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Impact of Protexin® on digestibility of corn starch by honey bee, *Apis mellifera* (Hymenoptera: Apidae)

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Abstract

This study was conducted to investigate the ability of bees to consume starch as a substitute for nectar and influence a commercial probiotic namely Protexin® on starch absorption. In the first experiment, 36 honey bee hives were randomly allocated into six similar groups and fed using one of the diets, first group received sugar syrup as control treatment, second group received sugar syrup supplemented with 1 g/L of Protexin® (P), third group received sugar syrup supplemented with 10% of the starch (S10), fourth group fed using sugar syrup supplemented with 20% of the starch (S20), fifth group received sugar syrup supplemented with 10% starch and one g/L of Protexin® (S10P) and sixth group fed using sugar syrup supplemented with 20% of starch and one g/L of Protexin® (S20P). In the second experiment, newly emerged worker bees were kept in laboratory cages and fed using the above-mentioned experimental treatments for 21 days (at 34 ± 1 ˚C and 50% R.H.). At the end of both experiments, 100 worker bees from each treatment were selected to evaluate the starch absorption, the microbial population at the bee's digestive tract, body weight, body protein, and lipid content. The results indicated that the starch absorption in the colonies fed by S20P treatment was significantly higher than that in the rest of the treated colonies ($P \leq 0.05$). The supplementation of diet with starch significantly enhanced their body weight, protein, and lipid content in both of the experiments ($P \leq 0.05$). Moreover, Protexin® increased the bee's gut microbial population at colony and cage conditions ($P \leq 0.05$). It is concluded that the dietary supplementation of the corn starch and Protexin® could have a beneficial effect on the health and strength of the bee colonies.

Key words: Microbial population, Probiotic, Starch absorption, Worker bees.

اثر پروتکسین بر قابلیت هضم نشاسته ذرت توسط زنبور عسل، *Apis melifera* (Hymenoptera: Apidae)

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چکیده

این پژوهش به منظور بررسی توان زنبور عسل در مصرف نشاسته به عنوان جایگزین شهد و اثر پروبیوتیک تجاری پروتکسین بر قابلیت هضم نشاسته در دستگاه گوارش زنبور عسل انجام شد. در آزمایش اول تعداد ۳٦ کندو به طور تصادفی در شش گروه همسان قرار گرفتند و هر گروه یکی از جیره‌های آزمایشی، تیمار اول جیره فقط بر پایه شربت شکر (گروه شاهد)، تیمار دوم جیره پایه حاوی یک گرم بر لیتر پروتکسین، گروه سوم جیره پایه حاوی ۱۰ درصد نشاسته، گروه چهارم جیره پایه حاوی ۲۰ درصد نشاسته (S20)، گروه پنجم جیره پایه حاوی ۱۰ درصد نشاسته و یک گرم بر لیتر پروتکسین (S10P) و گروه ششم جیره پایه حاوی ۲۰ درصد نشاسته و یک گرم بر لیتر پروتکسین (S20P) را دریافت کردند. در آزمایش دوم زنبورهای تازه ظاهر شده در شش گروه در داخل قفس‌هایی در انکوباتور قرار داده شده و تیمارهای اشاره شدهٔ بالا را به

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مدت ۲۱ روز دریافت کردند (در دمای ۱ ± ۳٤ درجه سلسیوس و رطوبت نسبی ۵۰ درصد). در پایان هر دو آزمایش تعداد ۱۰۰ عدد زنبور کارگر از هر تیمار انتخاب و از نظر میزان جذب نشاسته، وزن خشک، جمعیت میکروبی بخش‌های مختلف دستگاه گوارش، غلظت پروتئین و چربی بدن آن‌ها ارزیابی شدند. نتایج این آزمایش نشان داد، پروتکسین به طور معنی‌داری جذب نشاسته را در گروه S20P نسبت به گروه‌های دیگر افزایش داد ($P\leq0.05$). همچنین حضور نشاسته در جیره زنبورها سبب افزایش معنی‌دار وزن خشک، غلظت پروتئین و چربی بدن زنبورها شد ($P\leq0.05$). پروتکسین جمعیت میکروبی را در بخش‌های مختلف دستگاه گوارش زنبورها افزایش داد ($P\leq0.05$). بر اساس نتایج این آزمایش به نظر می‌رسد افزودن همزمان نشاسته و پروتکسین می‌تواند تاثیر مثبتی بر سلامتی و قدرت کلنی داشته باشد.

