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Research Article

Effects of the Dietary Supplement PQQ Consumed to Increase Activity and Cognition; A Study on Ants as Models

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Abstract

Pyrroloquinoline quinone (PQQ) is a newly used medicinal drug and dietary supplement the beneficial effects of which (e.g., improving humans' activity and brain functioning) begins to be rather well known, while its potential adverse effects are still unknown. For the latter reason and because we previously examined the side effects of Cognizing which contains a small amount of PQQ, we studied on ants as models the potential side effects of PQQ. We found nearly no one, but observed several beneficial effects such as improving the ants' activity, audacity, cognition, learning and memory. No adaptation and no habituation to, as well as no dependence on this dietary supplement occurred. Consequently, in addition to be a promising drug for patients, PQQ seems to be also useful for healthy humans. However, since information about its side effects is still lacking, this product should be cautiously used, at low doses during short time periods.

Keywords: *Adaptation; Habituation; Dependence; Memory; Mitochondria*

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees Per cm; mm/s: Millimeter Per Second; χ^2: Chi-Square; *vs:* Versus; n°: Number; cm: Centimeter; mm: Millimeter; mL: Milliliter; mg: Milligram; kg: Kilogram; s: Second; min: Minute; h: Hour; t: Time; %: Percentage

Introduction

The pyrroloquinoline quinone (PQQ) is a co-enzyme essentially (but not exclusively) present inside the mitochondria. It improves the functioning of the latter cellular element, protects them against oxidative damage, and activates their regeneration. PQQ has recently been used as a dietary supplement for increasing humans' activity and cognition, as well as for protecting them against health problems related to aging. Many recent scientific works prove these PQQ benefits [1-8]. A few more ones are reported in the discussion section.

If the wanted effects of PQQ becomes nowadays more and more rather well known, on the contrary, nothing has been investigated about potential adverse effects of this substance. Humans are simply advised to consume maximally 20 mg of the dietary supplements the active substance of which is PQQ (e.g. 2 tablets containing each one 10 mg), as well as to not treat children and pregnant women with this product (advise written on the dietary supplement packages).

According to the increasing use of PQQ and to the absence of information about its potential physiological and ethological side effects, we intended to examine these potential latter effects using ants as biological models. We all the more intended to conduct such a study because we previously examined the side effects of the dietary supplement Cognizing which contains essentially citicoline, but also a very small amount of PQQ, and we wondered if the latter product could have some impact on our results. Before reporting our work, we here below summarize why we use ants, on which ant species we worked and which knowledge we have of it. We also enumerate the different traits we aimed to consider.

Why using ants as models

Most of the biological processes (e.g. the genetical processes, the functioning of the nervous cells, the contraction of the muscles, the classical and the operant conditioning) are similar in every animal species including humans. This is why animals can be used for studying biological problems, processes, events, concerning, among others, the physiology and the behavior [9,10]. Since invertebrates have a small size, are anatomically very simple, and present a rapid development, they are generally preferentially used. For instance, many studies are made on the nematode worms, mollusks, beetles, the fruit flies and the bees. The most commonly used are the insects, and among them, the Hymenoptera [11,12]. We could thus work on ants for making our biological study. Ants can easily be kept in a laboratory, their maintenance is not expensive, and these insects have a lot of skills which could be affected by products used by humans. Among others, they live in colony, learn cues and use them to navigate, communicate with their congeners, olfactorily mark their territory, make brood and queens caring, construct nest, have cemeteries, can relocate their nest as soon as it is necessary [13].

The here used species, and the knowledge we have of it

The species we used was *Myrmica sabuleti* Meinert, 1861. Among others, investigation has been made about it eyes, vision, learning through conditioning, recruitment system, foraging, finding their way [14]. We have elucidated how they acquired several of their skills and knowledge [15]. When being about two to three years old, they present self-recognizing [16]. As in humans, their perception obeys to a distance and size effects, as well as to Weber's law [17,18]. They possess a lot of sophisticated skills, among others many numerical abilities (e.g. they detain a number line, know what is zero, can find the following number of a mathematical sequence, can memorize symbols, can associate visual and olfactory cues with their time period of occurrence [19-23]).

Which physiological and ethological traits we examined

We investigated on the same traits as those on which we studied the effects of Cognizing [24]. These traits were the food intake, activity, linear and angular speeds, orientation, reaction in front of an unknown apparatus, sensory perception, social interactions, state of stress and cognitive abilities, cognition, short and middle term memory, adaptation to side effects of PQQ, habituation to wanted effects of PQQ, dependence on its use, and decrease of the effect of PQQ after weaning. The methods we employed were similar to those used during previous studies. Until now, 55 products have been examined, the results on the 51 first ones having been briefly related in six summaries. The experimental protocols, related among others in [25-27], are again briefly explained for helping the readers understanding our work, but self-plagiarism could not be avoided.

Materials and Methods

Ants' collecting and rearing

The experimental work was performed on two colonies of the ant *M. sabuleti* collected in 09-2022 in an old quarry located in Belgium, Ardenne, Aise valley. These colonies nested in grass and under stones. They contained *ca* 600 workers, 2 queens, eggs, larvae and nymphs. They were kept in 1 - 3 glass tubes half filled with water, a cotton plug separating the part filled of water and the part devoted to the ants. The nest tubes of each colony were set in a tray (34 cm x 23 cm x 4 cm). The borders of these trays were covered with talc for preventing the ants from escaping. The trays served as foraging area: in them, we delivered twice per week a few cut *Tenebrio molitor* larvae (Linnaeus, 1758), and permanently set a tube containing an aqueous solution of sugar (15%). The lighting was either 110 or 330 lux, the temperature equaled *ca* 20°C, the humidity *ca* 80%, and the electromagnetism 2 μWm^2. All these conditions were suitable for the *M. sabuleti*. The words foragers or nestmates are here often used instead of ants as usually done by persons writing about social insects.

