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Effect of Milk Protein Nutrition (Cow casein, Buffalo casein and Whey protein isolate (WPI) on Brood Area and Hypopharyngeal glands development in Honeybee Colonies (*Apis mellifera* L.)

Mahmoud Gomaa Darwish[1] and Salma Mohamed Galal[2]

[1]Plant Protection Department - Faculty of Agriculture - Minia University, Egypt.
[2]Dairy Science Department - Faculty of Agriculture - Minia University, Egypt.

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ABSTRACT

This study was conducted to test the addition and feeding of milk casein and whey as a source of protein for honeybee individuals and its effect on honey productivity. Honeybee colonies collect pollen for adult longevity and the brood reading. However, pollens are poor in amino acids, so it's important to add artificial protein source. In this trial, cow casein, buffalo casein and whey were tested during autumn 2021. The results showed that the mean brood area in the tested colonies fed on whey diet (128.17 inch^2) increase significantly comparing with the other diets (110.88, 100.00 and 81.38 inch^2 for buffalo casein, cow casein and control diet, respectively. In the lab, caged worker bees consumed the highest amount of whey followed by buffalo and cow casein then the control ones (4.79, 3.64, 3.59 and 2.87 mg, respectively). The area of hypopharyngeal glands showed the greatest value in the worker bees fed on whey diet (0.039 mm^2). A positive relationship was conducted between consumption of protein diets and hypopharyngeal gland surface area development.

KEYWORDS: Honey bee, casein, whey protein and hypopharyngeal

1. INTRODUCTION

Pollen and nectar are the main sources of protein and carbohydrates, respectively to honeybee (Kaushik, 2012). Brood rearing in honeybee colony required protein level of 23 to 30% (Herbert *et al.*, 1977). Protein sources differed according to colony location, floral sources and strength of colony. So, if the flora is not available, it should be provision of pollen substitute and pollen supplement (Saffari *et al.*, 2004). Hence, pollen grains are poor in amino acids, so it's necessary to add a protein source rich in amino acids to bees' food (James and Robert, 2016). Protein is considered the main source for longevity of adult and brood rearing. Doull, (1980) found that honeybee colonies fed with diet from milk powder had a positive effect on brood area, foraging activity and bee longevity. Doug, (2005) revealed that fed honey bee colonies with whey improve bees' activity. There are many useful diets provided to bees, including casein powder (Doug, 2000). Hypopharyngeal and acid glands development depend on protein level in diets (Omar, *et al.*, 2017). Hrassnigg, and Crailsheim, (1998), recorded a positive relationship between diameter of HPG and protein consumption during workers life cycle. Moreover, proteinic diets have positive relationship with hypopharyngeal gland (Al-Ghamdi *et al.*, 2011).

2. MATERIALS AND METHODS

This study was carried out in the apiary of Faulty of Agriculture, Minia University – Minia, Egypt. Also, laboratory tests were done in Faculty of Agriculture labs, Minia University in autumn 2021.

2.1. Preparing diets:

Casein (cow and buffalo) prepared by 3.2 parts by weight, whey protein isolate (WPI) 0.8 parts by weight and 10 parts of pollen by weight. All diets mixed with candy (concentrated syrup with sugar powder). Doug, (2005) and Jozef, (2007). Control workers fed on just concentrated syrup with sugar powder mixed with candy.

2.2. Brood area:

Twelve colonies of 1st hybrid Carnioilan honeybees (*Apis mellifera carnica*) colonies having equal strength, equal stored food and headed by sister queens were chosen and divided to four groups with three replicates each. The treatments were cow, buffalo casein and whey the fourth one served as

control which supplied with sugar syrup 1:1. Each colony was given 100 g of the diet from 15, Sept. till 15, Dec. 2021 and renewing weekly (Jozef, 2007). Colonies were inspected at 12-day intervals over season. Brood area was measured using wired grad frame having 1.0 square inch divisions according to method of De Jong (1976).