واژه‌های کلیدی: پروبیوتیک، جذب نشاسته، جمعیت میکروبی، زنبورهای کارگر.

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Introduction

Honey bees are the most important pollinators of many crop plants which indirectly contribute to the diversity and availability of the human diet (Eilers *et al*., 2011). In addition, bee products such as honey and royal jelly are directly used as human dietary supplements due to their high nutritional values and antimicrobial properties. Therefore, bee's activities prevent the further risk of nutrient deficiencies for humankind (Ellis *et al*., 2015). Nutrition has a crucial role in honey bee colonies fitness, performance, and immunity (Brodschneider & Crailsheim, 2010; Papežíková *et al*., 2019). Like all other animals, bees require amino acids, lipids, carbohydrates, minerals, vitamins, and water to fulfill their nutritional requirements. It is reported that pollen is the main source of amino acids and lipids while honeydew or nectar is the major source of carbohydrates (Brodschneider *et al*., 2019). These factors are very crucial for colony performance, health, and survival and thus any inadequacy of these major nutrients, would negatively affect the colonies growth and development (Brodschneider & Crailsheim, 2010) which may lead to the colony starve as often happens in late winter or early spring (Somerville, 2000).

In colony conditions, the produced honey is harvest by the beekeepers and the colonies nutritional requirements will supply by replacing the supplemental food (Papežíková *et al*., 2019). Bee colonies should have at least 5 kg of permanent carbohydrates supply for overwintering (Naug & Gibbs, 2009). Therefore, provision of the bee's nutritional requirements especially the high-quality saccharide, can strongly grant the colony overwintering success, fitness, and productivity over the next season (Semkiw & Skubida, 2016). The feed supplements should have an adequate amount of amino acids and carbohydrates to keep the colony alive and growing (Somerville, 2000). Thus, the question arises if and how bees should be provided with supplemental food when nutritional deficits occur (Brodschneider & Crailsheim, 2010).

To supply winter food, beekeepers can use sugar syrup (Gemeda *et al*., 2018), sucrose inverted syrup, starch syrup (Von der Ohe & Schönberger, 2002; Semkiw & Skubida, 2016) or high-fructose corn syrup (HFCS) (Sammataro & Weiss, 2013). Honeybees fed by sugar

syrup (a combination of water and pure sucrose from sugar beets or sugar cane) for many years (Free & Spencer-Booth, 1961; Barker & Lehner, 1978). Sugar syrup showed to be very beneficial for the colony, however, the use of granulated sugar has some disadvantages such as the higher required amount colonies feeding, relatively high cost (Semkiw & Skubida, 2016), and the risk of fermentation during long-term storage (Goodwin, 1997; Sammataro & Weiss, 2013). Thus, it is necessary to find a suitable alternative for sugar syrup, especially at relatively lower prices.

The high-fructose corn syrup (HFCS) and starch syrup were reported to have a lower price in comparison to the sucrose in the North America (Hanover & White, 1993; Sammataro & Weiss, 2013) and Western Europe (Von der Ohe & Schönberger, 2002). Despite the starch is a natural component of pollen, it is not a major component of the bee's diet (Linskens & Jorde, 1997). It is reported that each bee colonies consume an average of 20 kg of pollen and 60 kg of honey in a year (Seeley *et al*., 1991). On the other hand, some pollens have more than 10% of starch content (Linskens & Jorde, 1997). Thus, the starch can be a potential replacement of sugar syrup while it is a part of the bee's normal diet. With this background, Semkiw & Skubida (2016) fed the colonies with a different source of starch and compared them with normal sugar syrup for their effect on colonies overwintering. They further concluded that the treatments did not showed significant differences in bee's mortality, food consumption, colony strength, brood area, and honey yield during overwintering. Thus, they suggested that the different starch sources can be used as an alternative for sugar syrup for bee colonies feeding.

