0.833)	29± 1.552 (12.917) *	29± 1.185 (15) *
Pupa	36.8±0.994	39±1.077 (0.815)	38±0.543 (0.543)	43± 1,683 (11.957) *	43± 1.416 (12.818) *
Worker	17.6+1.'265	18 ±0.58 (1.136)	19±0.642 (0.568)	21± 0.724 (15,341) *	21± +1.157 (17.614) *

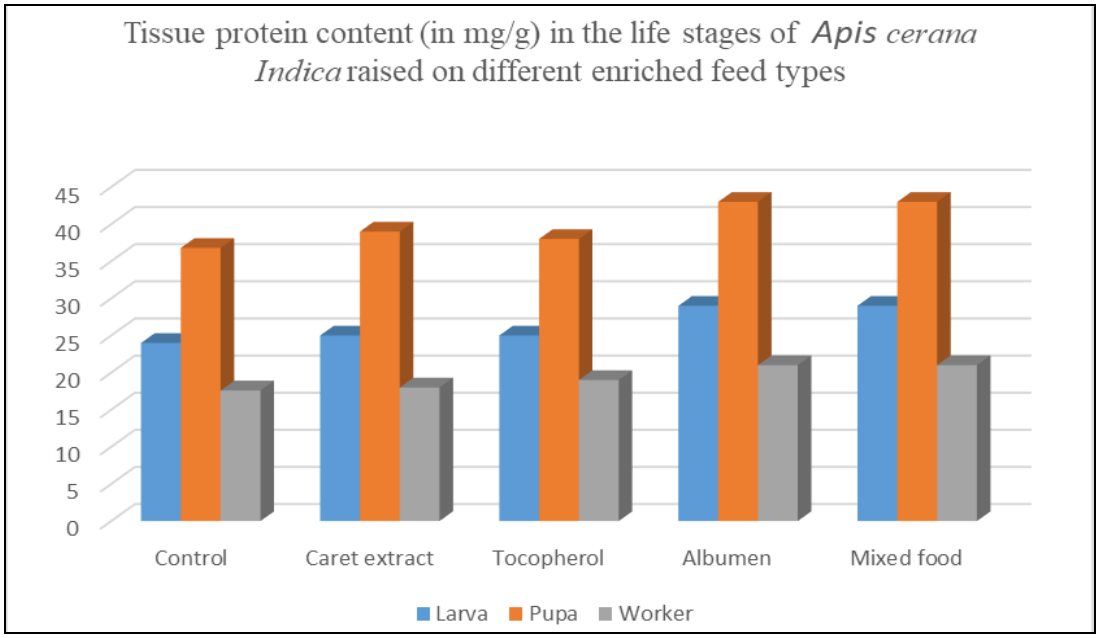


Fig 1

[2] Reported the water dispersible, substantially homogeneous artificial diet and diet formulations which provide honeybees with a fully nutritious complex mixture of proteins, carbohydrates, fats, minerals and vitamins in an easily digestible form. These diets support growth and development of honey bees sustain brood rearing and maintain hive vigor and facilitate continuous rearing of bees using an artificial diet. [4] Reported feed bee, bee-pro, pollen and acadia pod flour diets increased protein titers in the hemolymph by factor of 2.65, 2.51, 1.76 and 1.69 respectively, over protein titers in bees fed only sucrose solution.

The total free amino acid in the body tissues of *Apis cerana indica* increased with fortification treatment. In all stages of *Apis cerana indica* fortified with mixed food had More Free amino acid content in their body tissue, compared to other fortified food. The free amino acid content in larvae fed with carrot extract is 0.24 ± 0.002 mg/g, pupae, 0.14 ± 0.03 mg/ g and adult workers 0.05 ± 0.001 mg/g. In vitamin E consumed *Apis cerana indica*, the free amino acid content in larva is 0.26 ± 0.02 mg/ in pupae 0.15 ± 0.017 mg/g and in worker it is 0.05 ± 0.001 mg/g. In larvae, fed with albumin the free amino acid content is 0.357 ± 0.056 mg/g, in pupa it is 0.17 0.014 mg/g and in worker it is 0.07 0.001 mg/g. In all Stages except carrot treated pupa, a significant increase in free amino acid content is noticed in the body tissues.