Solution of PQQ given to the ants

A package of PQQ (pyrroloquinoline quinone) produced by DeBa Pharma (Dumolinlaan 13, 1500 Kortrijk) was furnished by the pharmacist Wera (Brussels, Belgium). Humans are advised to consume two tablets (containing each one 10 mg of PQQ) of this dietary supplement per day. A human intakes *ca* 1 liter of water per day. He thus intakes the two tablets of PQQ with one liter of water. The insects, due to their excretory apparatus and cuticle, consume about ten less water than mammals. Therefore, to set ants under a PQQ diet identical to that of humans, they must be provided with a solution of the content of two tablets of PQQ in 100ml of sugared water, or a solution of the content of one tablet into 50 ml of sugar water. The realization of such a solution is illustrated in figure 1. This solution of PQQ was delivered to the ants in cotton plugged tubes as was their usual sugar water. The plug was humidified every 2 days, and the content of each tube was replaced every 4 days. The solution had an orange color, and a blue one after five days: it was renewed before this change of color. Many times per day and night, we looked if ants drunk the given solution, and they did. We firstly made the control experiments on ants living under normal diet. Then, we replaced the tubes filled of sugar water by those filled of the sugared solution of PQQ and we started the test experiments one day after.

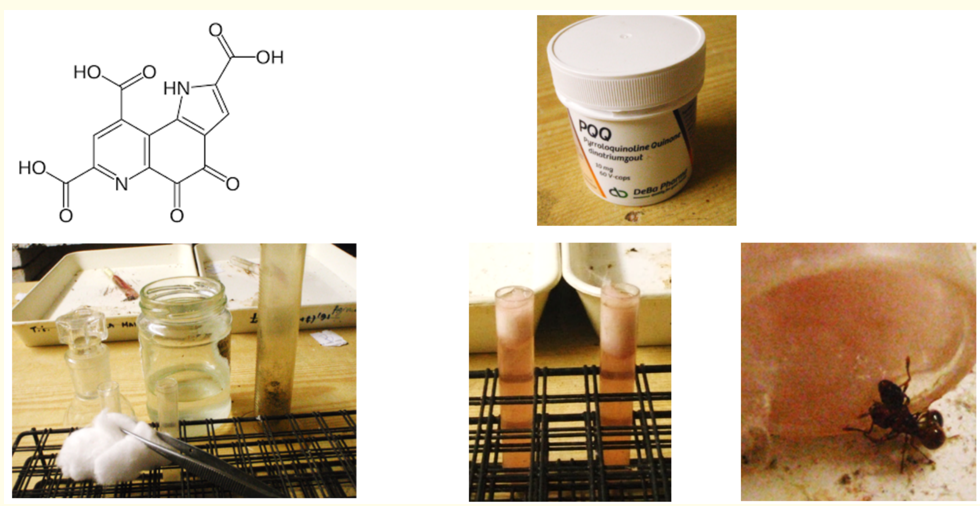

Figure 1: *Realization of the PQQ solution given to the ants. Upper part: chemical structure of PQQ, a package of this dietary supplement; lower part: the material used for making the solution, the tubes filled with the sugar water solution of PQQ given to the ant, two ants drinking this solution.*

Food intake, activity

As previously [25-27], during six days, we counted four times over the day and the night the workers which were on their meat food, were at their sugar water tube entrance, and which were active anywhere (i.e. in their foraging area, in the nest, on the food sites). This was done for each two colonies, first while they were under normal diet, then while they consumed PQQ. Each time, for each three examined traits, we established the mean of the eight made counts (2 colonies x 4 counts) (Table 1, lines I to VI). The total n° of made counts equaled 8 counts x 6 days = 48 counts. We also calculated the means of the six obtained daily means (Table 1, line I- VI). For the ants' meat intake, sugar water intake, and activity, the six daily means recorded while ants lived under normal diet were compared to the six daily means recorded while they consumed PQQ using the non-parametric test of Wilcoxon [28], setting the level of probability at 0.05.

Speeds, orientation

As in previous works [25-27], these traits were assessed on ants walking in the foraging area, the speeds simply while they were walking, the orientation while they were in the presence of a tied nestmate (Figure 2A). Such a tied worker emits an attractive alarm pheromone issued from its mandibular gland. The ants moving in its surrounding became attracted by this secretion and oriented themselves towards the tied nestmate. For quantifying the speed of the ants, and then their orientation, forty trajectories were registered and analyzed thanks to an adequate software set up on the basis of the here reported definition [29]. The linear speed (in mm/s) equals length of the trajectory/time for travelling it; the angular speed (in ang.deg./cm) equals sum of the angles present over the trajectory/length of the trajectory; the orientation (in ang.deg.) equals sum of the angles between the direction of the trajectory and that to the tied nestmate/number of measured angles. A value of orientation lower than 90 degrees signifies that the ant orients towards the tied nestmate; a value higher than 90 degrees signifies that the ant walked away from the tied nestmate. For each of the three considered traits, the median and quartiles of the obtained 40 values were established (Table 2, lines 1, 2, 3), and each distribution of values obtained for ants under normal diet was compared to each one obtained for ants consuming the dietary supplement using the non-parametric χ^2 test [28].