Honey bees gut microbial population has become appreciated recently (Alberoni *et al*., 2018; Mortensen *et al*., 2019). Balanced gut microbiota is necessarily associated with bee health since it provides countless enzymatic activities to break down the complex sugars of the honey bee's diet (Alberoni *et al*., 2018; Di Gioia & Biavati, 2018). Gut symbiotic are persistently included within the bioconversion and preservation of pollen material, nectar, honey, and beebread. Moreover, Vásquez & Olofsson (2009) suggested that the fermentation process of beebread in the honeybee stomach by lactic acid bacteria (LAB) led to improving its nutritive value by vitamin production. Furthermore, according to Di Gioia & Biavati (2018), some probiotics have an important impact on different sugars metabolization in the bee's gut.

Protexin® is a probiotic containing five species of beneficial bacteria (*Lactobacillus acidophilus* (Moro), *Lactobacillus plantarum* (Orla-Jensen), *Bifidobacterium bifidum* (Orla-Jensen), *Enterococcus faecium* (Orla-Jensen), *Lactobacillus rhamnoses* (Hansen)) and two species of fungi (*Aspergillus oryzae* (Ahlburg) and *Candida pintolopesii* (Berkh)) (Azadegan-mehr *et al*., 2007; Borges, 2015). This product protects the bee colonies against *Nosema ceranae* by improving the gut microbiota condition (Klassen, 2018). It is also reported that Protexin® is able to significantly increase the adult bee population and the

worker bee's life span (Borges, 2015). To date, there are a few studies in feeding of the honey bee colonies with complex carbohydrates like starch and the effect of probiotics on their digestibility. This study was therefore aimed to investigate whether bee colonies can use the starch as a low-cost replacement of honey or sugar syrup and how Protexin® can influence the starch breakdown digestibility or absorption by honey bees either in colony or cage conditions.

Materials and Methods

Reagents and Chemicals

Protexin® was purchased from Nicotech Company (a local branch of Probiotics International Ltd, London, UK) and corn starch powder was provided by Glucosan Company (Karaj, Iran). All other chemicals were obtained from Sigma-Aldrich unless indicated.

Experimental Design

First experiment

The first experiment was conducted in the experimental farm of the department of animal science, University of Tehran, Karaj, Iran from 7th October to 22th November, 2018. Though, 36 colonies (*Apis mellifera* L.), without symptoms of clinical disease were kept in wooden beehives (frame size 43.5 cm x 30.0 cm) in the farm. The colonies were randomly allocated within the six experimental groups. The first group which considered as control and only received sugar syrup (50% sugar dissolved in water) as a basal diet, the second group received basal diet supplemented with 1 g/L of Protexin® (P), the third group received basal diet supplemented with 10% of the starch (S10), the fourth group received basal diet supplemented with 20% of the starch (S20), the fifth group received basal diet supplemented with 10% of the starch and one g/L of the Protoxin® (S10P), and the sixth group fed by basal diet which supplemented with 20% of the starch and one g/L of the Protoxin® (S20P). All of the experimental groups received 500 mL of the prepared treatments with one day time interval for 45 days. At the end of the experiment, 100 worker bees from each treatment were selected for evaluating the starch digestibility and absorption, gut microbial population, body weight, and body protein and lipid content.

Second experiment

In the second experiment, the newly emerged bees were incubated at 34.0±1.0 ˚C and 50% R.H.. Bees were fed on the above mentioned experimental treatments for 21 days. This experiment was conducted to prevent bees to use pollen as the major natural source of the starch. Five cages with at least 200 bees were used for each of the examined treatments. At the end of the experiment, at least 100 bees from each treatment were selected to evaluate the starch digestibility and absorption, gut microbial population, body weight, body protein and lipid content.

Assessment of starch digestion

The starch concentration was assessed using the anthrone-sulfuric acid colorimetrical method (Rose *et al*., 1991; Chow & Landhäusser, 2004). Briefly, the selected bee's gut was removed and their foregut, midgut, and hindgut were separated to extract the digesta. Then the samples of each section in each treatment were pooled, dissolved in ethanol (60 ˚C) and centrifuged (for 10 min at 4500 rpm) to exclude soluble carbohydrates. After centrifugation, the supernatant was removed and the pellets were dissolved in a mixture of distilled water (5 mL) and perchloric acid (6.5 mL, 52%, $HClO_4$) and were kept in the refrigerator (at temperature 0 ˚C) for next 20 min. Then the samples were re-centrifuged (for 10 min at 4500 rpm) and the supernatant was used for further assessment. Next 200 µL of the supernatant was mixed with 4 mL of anthrone-sulfuric acid solution (200 mg anthrone dissolved in 200 mL of sulfuric acid) and incubated at 60 ˚C for further eight minutes. After quick cooling, the starch concentration was assessed via a spectrophotometer (UV-1200, Shimadzu, Japan) at 630 nm. The difference in starch concentration between the foregut and midgut have considered as absorbed starch.