2.3. Diets consumption:

Lab test was carried out in wooden cages of 15×14×6 cm, glass side and other side from wire. Each cage contained 100 newly emerged honeybee workers. Diet consumption was calculated as mg for bee/3 days until 21 days old, each cage given 5 g from diet. Diets were renewing every three days with one tap water interval (Williams, *et al.* 2013). By subtracting the weight before and after, the consumption was calculated. Colonies served as control fed on just sugar solution 1:1 (w/v).

2.4. Development of hypopharyngeal gland:

Development of hypopharyngeal glands was determined in honeybee workers aged 3, 6, 9, 12 15 and 18 days by surface area according to Maurizio, (1954) method.

2.5. Cow casein, buffalo casein and whey analysis:

All cow and buffalo casein samples were analyzed in Dairy Science Department, Faculty of Agriculture, Minia University.

Table 1. Chemical analysis of tested diets (cow casein, buffalo casein and whey).

	Total protein%	Fat %	Ash %	Moisture %	pH
Cow casein	84.5	1.5	1.9	11.2	4.7
Buffalo casein	87.6	1.6	2.6	8.3	4.9
Whey	94	0.4	2.1	3.5	3.9

3. RESULTS AND DISCUSSIONS

Data in Table (2) revealed that colonies fed on whey diet reared the highest mean brood area (128.17 inch^2) while the lowest mean brood area was measured in the control colonies which showed 81.38 inch^2.
Moreover, there were significantly differences between the mean brood area in the colonies in different dates. Whereas, data in table (2) included non-significantly differences between mean brood area in 21 September, 3 October and 27 October also, 20 November and 2 December finally, between 21 September, 15 October and 27 October.

In the same time, the results illustrated in Fig. (1) showed that the whey diet placed on the top by mean brood area 133.25 inch^2 at 15 October while the bottom control at 15 December 55.67 inch$^{2.}$. This data agreed with that of Doug (2005) who found that whey in honey bee bread improve bee brood because increase protein percentage.

Table 2. Mean brood area (inch$^{2)}$ of colonies fed with cow casein, buffalo casein and whey (WPI) diets from 21 September to 15 December 2021.

Diets	Mean brood area (inch^2)				General Mean
Dates	**Cow casein**	**Buffalo casein**	**Whey**	**Control**	
21 September	121.0	142.7	154.3	100.3	129.58 ab
3 October	111.3	125.7	150.7	98.0	121.42 bc
15 October	131.3	141.3	153.3	107.0	133.25 a
27 October	126.7	128.7	148.0	106.3	127.42 ab
8 November	120.7	114.0	132.7	96.0	115.83 c
20 November	74.0	89.7	102.7	63.3	82.42 d
2 December	70.7	88.7	92.7	49.0	75.25 d
15 December	44.3	56.3	91.0	31.0	55.67 e
General mean	100.00 c	110.88 b	128.17 a	81.38 d	
L.S.D.	8.53				