Audacity

This trait was indirectly assessed through the ants' coming onto an unknown apparatus, as was done in previous works [25-27]. A cylindrical tower having a height of 4 cm and a diameter of 1.5 cm and being attached to a squared platform (9 cm^2) (these two parts being of white Steinbach® paper) was deposited in the ants' foraging area. For each two colonies, we counted the ants sighted on this apparatus 20 times during 10 min, obtaining thus = 20 x 2 colonies = 40 numbers (Figure 2B). The numbers recorded for each two colonies were correspondingly summed, and their mean and extremes were established (Table 2, line 4). Also, the numbers corresponding to two successive minutes were added and the 10 sums obtained for ants normally maintained were compared to the ten sums obtained for ants consuming PQQ using the non-parametric test of Wilcoxon [28].

Tactile (pain) perception

This trait was evaluated by assessing the ants' moving on a rough substrate, as it was done in previous works [25-27]. Ants able to perceive the rough uncomfortable character of a substrate walk slowly, sinuously, with difficulty, often touching the substrate with their antennae (Figure 2C). Ants not well perceiving the rough character of a substrate walk on it rather quickly, not very sinuously, and only seldom touch it with their antennae. A piece (3 cm x 2 + 7 + 2 = 11 cm) of emery paper n° 280 paper was dully folded then tied to the bottom and the borders of a tray (15 cm x 7 cm x 4.5 cm), which were so divided into a first 3 cm long area, a second 3 cm long area covered with the emery paper, and a last 9 cm long area. For performing an experiment on a colony, 20 ants were deposited inside the first area of the apparatus, and 20 trajectories were recorded when these ants moved on the emery paper. The total number of recorded trajectories equaled 20 x 2 colonies = 40. Thanks to them, the ants' speeds were assessed in the same way they were while moving in their foraging area (see the subsection relative to the speeds). For each kind of speed, the median and quartiles of the recorded values were established (Table 2, lines 5, 6), and the distributions obtained for ants normally maintained were compared to those obtained for ants consuming PQQ using the non-parametric χ^2 test [28].

Brood caring behavior

As in previous works [25-27], for each colony, a few larvae were prevailed from the nest and deposited near the nest entrance. For each two colonies, the ants' behavior in front of five of these larvae was observed for five minutes (number of observations = 2 x 5 = 10), and the larvae of these 10 ones not re-entered in the nest were counted after 30 seconds, 1, 2, 3, 4 and 5 minutes (Figure 2D). The numbers recorded for each two colonies were correspondingly added (Table 3, line 1). The six added numbers obtained for ants normally maintained were compared to the six added numbers obtained for ants consuming PQQ using the non-parametric test of Wilcoxon [28].

Social interrelations

The ants of one colony (= nestmates) do not attack their congeners. Several circumstances may affect this peaceful social behavior. To assess the ants' potential aggressiveness against nestmates induced by PQQ, five dyadic encounters were performed as it has been done previously [25-27] The numbers of encountering made for each kind of diet equaled 5 x 2 colonies = 10. Each of them was performed in a cylindrical cup, the diameter of which equaled 2 cm and the height of which equaled 1.6 cm. The border of the cup was covered with talc to prevent ants from escaping. For each encountering, one ant was cautiously observed for 5 minutes and its behavior was described through the numbers of times it presented no reaction (level 0 of aggressiveness), contacted the other ant with its antennae (level 1), opened its mandibles (level 2), gripped the opponent (level 3), and stung it (level 4) (Figure 2E). The numbers of these five levels of aggressiveness recorded for the two colonies were correspondingly added (Table 3, line 2). The distribution of the five added numbers corresponding to ants consuming PQQ was compared to that obtained for ants normally maintained using the non-parametric χ^2 test [28]. In addition, the ants' aggressiveness was evaluated thanks to a variable 'a' equaling the number of levels 2 + 3 + 4 divided by the number of levels 0 + 1.

Stress and cognition

As in previous works [25-27], this trait was evaluated via the ants' ability to go out of an enclosure. For escaping, an enclosed individual must be calm, researches for an exit, and has its cognitive abilities intact. For each colony, six workers were set under an enclosure, i.e. a reversed polyacetate cup (height = 8 cm, diameters = 7 cm for the bottom, 5 cm for the ceiling; the inner face being covered with talc) set in the foraging area. To allow the ants escaping, a notch (3 mm height, 2 mm broad) was made in the rim of the bottom of the cup (Figure 2F). The ants which could escape after 2, 4, 6, 8, 10 and 12 minutes were recorded for each colony, and the numbers corresponding to the two colonies were correspondingly added (Table 3, line 3). The six added numbers obtained for ants consuming PQQ were compared to the six ones obtained for ants not consuming this dietary supplement using the non-parametric Wilcoxon test [28].

Cognition

This trait was evaluated as previously [25-27] through the ants' skill to navigate a twists and turns path. A simple apparatus was constructed for each colony. Two pieces of white extra strong paper (Steinbach®, 12 cm x 4.5 cm) were folded then inserted inside a tray (15 cm x 7 cm x 4.5 cm) in order to divide the tray into a first area in front of the area containing the folded paper which created a twists and turns path, and a last area beyond this difficult path (Figure 3A). To conduct an experiment on a colony, 15 workers were transferred into the first area of their own apparatus, and the ants still in this area as well as those present in the last area beyond the twists and turns path were counted after 2, 4, 6, 8, 10 and 12 minutes. The numbers recorded for the two colonies were correspondingly added (Table 3, line 4), and the six sums obtained for ants consuming PQQ were compared to the six sums obtained for ants not consuming this dietary supplement using the non-parametric Wilcoxon test [28].