Assessment of bee's body composition

Body weight

In order to assess the dry body weight, at least 50 worker bees were removed from the hives or cages and dried in the incubator (at 85 ˚C for 48 hours). The weighing was conducted using a digital scale (GF600, A&D, Japan).

Body crude protein and lipid content

Dried bees (n=50) were powdered and mixed to provide a homogenous sample to assess the crude protein (CP) and crude lipid content. Body crude protein and crude lipids were assessed according to the AOAC method (Latimer Jr, 2016).

Assessment of gut microbial population

The microbial population of digesta from a different section of the bee's gut was measured using optical density (OD) measurements based on (Patton *et al*., 2006) with a slight modification. For this purpose, 200 µL of digesta sample was dissolved in 800 µL of Lysogeny Broth (LB) medium and OD was determined using a spectrophotometer at 620 nm (*T0*). The samples were incubated in dark for 24 hours in a shaker at 100 rpm. After 24 hours the samples were read again at 620 nm using the spectrophotometer (T24). The OD for each replicate at T0 was subtracted from the OD for each replicate at T24 and the highest value represents the higher microbial population in the sample.

Statistical analysis

The GLM procedure was applied for data analysis by using SAS software (SAS Institute Version 9.4). The data were checked for normality using Shapiro–Wilk test. The results were expressed as means ± SE and Duncan's multiple range test used for statistically grouping the means at ≤0.05 probability level.

Results

First experiment

The effect of Protexin® on starch digestion and absorption at colony conditions is resented in Table 1.

The results of this experiment indicated that S20P and S20 treatments caused significantly higher starch concentration in bee's foregut and hindgut ($P \leq 0.05$). Moreover, the result presented in Table 1 shows that Protexin® significantly ($P \leq 0.05$) increased starch absorption in the bees fed by S20P in comparison with the other groups (Table 1). The S10P group showed higher starch absorption than S10 and the ($P \leq 0.05$, Table 1).

The effect of starch and Protexin® on dry weight, body protein and lipid content are presented in Figures 1-3. Supplementation of the diet with starch could significantly ($P \leq 0.05$) enhanced bee's body weight and body protein content. Moreover, the S10P and S20P showed significantly ($P \leq 0.05$) higher body weight, body protein and lipid content in comparison with control, P, and S10 groups. The group P increased significantly the body weight ($P \leq 0.05$), but did not affect body protein and lipid content ($P \geq 0.05$) in comparison with the control.

Table 1. Starch concentration in different section of bee's gut and the effect of Protexin® on starch absorption at colony condition (Mean ± SE).

Starch concentration	Treatments					
	Control	P	S10	S20	S10P	S20P
Foregut (mg/g)	23.21 ± 1.36^{e}	32.60 ± 4.05^{e}	134.2 ± 11.10^{d}	291.63 ± 4.96^{a}	186.52 ± 12.12^{c}	239.30 ± 5.35^{b}
Midgut (mg/g)	15.56 ± 0.94^{d}	21.96 ± 2.40^{d}	87.01 ± 9.00^{c}	189.20 ± 6.65^{a}	110.73 ± 9.04^{b}	113.06 ± 5.78^{b}
Hindgut (mg/g)	31.35 ± 2.12^{e}	43.75 ± 4.81^{e}	163.37 ± 12.39^{c}	270.50 ± 11.13^{a}	136.67 ± 7.58^{d}	244.77 ± 9.12^{b}
Starch Absorption (mg)	7.65 ± 0.42^{e}	10.64 ± 1.66^{e}	47.18 ± 5.38^{d}	102.43 ± 3.23^{b}	75.79 ± 5.15^{c}	126.23 ± 9.32^{a}

P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one g/L Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one g/L Protexin®.