Table 3: Tissue free amino acid content (in mg/g) in the life *stages of A. cerana indica raised* on different enriched feed types

Life stage	Control	carrot extract	Tocopherol	Albumen	Mixed food
Larva	0.3±0.02	0.24±0.02 (10) *	0.26±0.02 (25) *	0.359±0.06 (78.5) *	0.39±0.05 (90) *
Pupa	0.13±0.02	0.14±0.03 (8.333)	0.15±0.02 (16.667) *	0.19±0.01 (41.667) *	0.20±0.01 (58.333) *
Worker	0.04±0.01	0.05±0.01 (33.333) *	0.05±0.01 (33.533) *	0.07±0.01 (133.333) *****	0.10±0.01 (200) *

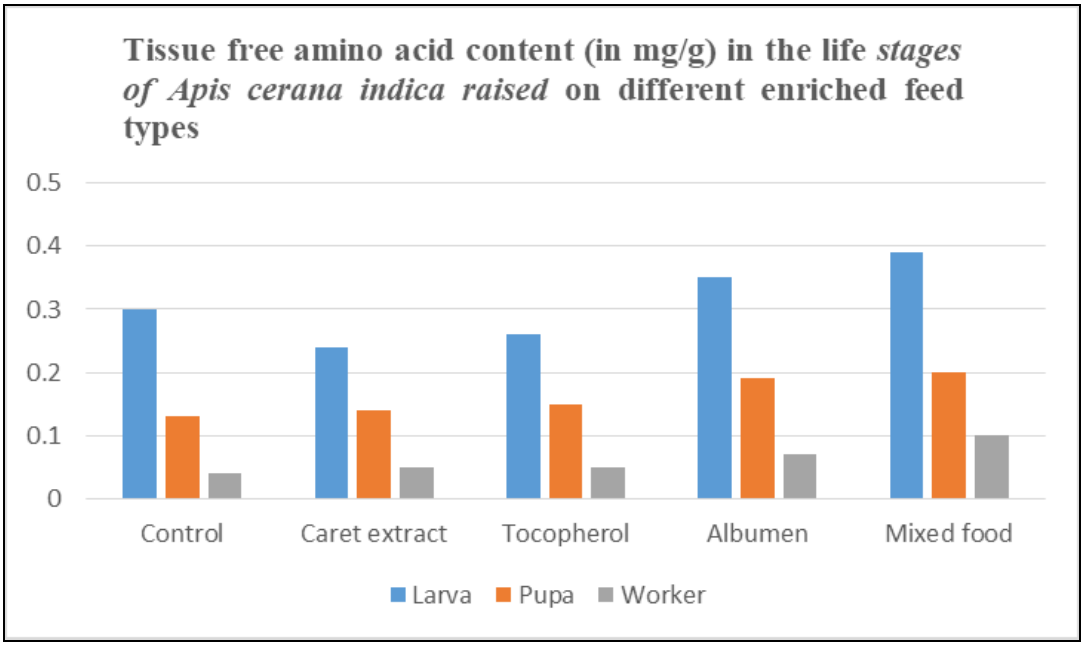
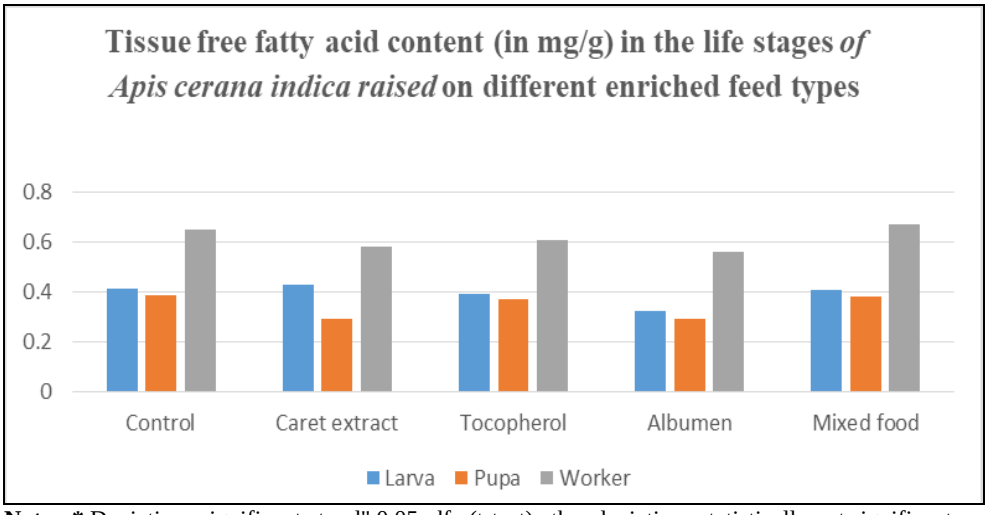