Conditioning acquisition and memory

This trait was assessed as in previous works [25-27]. For each colony, a green hollow cube (constructed in strong green paper Canson®) was set on the sugar water tube entrance, and the *T. molitor* larvae were transferred near this cube (Figure 3B2 a). Since this setting and relocation, the ants underwent visual conditioning. The control experiment has been previously made on a similar colony, collected at the same time from the same site, since when an individual has acquired a conditioning to a given stimulus, it keeps its conditioning for a rather long time, and after having lost it, it more quickly than initially acquires it again (i.e. it keeps some episodic memory of its learning). Consequently, its native ability to acquire conditioning can no longer be quantified. During the ants' conditioning acquisition, then their loss of it after the cue removal, the ants of each colony were tested in a Y-maze (Figure 3B2 b). Each colony had its own tray which were placed in a tray (30 cm x 15 cm x 4 cm). These Y mazes were in strong white paper, and had their sides covered with talc. A thin paper was deposited on their floor and were renewed after each test. Also, a green cube was set in one of the Y-maze branches. To perform a test on a colony, 10 workers were deposited one by one in the Y-maze in front of its two branches. The choice of one branch of the Y-maze by each ant was recorded. A correct response consisted in choosing the branch into which a green cube had been set. After having been tested, an ant was deposited in a cup until 10 workers of its colony were tested for avoiding testing several times the same ant. As soon as 10 ants were tested, they were transferred back in their foraging area. For each test, the workers which gave the correct and the wrong responses were counted. For the colonies A and B, the recorded dated were correspondingly added, while for the control experiment, these data were used as they were. On the basis of these data, the conditional score obtained at each experimental time was calculated (Table 4). The twelve scores obtained for workers living under a PQQ diet were compared to the twelve scores recorded for workers having no PQQ in their diet thanks to the non-parametric Wilcoxon test [28].

Adaptation to side effects of PQQ

Adaptation to a substance consists in less and less suffering from the adverse effects of this substance in the course of its use. For studying the occurrence of adaptation, a trait affected by the substance must be evaluated just after this substance was used, then once more later after the substance was used during some time, and the two evaluations must be compared. In the present work, the ants' meat and sugar consumption as well as their speeds were impacted by PQQ. Therefore, these traits were again quantified after the ants consumed PQQ for seven days (as they were quantified after 1 - 6 days of such a consumption), and the results of these two quantifications were compared to each other using the adequate non-parametric tests [28].

Habituation to wanted effects of cognizing

An individual becomes habituated to a substance if, over his use of the substance, he less and less perceives the expected beneficial effect of the substance. For studying a habituation, a trait improved by the substance must be assessed just after the use of this substance, then several time after its use, and the results of the two assessments must be compared. In the present work, the ants' activity, orientation, escaping ability and memory appeared to be improved by PQQ. Since the ants' memory was examined during 6 days, the ants' potential habituation to the impact of PQQ on the memory could be evaluated. We also again assessed the ants' activity, orientation and escaping ability after they consumed the dietary supplement for 8 days, as we did after 1 - 6 days of consumption, and we compared the two made assessments using the appropriate non-parametric tests.

Dependence on cognizing consumption

Dependence on a substance consists in enjoying using this substance, wanting to have it permanently at disposal, using it whatever its adverse effects, and in fine becoming unable to live without using the substance. In the present work, we examined if ants developed dependence on PQQ (doing so as we did for other products [25-27]), after the ants consumed it for 9 days. At the end of the 9th day, for each colony, 15 workers were deposited in a tray (15 cm × 7 cm × 5 cm; the borders covered with talc) containing two cotton-plugged tubes (h = 2.5 cm, diam. = 0.5 cm), one filled with the ants' usual sugared water, the other filled with the solution of PQQ used all along the experimental work (Figure 3E2 a, b). The tube filled with PQQ was set on the right for colony A, and on the left for colony B. Then, for each colony, the ants going near each tube were counted 20 times in the course of 10 minutes, and the numbers recorded for the two colonies were correspondingly added (Table 5, lower part). On the basis of these two sums, the proportions of ants having gone near the drug and the drug-free solutions were calculated. In addition, the two obtained sums of counted ants' visits were compared to the two numbers which would be obtained if the workers had randomly gone near each tube using the non-parametric χ^2 goodness-of-fit test [28].