Different superscripts show significant differences among treatments ($P \leq 0.05$).

Table 2. Starch concentration in different sections of bee's gut and the effect of Protexin® on starch absorption in cage condition (Mean ± SE)

Starch concentration	Treatments					
	Control	P	S10	S20	S10P	S20P
Foregut (mg/g)	2.05 ± 0.31^{c}	1.92 ± 0.44^{c}	133.20 ± 7.95^{b}	223.67 ± 10.00^{a}	142.43 ± 3.91^{b}	233.43 ± 7.35^{a}
Midgut (mg/g)	1.86 ± 0.25^{d}	1.69 ± 0.29^{d}	76.90 ± 6.47^{c}	176.63 ± 2.79^{a}	81.00 ± 1.73^{c}	161.50 ± 13.43^{b}
Hindgut (mg/g)	3.56 ± 0.61^{d}	3.78 ± 0.48^{d}	162.00 ± 13.30^{c}	269.73 ± 15.29^{a}	143.10 ± 12.05^{c}	244.16 ± 13.34^{b}
Starch Absorption (mg)	0.38 ± 0.08^{d}	0.24 ± 0.17^{d}	56.29 ± 3.01^{b}	47.03 ± 7.79^{c}	53.76 ± 2.76^{bc}	71.93 ± 6.63^{a}

P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one mg/L of Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one mg/L of Protexin®.

Columns with different superscript indicate differences ($P \leq 0.05$).

a, b: Different superscripts show significant differences among treatments ($P \leq 0.05$).

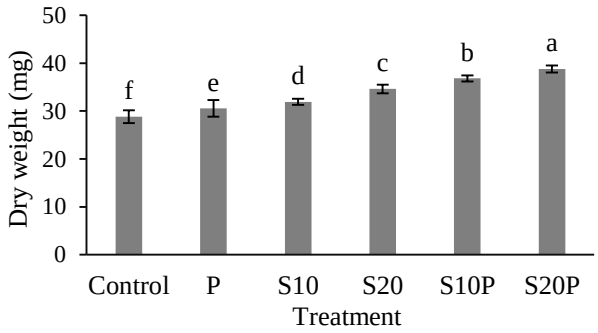

Fig. 1. The effect of starch feeding and Protexin® supplementation on the dry weight of worker bees under hive condition (Mean ± SE).

P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one g/L Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one g/L Protexin®.
Columns with different superscript indicate significant differences ($P \leq 0.05$).

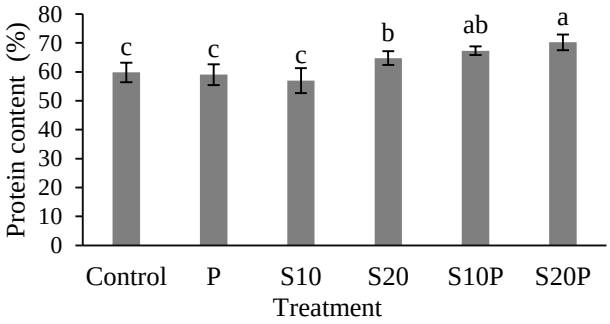

Fig. 2. The effect of starch feeding and Protexin® supplementation on the body protein content of worker bees under hive condition (Mean ± SE).

P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one g/L Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one g/L Protexin®.
Columns with different superscript indicate significant differences ($P \leq 0.05$)

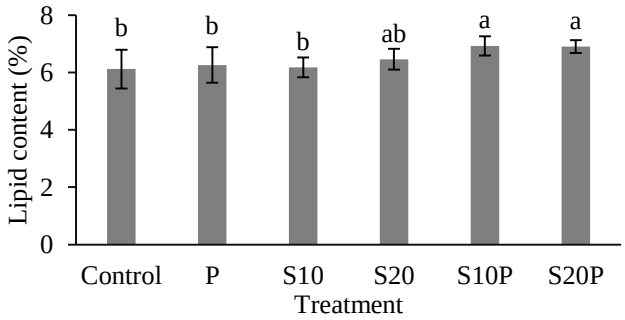

Fig. 3. The effect of starch feeding and Protexin® supplementation on the body lipid content of worker bees under hive condition (Mean ± SE).