*Means followed by the same letter are not significantly different

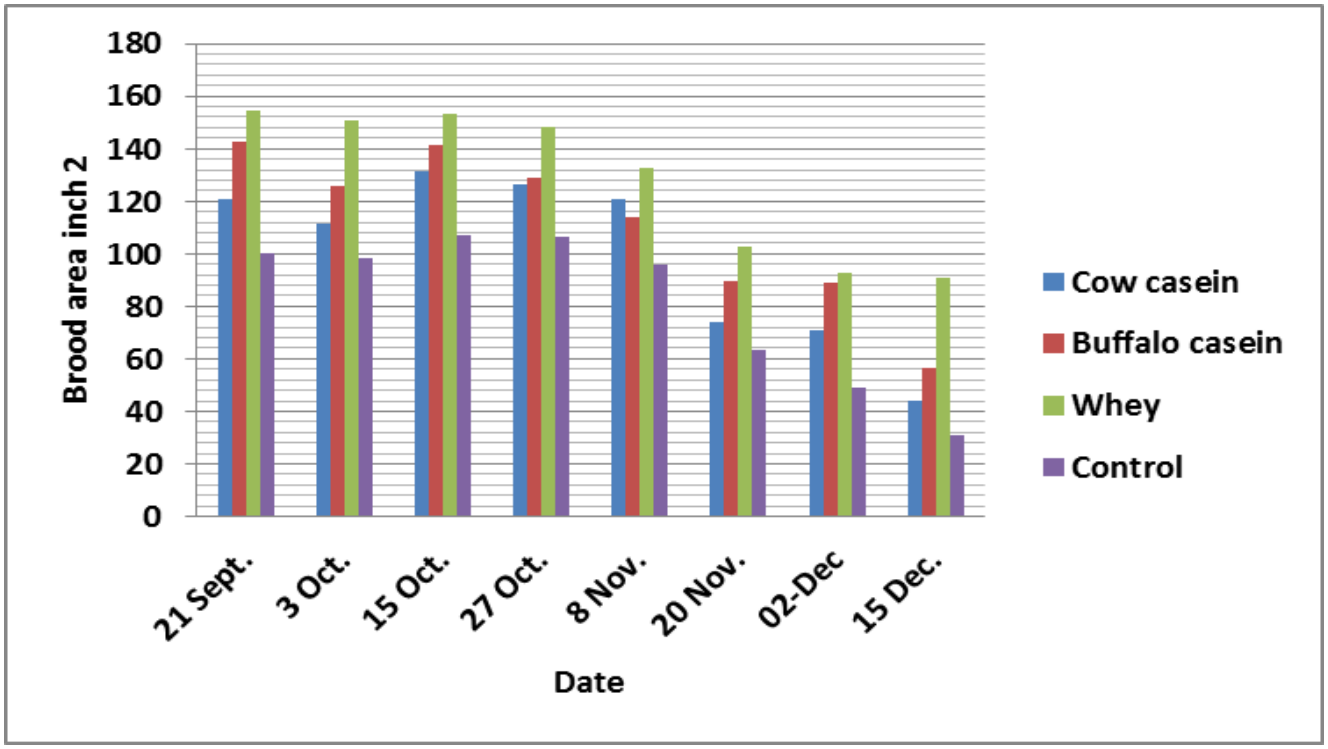

Fig. 1. Mean brood area of colonies fed with cow casein, buffalo casein and whey (WPI) diets from 21 September to 15 December 2021.

Data in Table (3) and Fig. (2) showed that there were significant differences between daily mean amounts of consumed diets (cow casein, buffalo casein, whey). Also, there were significant differences between worker ages 3, 6, 9, 12, 15, 18 days. While non-significant differences between age 18 and 21 days were recorded. On the other side the highest mean consumption of diets was recorded in whey diet by 4.79 mg followed by buffalo casein diet 3.64 mg then cow casein diet 3.59 mg and finally control diet by 2.87 mg followed by significant differences between the four diets. These data came in agreement with Madras-Majewska *et al.*, (2005) & Avni *et al.* (2009) and DeGrandi-Hoffman *et al.* (2010).

Table 3. Consumption of some diets (cow casein, buffalo casein, whey (WPI) and control) by honey bees during 21 days.

Diets / Age	Mean diet consumption (mg/bee/3days) of caged bees				General Mean
	Cow casein	Buffalo casein	Whey	Control	
3 days	2.70	2.27	4.40	2.23	2.90 e
6 days	3.27	3.50	4.60	2.57	3.48 d
9 days	3.57	3.70	4.67	2.67	3.65 c
12 days	3.60	4.03	4.93	3.03	3.90 b
15 days	3.87	3.30	5.00	3.07	3.81 b
18 days	4.03	4.23	4.97	3.30	4.13 a
21 days	4.07	4.43	5.00	3.23	4.18 a
General mean	3.59 b	3.64 b	4.79 a	2.87 c	
L.S.D.	0.137				