Decrease of the effect of PQQ after weaning

This decrease was studied after the ants consumed PQQ during 11 days, as this was examined for other substances in several previous studies [25-27]. At the 11th day, the ants were provided with a fresh solution of PQQ, and several hours later, their orientation to a tied nestmate as well as their general activity were assessed as they had been after 1 and 7 days (for the orientation), during the 1 - 6 days of the dietary consumption (for the activity), except that, for the orientation, only 20 trajectories instead of 40 ones were recorded and analyzed, and, for the activity, 8 data were recorded, for being able to achieve every assessment over the decrease of the effect of PQQ, and thus to estimate the decrease at any time. After the assessments made at the 11th day, at the 12th day, weaning started, i.e., the tubes filled with the sugared solution of PQQ were replaced by tubes filled with a sugared aqueous drug-free solution. This replacement constituted the start of the examined decrease, and was thus done at t = 0. From this time, the ants' orientation and general activity were assessed every three hours until these traits became identical to those of ants normally maintained, i.e., to the controls. For each time, the median and quartiles of the 20 obtained orientation values, and the mean of the 8 recorded values of activity were established (Table 6). In addition, for each testing time, the obtained distribution of orientation values was compared to the distribution recorded at t = 0 and to the control distribution using the non-parametric χ^2 test, while the 8 values of activity were compared to those obtained at t = 0 using the non-parametric test of Wilcoxon [28]. These results are given in Table 6 and are also graphically presented in Figure 4. The mathematical function which best described the loss of the effect of PQQ after weaning was tried to be defined, and is given in the text, results and discussion section.

Results

Food intake, general activity

Numerical results can be found in Table 1. PQQ appeared to slightly decreased the ants' meat consumption; the difference with the ants normally maintained was however at the limit of significance: N = 4, T = 10, P = 0.063. The dietary supplement increased the ants' sugar water intake, and this difference with the ants normally maintained was significant: N = 5, T = 15, P = 0.031. What was very obvious to observers was the increase of activity induced by PQQ. The ants consuming this substance were far more active than those living under normal diet, a difference statistically significant: N = 6, T = 21, P = 0.016. This last effect is surely one of the effect wanted when intaking PQQ.

Days	Normal diet			Diet with PQQ		
	Meat	Sugar	Water Activity	Meat	Sugar	Water Activity
I	0.63	0.63	10.50	0.25	1.00	19.25
II	0.38	0.25	10.25	0.25	0.50	18.50
II	0.75	0.63	10.00	0.38	0.75	24.87
IV	0.38	0.25	10.00	0.38	0.63	32.75
V	0.25	0.38	10.50	0.25	0.75	25.13
VI	0.38	0.50	10.75	0.50	0.50	24.50
I-VI	0.46	0.50	10.33	0.33	0.69	18.90

Table 1: *Impact of PQQ on the ants' food consumption and general activity. The table gives the mean numbers of workers eating meat, drinking sugared water, and being active during six days (the six first lines), and the means of these six means (the last I-VI line). The dietary supplement decreased the meat consumption, but increased the sugar water intake and essentially the activity.*

Linear and angular speeds

Though the ants consuming PQQ walked a little more quickly and more sinuously than those living under normal diet (Table 2, lines 1 and 2), this difference of locomotion between the ants under one and the other kinds of diet was not significant: linear speed: $\chi^2 = 0.14$, df = 2, 0.95 < P < 0.90; angular speed: $\chi^2 = 3.53$, df = 2, 0.10 < P < 0.20. Effectively, PQQ is devoted to improve the individuals' activity, but not particularly their speed of locomotion. A slight increase of the latter trait under PQQ diet was thus a result in agreement with what could be expected.

Orientation to a tied nestmate

This ethological trait was improved by PQQ consumption (Table 2, line 3; Figure 2A). Under this dietary supplement, the ant oriented themselves far better than while living under normal diet. The difference as for their orientation ability between the workers maintained under one or the other kinds of diet was statistically significant: $\chi^2 = 14{,}68$ df = 2, P < 0.001. This difference could be considered as being a wanted effect of PQQ: the individuals' performances are ameliorated.

Audacity

PQQ accentuated this trait (Table 2, line 4; Figure 2B). Indeed, the workers consuming this dietary supplement were more inclined to approach the unknown apparatus and to walk on it than those normally maintained, and this was statistically significant: N = 8, T = 30.5, P = 0.047. A not assessed observation was that, under PQQ diet, the ants more often climbed on the tower than while not consuming this dietary supplement. They were thus more active, more ready to accomplish new tasks, and more prompt to face difficulties. This is probably one effect wanted when intaking PQQ, which may be due to the effect of PQQ on the nervous system via its impact on the mitochondria.

Tactile (pain) perception

Results are given in Table 2, lines 5, 6, and photos are shown in figure 2C. Briefly, PQQ did not impact the ants' sensory perception. Ants under normal diet walked on a rough substrate more slowly and more sinuously than in their foraging area, and this difference was statistically significant: linear speed: $\chi^2 = 80{,}00$, df = 1, P < 0.001; angular speed: $\chi^2 = 72{,}38$, df = 1, P < 0.001. In the same way, ants consuming PQQ walked on a rough substrate at a lower linear speed and a higher angular speed than on their foraging area, and these two differences were also statistically significant: for each two speeds: $\chi^2 = 80{,}00$, df = 1, P < 0.001. They even did so to a larger extend, but this accentuation was not highly significant: linear speed: $\chi^2 = 7{,}16$, df = 1, 0.01 < P < 0.02; angular speed: $\chi^2 = 1{,}38$, df = 1, 0.20 < P < 0.30. The non-impact of PQQ on the ants' sensory perception was in agreement with their perfect orientation towards a tied nestmate, i.e. they perfectly perceived the alarm pheromone emitted by the tied nestmate.