P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one g/L Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one g/L Protexin®.
Columns with different superscript indicate significant differences ($P \leq 0.05$).

The results of the effect of Protexin® on bees gut microbial population at colony condition are presented in Figure 4. Protexin® significantly increased microbial population in bee's gut ($P \leq 0.05$). However, the starch did not affect the microbial population ($P \geq 0.05$, Figure 4).

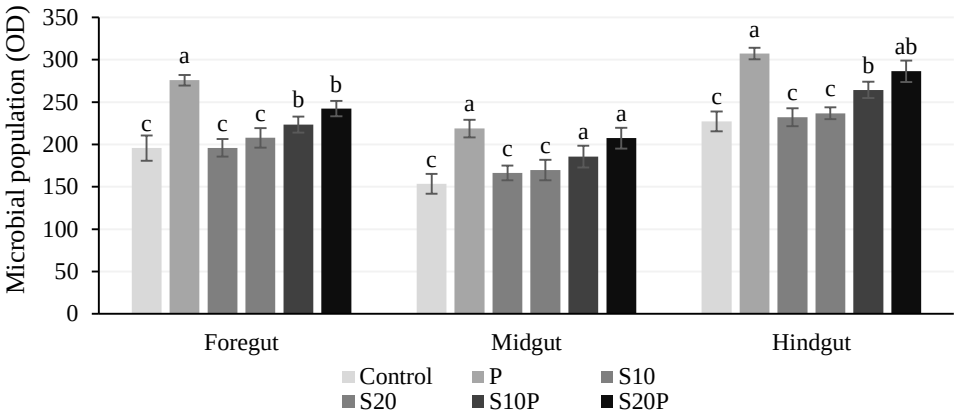

Fig. 4. The effect of starch feeding and Protexin® supplementation on the microbial population of worker bee's gut under hive condition (Mean ± SE).

OD: optical density, P: the group which received one g/L Protexin® probiotic, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one mg/L of Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one mg/L of Protexin®.
Columns with different superscript indicate differences ($P \leq 0.05$).

Second experiment

The effect of Protexin® on starch digestion and absorption under cage conditions are presented in Table 2.

The effect of starch feeding and Protexin® supplementation on dry weight, body protein and lipid content of the worker bees under cage condition are presented in Figures 5 to 7. The dietary starch supplementation significantly enhanced bee's body weight and body protein content ($P \leq 0.05$). Moreover, the bees treated with S10P1, S20P1, and S20 showed higher body weight and body lipid content ($P \leq 0.05$). The treatment P significantly increased the body protein content ($P \leq 0.05$), but did not affect body weight and lipid content ($P \geq 0.05$) as compared to the control.

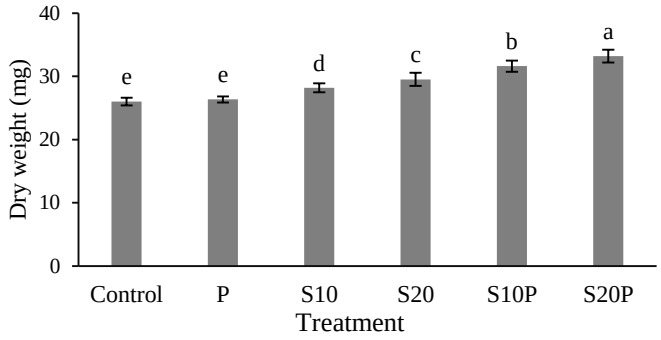

Fig. 5. The effect of starch feeding and Protexin® supplementation on the dry weight of worker bees under cage condition (Mean ± SE).
P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed by a diet supplemented with 10 % of starch and one mg/L of Protexin®, S20P: the group which fed by a diet supplemented with 20 % of starch and one mg/L of Protexin®.
Columns with different superscript indicate differences ($P \leq 0.05$).