*Means followed by the same letter are not significantly different

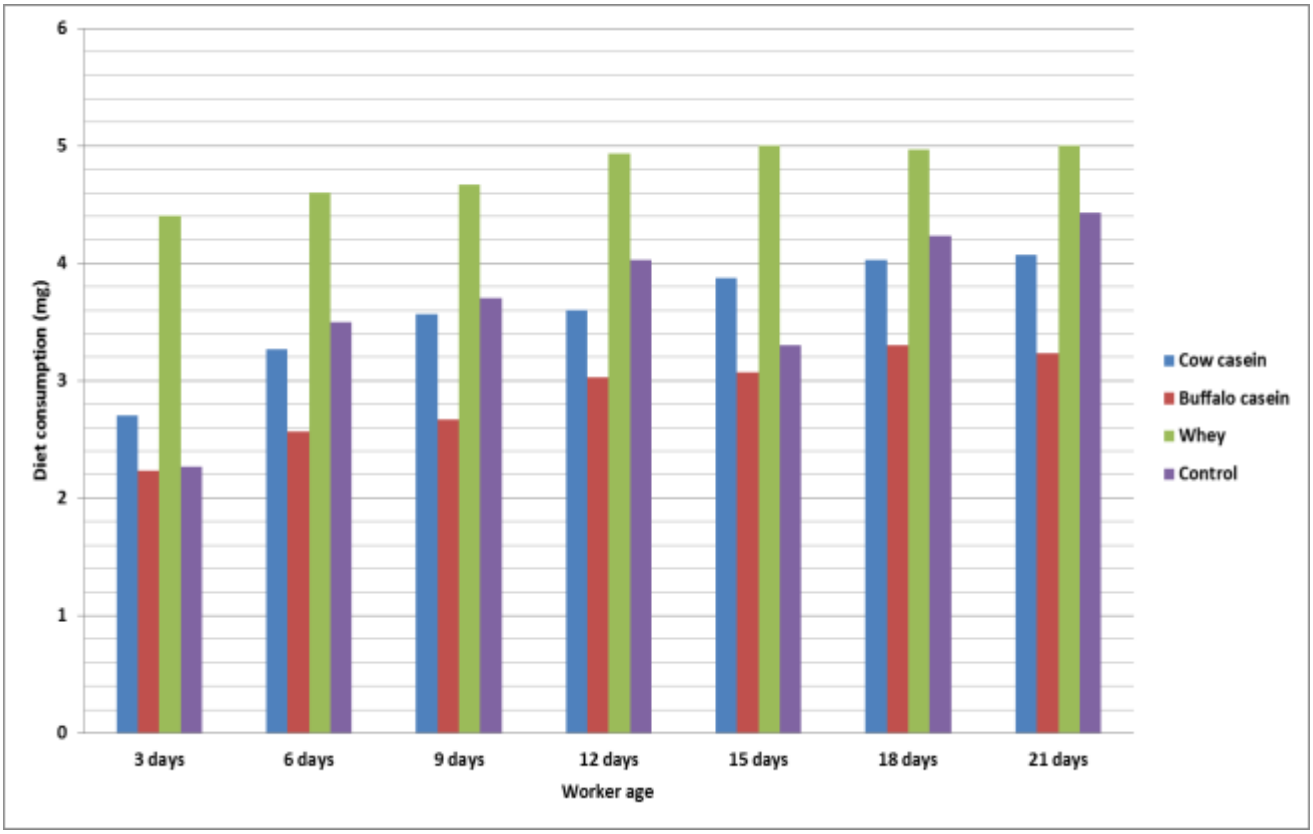

Fig. 2. Consumption of some diets (cow casein, buffalo casein and whey) by honey bees during 21 days.

Data of Table (4) and Fig. (3) showed the efficiency of the tested diets on hypopharyngeal gland development. Of the tested diets, whey diet resulted the highest mean surface area of 0.039 mm^2. Cow casein diet efficacy occupied the second (0.036 mm^2), then buffalo casein diet (0.032 mm^2). While control diet occupied the last position (0.020 mm^2).

Statistical analysis of the obtained data showed that there were significant differences among efficacy of different diets also, between workers age.

These results were in agreement with Al-Ghamdi *et al*. (2011) who found that positive relationship between consumption of proteinic diets and hypopharyngeal gland surface area development.

Table 4. Effect of fed honeybee workers with cow casein, buffalo casein and whey diets on surface area of hypopharyngeal gland.