Traits	Normal diet	Diet with PQQ
Linear speed (mm/s)	9.6 (8.9 - 10.8)	9.8 (8.7 - 11.1)
Angular speed (ang.deg./cm)	95 (76 - 119)	116 (99 - 128)
Orientation (ang.deg.)	30.9 (26.3 - 40.9)	21.5 (15.7 - 24.1)
Audacity (n° of ants)	4.42 [3 - 6]	4.98 [4 - 7]
Tactile perception through		
The ants' linear speed	3.8 (3.3 - 4.2)	2.8 (2.4 - 3.5)
and angular speed on a rough substrate	280 (251 - 330)	316 (261 - 360)

Table 2: *Impact of PQQ on five biological traits. The table reports the median (and quartiles) as well as the mean [and extremes] of the recorded values. Briefly, the dietary supplement affected the ants' locomotion, improved their orientation, slightly increased their audacity, and did not affect their tactile perception.*

Brood caring

This ethological trait was not affected by PQQ consumption (Table 3, line 1; Figure 2D). While consuming this substance, the ants re-entered the larvae removed from the nest as quickly as did ants living under normal diet. They only slightly, not statistically significantly did so somewhat more rapidly. The difference between the two kinds of ants as for this brood caring behavior was not significant: N = 1, NS. The next experiment checked again the potential impact of PQQ on the ants' social relationships.

Social relationships

This ethological social trait was not statistically affected by PQQ intaking (Table 3, line 2; Figure 2E). Ants under this dietary supplement as well as ants living under normal diet never attacked their congeners. They stayed near each other peacefully, doing nothing or contacting themselves with their antennae and slightly opening their mandibles. Those consuming PQQ did the two latter behaviors a little more often than the ants normally maintained, but this difference was not statistically significant (χ^2 = 2,50 df = 2, 0.20 < P < 0.30). However, due to this non-significant difference, the variable assessing the ants' aggressiveness differed between the two kinds of ants: it equaled 0.11 for ants under PQQ diet and 0.05 for ants normally maintained. Such a slight difference does not require rejecting using PQQ.

Stress and cognition

These biological traits were not impacted by PQQ consumption, on the contrary (Table 3, line 3; Figure 2F). The ants normally maintained walked erratically for one to two minutes, then more calmly and essentially along the rim of the enclosure. They so found the exit and often then went out. While consuming the dietary supplement, the ants walked quickly and sinuously all around the enclosure, then rather more along its rim but still quickly, nervously, and went often over the exit. However, as soon as they found it, they went out of the enclosure. Consequently, even if staying excited, nervous (but not stressing), the ants consuming PQQ succeeded in escaping even somewhat better that those living under normal diet. Statistically, the difference relative to this ability between the ants maintained under one or the other kinds of diet was at the limit of significance, no more because the sample was small (N = 4, T = 10, P = 0.063). Conclusively, the dietary supplement did not affect the ants' cognition (on the contrary, it improved this physiological trait), not their state of stress. The next experiment checked again the potential effect of PQQ on the ants' cognition (See below).

Cognition

PQQ did not affect this biological function (Table 3, line 4; Figure 3A). Ants living under this dietary supplement diet could cross the twists and turns path as quickly, as efficiently as those normally maintained. There was no statistical difference between the two kinds of ants as for the numbers of ants sighted over time in the areas lying at either side of the difficult path: in front: N = 4, T = 10, P = 0.063; beyond: N = 2, NS. Since the ants consuming the PQQ were rather excited, nervous, and often came back on their way while navigating the twists and turns path and, however, succeeded to travel as well as the ants not consuming this dietary supplement, it could be estimated that their cognitive ability was somewhat improved, a result in agreement with that of the previous experiment (see the above subsection).

Conditioning acquisition, memory

PQQ appeared to improve or a least to not impact the ants' conditioning acquisition (Table 4, Figure 3B). The difference between the ants consuming this dietary supplement and those living under normal diet as for their conditioning acquisition ability was at the limit of significant in favor of the former ants: N = 4, T = 10, P = 0.063. Considering the excitation, the nervousness of the ants consuming PQQ, it could be estimated that these ants' conditioning acquisition was somewhat improved by the dietary supplement. After the cue removal, the ants went on presenting a conditioning score of 90% during 55 hours and finally a score of 85% seventeen hours later. The difference as for the ants' memorization between those consuming PQQ and those living without this dietary supplement was significant: N = 6, T =21, P = 0.016.

Traits	Normal diet	Diet with PQQ
Brood caring: n° of not re-entered larvae over time	30'' 1' 2' 3' 4' 5' 10 8 5 2 0 0	30'' 1' 2' 3' 4' 5' 10 8 4 2 0 0
Social relationships: n° of aggressive levels 0-4, variable 'a'	Levels: 0 1 2 3 4 'a' 64 47 6 0 0 0.05	Levels: 0 1 2 3 4 'a' 52 47 11 0 0 0.11
Stress and cognition: n° of ants escaped over time	2' 4' 6' 8' 10' 12' 2 5 8 10 11 12	2' 4' 6' 8' 10' 12' 2 6 9 11 12 12
Cognition: n° of ants in front (f) and beyond (b) a twists and turns path over time	2' 4' 6' 8' 10' 12' f: 21 16 9 7 6 5 b: 0 2 9 11 13 17	2' 4' 6' 8' 10' 12' f: 23 16 13 11 7 5 b: 0 2 7 11 16 17

Table 3: *Impact of PQQ on four biological traits. The table reports the amounts of counted larvae, levels of aggressiveness, or workers. Details can be found in the text, and photos seen in figure 2 and 3. The dietary supplement did not affect the ants' social relationships, and slightly improved their cognition.*