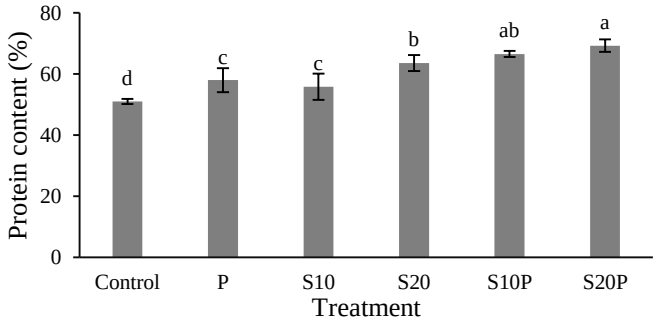

Fig. 6. The effect of starch feeding and Protexin® supplementation on the body protein content of worker bees under cage condition (Mean ± SE).
P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed by a diet supplemented with 10 % of starch, S20: the group which fed by a diet supplemented with 20 % of starch, S10P: the group which fed with a diet supplemented with 10 % of starch and one mg/L of Protexin®, S20P: the group which fed with a diet supplemented with 20 % of starch and one mg/L of Protexin®.
Columns with different superscript indicate differences ($P \leq 0.05$).

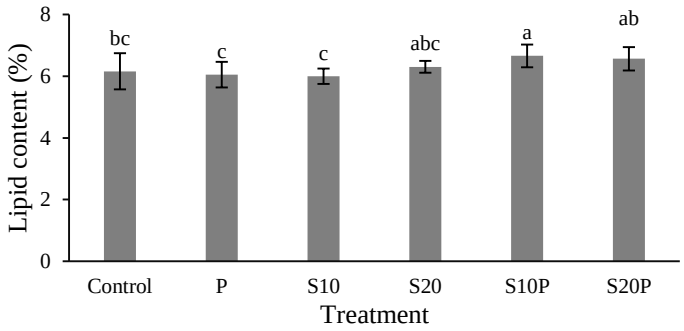

Fig. 7. The effect of starch feeding and Protexin® supplementation on the body lipid content of worker bees under cage condition (Mean ± SE).
P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed with a diet supplemented with 10 % of starch, S20: the group which fed with a diet supplemented with 20 % of starch, S10P: the group which fed with a diet supplemented with 10 % of starch and one mg/L of Protexin®, S20P: the group which fed with a diet supplemented with 20 % of starch and one mg/L of Protexin®.
Columns with different superscript indicate differences ($P \leq 0.05$).

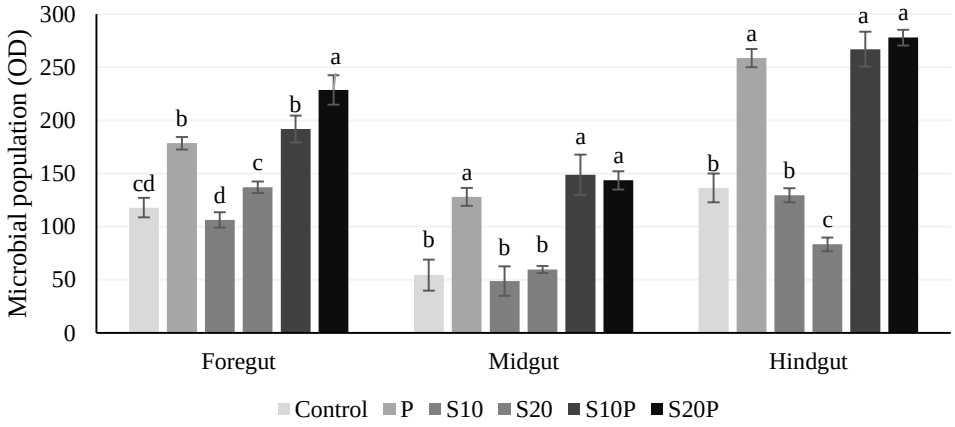

Fig. 8. The effect of starch feeding and Protexin® supplementation on the microbial population in worker bees under cage condition (Mean ± S.E.).

OD: optical density, P: the group which received one g/L Protexin®, S: Starch, S10: the group which fed with a diet supplemented with 10 % of starch, S20: the group which fed with a diet supplemented with 20 % of starch, S10P: the group which fed with a diet supplemented with 10 % of starch and one mg/L of Protexin®, S20P: the group which fed with a diet supplemented with 20 % of starch and one mg/L of Protexin®.
Columns with different superscript indicate differences ($P \leq 0.05$).