Fig 2

[9] reported amino acid and lipid spectra of larvae of *Apis cerana indica* Fabricius supplied with mustard pollen. Amino acid and lipid composition of the honey bees confined to cage mustard pollen increased significantly. Foraging on caged non-mustard plants was less effective.
[5] reported in the queen honey bees (A. mellifera L.) the free amino acid content in the hemolymph depends on the physiological and social environment of the individual. While in drones and workers the content of free amino acid increases during emergence and reaches a peak in 5-day old animals and decrease afterwards. The amino acid content increases to its highest level (760 nmol/microl hemolymph) with the onset of egg laying (10 d of age). Most probably the high amino acid concentration in the hemolymph is the basis for the high protein synthesis. In *Apis cerana indica*, vitamin E supplementation significantly increased the total free amino acid and total free fatty acid content in the body tissue in all stages of development. No significance difference was noticed in the tissue protein content.

Table 4: Tissue free fatty acid content (in mg/g) in the life stages *of Apis cerana indica raised* on different enriched feed types

Life stage	Control	carrot extract	Tocopherol	Albumen	Mixed food
Larva	0.414±0.02	0.431±0.044 (8.599)	0.39±0.051 (17.834) *	0.325±0.044 (3.503)	0.41±0.022 (24.204) *
Pupa	0.385±0.01	0.29±0.015 (0.702)	0.37±0.02 (22.807) *	0.289±0.015 (0.351)	0.381±0.016 (27.368) *
Worker	0.65±0.05	0.582±0.071 (3.818)	0.61±0.033 (9.09) *	0.561±0.049 (1.091)	0.67±0.049 (16.364) *



Note-. * Deviations significant at p d" 0.05; dfo (t-test) other deviations statistically not significant

Fig 3

[8] Reported vitamin E supplement in honey bee colonies increased cell acceptance rate and royal jelly production. They gave different colony feeding like pollen substitute (PS), pollen substitute and vitamin. E (PS + Ve), Sugar syrup (SS), Sugar syrup and vitamin E (SS +Ve) and no supplementary feed (control C). Royal jelly production (g per cell) was 0.25, 0.26, 0.23, 0.25 and 0.21; average colony yield per harvest was 32.1, 33.2, 31.0, 34.6 and 25.0 g and

the total colony production was 96.2, 99.6, 93.0, 103.9 and 75.0 g in groups PS, PS + Ve, SS, SS +Ve and C.

Conclusion

The honey product is enriched with additional ingredients, some of which include fruit extract, natural dyes, Siberian ginseng, ginger root and ginkgo, as well as spices like cinnamon, clove and cardamon. These particular ingredients were chosen to fortify the product become of studies that have shown their abilities to fight fatigue, regulate the immune system, tackle digestive issues and supply the body with antioxidants. By fortifying the diet complex of honey bee to the benefits of adult bees as well as the developing larvae and contribute to the development of colony with enhanced honey production benefits the farmer.

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