Time (hours)	Normal diet conditioning scores	Diet with PQQ: n° of correct *versus* wrong responses given by colonies A; B → conditioning scores
7h	60%	6 *vs* 4; 6 *vs* 4 → 60%
4h	60%	8 *vs* 2; 7 *vs* 3 → 75%
31h	70%	8 *vs* 2; 8 *vs* 2 → 80%
48h	70%	9 *vs* 1; 9 *vs* 1 → 90%
55h	80%	9 *vs* 1; 9 *vs* 1 → 90%
72h	85%	9 *vs* 1; 8 *vs 2* → 85%
Cue removal		
7h	85%	9 *vs* 1; 9 *vs* 1 → 90%
4h	80%	9 *vs* 1; 9 *vs* 1 → 90%
31h	80%	9 *vs* 1; 9 *vs* 1 → 90%
48h	80%	9 *vs* 1; 9 *vs* 1 → 90%
55h	80%	9 *vs* 1; 9 *vs* 1 → 90%
72h	80%	8 *vs* 2; 9 *vs* 1 → 85%

Table 4: *Impact of PQQ on the ants' conditioning acquisition and memory. The table gives the control conditioning scores, and the numbers of right and wrong responses given by colonies A and B as well as the corresponding conditioning scores obtained over the ants' conditioning acquisition and loss. PQQ improved the conditioning acquisition and the memory (ants kept 90% of their learning for 55 h).*

Adaptation to the side effects of PQQ

Numerical results are given in Table 5, upper part. The ants presented no adaptation to the effect of PQQ on their meat consumption. After 7 days on this dietary supplement, the ants still eat less meat that usually (difference between their meat intake after 7 days and during the first six days: N = 3, NS). Also, they did not adapt themselves to the effect of the dietary supplement on their sugar water, on the contrary, they even drunk much sugar water after having lived for 7 days on PQQ diet than before this 7th day, and this was statistically significant (N = 6, T = 21, P = 0.016). Concerning the ants' adaptation to the impact of PQQ on their locomotion, no adaptation occurred. After having been on a PQQ diet for 7 days, the ants walked at the same linear speed and nearly the same angular speed than after having lived for one day under this dietary supplement diet. The difference between the ants having been maintained under a PQQ diet for 7 or 1 days as for their locomotion was not statistically significant: linear speed: χ^2 = 0,08, df = 1, 0.70 < P < 0.80; angular speed: χ^2 = 4,30, df = 2, 0.10 < P ≤ 0.20. It could thus be definitively concluded that the ants presented no adaptation to the few adverse effects of PQQ.

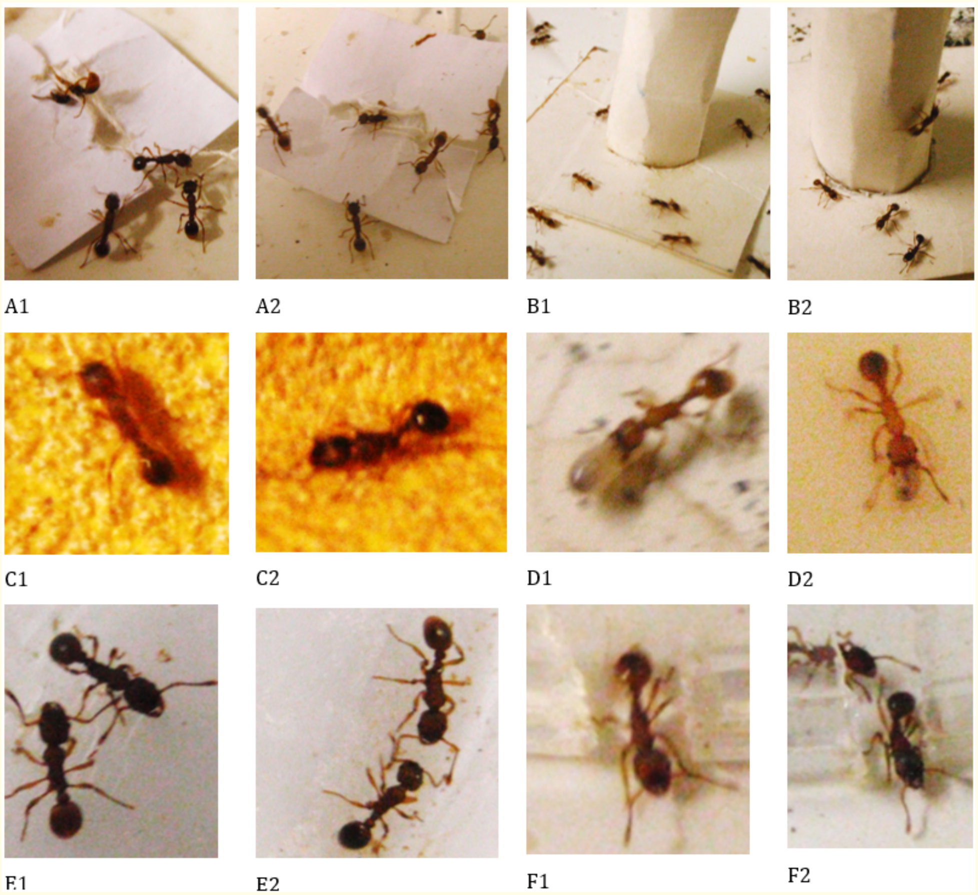

Figure 2: *Some views of the experiments made to know the impact of PQQ on six physiological and ethological traits 1: ants under normal diet; 2: ants under a diet with PQQ. A: ants attracted to a tied nestmate. B: ants coming onto an unknown apparatus. C: ants walking with difficulty on a rough substrate. D: ants transporting a larva towards the nest. E: two nestmate staying peacefully near each other. F: ants escaping from an enclosure.*