The results showed that the dietary supplementation by Protexin® could increase the microbial population in different sections of the bee's gut under cage condition ($P \leq 0.05$, Figure 8). However, the starch supplementation did not affect the microbial population ($P \geq 0.05$).

Discussion

Present study was conducted to test the effect of Protexin® on starch digestibility and absorption in honey bees. The supplementation of starch as an alternative for normal sugar and inverted sucrose syrup gained a lot of interest due to its competitive price. In the first experiment, the bee colonies were located under farm condition with free accessibility to nectar and pollen as a natural source of the starch. Moreover, the second experiment was conducted to inhibit bees from use of pollen as the major natural source of the starch. The results of this experiment indicated that Protexin® supplementation increased the digestibility and absorption of the starch in bee colonies. The colonies which received either 10 or 20 percent of corn starch showed higher starch concentration in different sections of their digestive tract which means that the bees properly used and absorbed starch syrup as a sugar alternative.

Several researches have performed to study the effects of various saccharide supplements on bee's health and performance. In accordance with the results of our study, Semkiw & Skubida (2016) showed that the feeding of different sources of starch syrup did not cause negative effect on bee's condition after overwintering. They indicated that starch syrups turned out to be as suitable as sugar or inverted sucrose syrup for winter feeding of

colonies. Similarly, Severson & Erickson (1984) demonstrated that use of HFCS as carbohydrate supplements does not adversely affect honey bee colonies performance. Moreover, it is concluded in a study that the worker bees can utilize and absorb starch very quickly (Hrassnigg *et al*., 2005). They also suggested that workers not only forage for food but also predigest complex carbohydrates for other members of the colony. They concluded that the workers are well equipped with enzymes to efficiently degrade amylose to the glucose. But in contrast, Papežíková *et al*. (2019) demonstrated that the using wheat starch syrup negatively affected the colonies performance and caused a higher incidence of infected bees. They indicated that the different sources of starch are less suitable for bee colonies due to the content of indigestible complex saccharides. In another study, Sammataro & Weiss (2013) described the significantly lower performance in colonies used HFCS for overwintering compared to those fed with normal sugar syrup. Moreover, Barker & Lehner (1978), performed an experiment on caged bees and showed that the lifespan of bees fed by HFCS was significantly lower than those fed by sucrose syrup.

The results of the present study showed that supplementation of bee's diet with Protexin® can enhance starch degradation and absorption. Furthermore, the dietary supplementation of Protexin® increased the bee's dry weight and body protein content both in colony and cage conditions. To the best of our knowledge, there is a lack of information about the use of Protexin® in honey bee's diet. According to the results of present study, it is assuming that Protexin® can enhance the degradability and absorption of the starch in bee colonies. The increasing of the available amino acids after Protexin® treatment might be a potential reason for increasing of the bee's body weight and protein content. Interestingly, it is reported that Protexin® reduced *Nosema ceranae* infections, increased honey production (Borges, 2015), increased adult bee populations, and eliminated colony winter mortality (Klassen, 2018). It is also showed that Protexin® was able to significantly increase the adult bee population and worker bee's life span (Borges, 2015). Moreover, our results showed that the microbial population of bee's gut, was enhanced by Protexin® supplementation both in colony and cage condition. This may lead to increase the beneficial microbial population in the bee's gut resulting to increase the digestibility and absorption of the starch.

In a recent review, researchers summarized the effect of probiotics on protein digestion and concluded that probiotics can enhance digestion and absorption of the proteins by regulating of the intestinal microflora and thereby influence intestinal bacteria related to proteolysis, induction host digestive protease and peptidase activity, releasing exoenzymes involved in the digestion of proteins, improving the absorption of small peptides and amino acids, improving the absorption ability of the epithelium and finally by reducing of the harmful protein fermentation and thus decrease the toxicity of metabolites (Wang & Ji, 2019). Thus, it may be assumed that the increasing of the availability of different amino acids resulted in higher body protein content as well as the higher body weight.

In conclusion, the results of the present study indicated that beekeepers can feed their honey bee colonies by corn starch as an efficient and cheaper alternative for sugar syrup. Furthermore, the supplementation of bee's diet with Protexin® probiotic enhanced the degradability and absorption of the corn starch. However, further studies are needed to test the exact mechanism of Protexin® in bee's nutrition.

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