Diets	**Mean hypopharyngeal gland surface area (mm^2)**				**General Mean**
Age	**Cow casein**	**Buffalo casein**	**Whey**	**Control**	
3 days	0.028	0.025	0.029	0.015	0.0243 c
6 days	0.045	0.040	0.049	0.023	0.0393 b
9 days	0.045	0.042	0.051	0.029	0.0417 a
12 days	0.045	0.042	0.046	0.024	0.0393 b
15 days	0.025	0.022	0.037	0.017	0.0253 c
18 days	0.026	0.021	0.024	0.013	0.0211 d
General mean	0.036 b	0.032 c	0.039 a	0.020 d	
L.S.D.	0.002				

*Means followed by the same letter are not significantly different

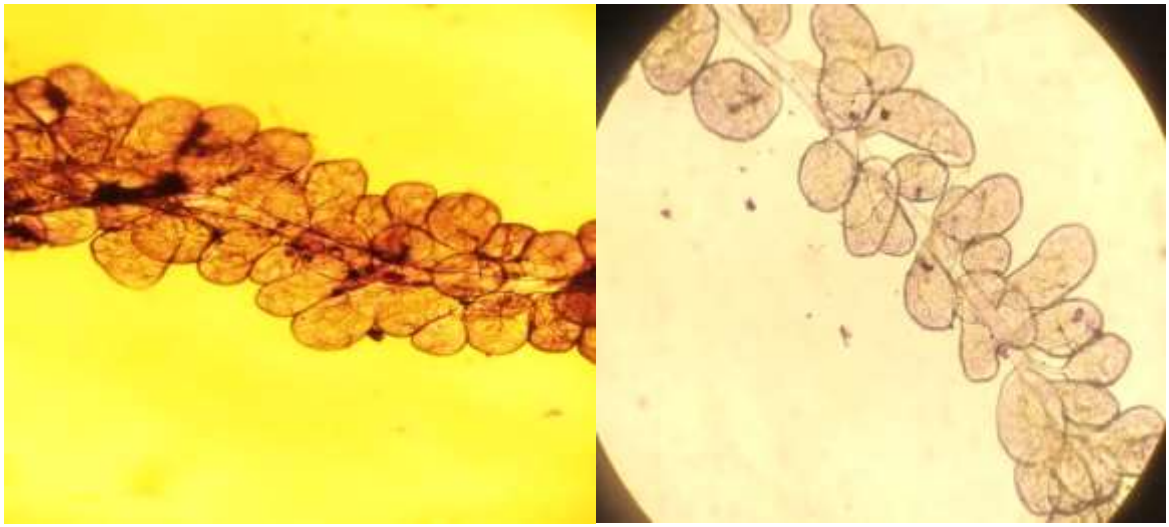

Photo 1. Hypopharyngeal glands of the nurse bees

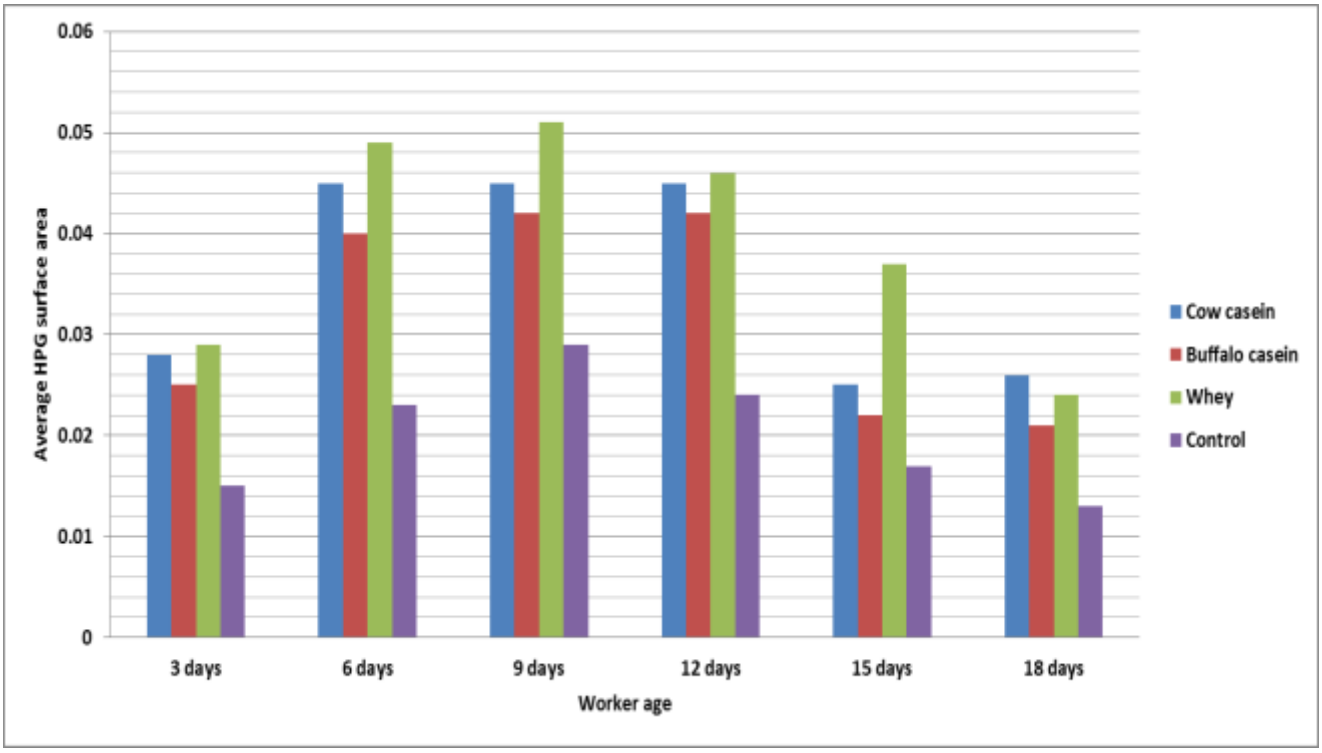

Fig. 3. Effect of fed honeybee workers with of some diets (cow casein, buffalo casein and whey) on surface area of hypopharyngeal gland.

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الملخص العربي

تأثير التغذية ببروتين اللب (كازين البقر ، كازين الجاموس كازين وبروتين الشرش) على مساحة الحضنة وتطور الغدد البلعومية في طوائف نحل العسل

محمود جمعة درويش[١] و سلمى محمد جلال[٢]

[١]قسم وقاية النبات – كلية الزراعة – جامعة المنيا

[٢]قسم الالبان – كلية الزراعة – جامعة المنيا

تجمع طوائف نحل العسل حبوب اللقاح لأنها تؤثر على طول عمر النحل البالغ ومساحة الحضنة المرباة بالرغم من أن حبوب اللقاح فقيرة فى الأحماض الأمينية ، لذلك من المهم إضافة مصدر بروتين صناعي . في هذه التجربة ، تم اختبار كازين البقر والجاموس وبروتين الشرش خلال خريف ٢٠٢١. وأظهرت النتائج أن متوسط مساحة الحضنة في الطوائف المختبرة التي تم تغذيتها على وجبة بروتين الشرش بلغت ١٢٨.١٧ بوصة مربعة حيث ازدادت بشكل ملحوظ مقارنة بالوجبات الأخرى التى سجلت ١١٠.٨٨ و ١٠٠.٠٠ و ٨١.٣٨ بوصة مربعة لكازين الجاموس وكازين البقر والكنترول على التوالي . استهلك النحل المعامل في الأقفاص في المعمل أكبر كمية من بروتين الشرش يليه كازين الجاموس ثم كازين الأبقار ثم الكنترول (٤.٧٩ ، ٣.٦٤ ، ٣.٥٩ ، ٢.٨٧ مجم ، على التوالي). أظهرت مساحة الغدد البلعومية أكبر قيمة في النحل الكامل الذي تغذى على بروتين الشرش (٠.٠٣٩ مم^{٢}).

الكلمات المفتاحية: عسل النحل – الكازين – بروتين الشرش – الغدد البلعومية