Habituation to the wanted effects of PQQ

Numerical results are reported in Table 5 middle part. No ants' habituation to the effect of PQQ on their general activity occurred. There was no statistical difference between the numbers of active ants counted during the 7th day of their maintenance under a PQQ diet and those counted before that day: N = 6, T = 11, -10, P = 0.50. As for the impact of the dietary supplement on their orientation ability, the ants also presented no habituation. After 7 days on PQQ diet, they went on orienting themselves very well towards a tied nestmate; there was no statistical difference between their orientation value obtained after they consumed PQQ for 7 or for 1 day (χ^2 = 0,80 df = 1, 0.30 < P < 0.50). Concerning the ants' escaping ability, after 7 days on PQQ diet, they were still able to soon go out of the enclosure. The numbers of escaped ants over 12 minutes were 2, 6, 8, 10, 12, 12, what was not statistically different from what was previously observed (see Table 3, line 3). The ants presented thus no habituation to the effect of PQQ on their cognition. Note that, during the experiment made on ants having consumed PQQ for seven days, the ants were excited, nervous as they were previously. No habituation occurred, but no adaptation also occurred. Finally, let us add that the ants' conditioning acquisition and memory was assessed over six consecutive days, and that the ants still presented a score of 85% on the sixth day. They were thus not habituated to the favorable effect of PQQ on their memorization.

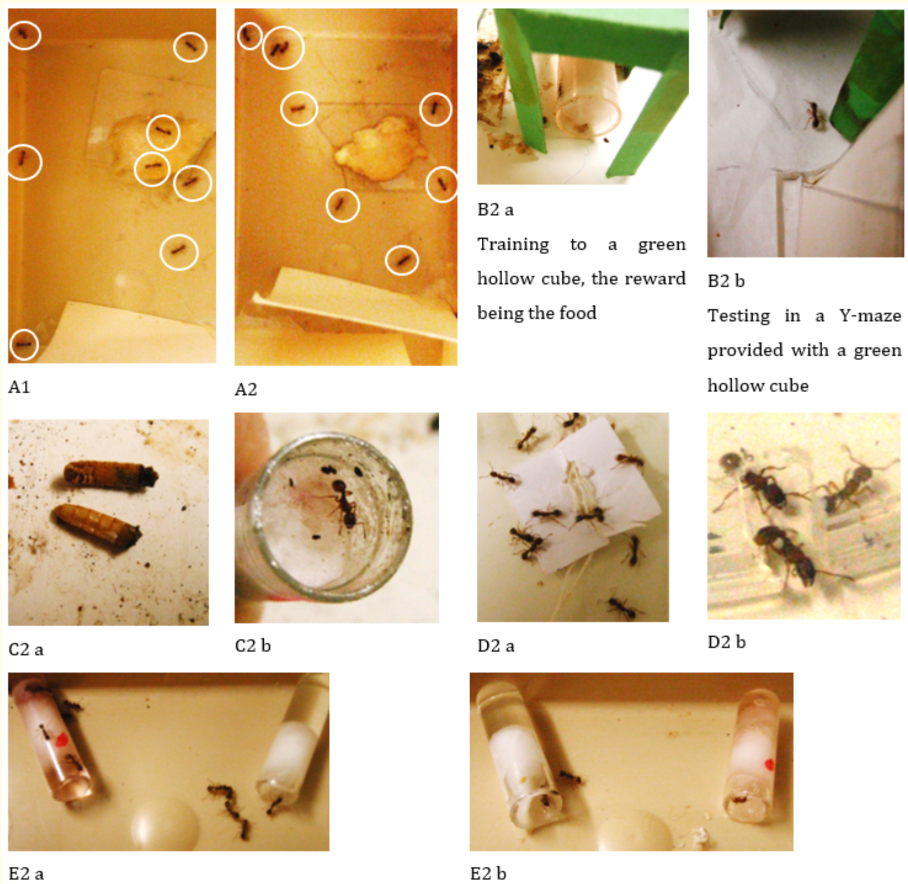

Figure 3: *Some views of the experiments made to know the impact of PQQ on cognition (A), learning and memory (B), the ants' adaptation (C) and habituation (D) to the effect of this dietary supplement, and the ants' dependence on its consumption (E). PQQ did not affect the ants' cognition nor their learning (so, their memory). The ants did not adapt themselves to the impact of PQQ on their meat and sugar consumption. The did not habituate themselves to the effect of PQQ on their orientation and escaping ability. They developed no dependence on that dietary supplement consumption.*

Dependence on PQQ consumption

The ants did not develop dependence on PQQ consumption (Table 5, lower part; Figure 3E a, b). During the experiment, ants of colony A were seen 38 times on the tube containing the PQQ-free solution, and were seen 47 times on the tube containing the PQQ solution. At the same time, ants of colony B were seen 39 times on the PQQ-free solution and 34 times on the PQQ solution. In total 77 ants' visits occurred for the drug-free solution while 81 ones occurred for the drug solution. This corresponded to 48.73% of visits to the PQQ-free tube and 51.26% of visits to the tube containing PQQ. The recorded numbers of counted ants' visits (77, 81) was not significantly different from those resulting from random ants' visits to the two provided tubes (79, 79): χ^2 = 0,014, df = 1, 0.90 < P < 0.95. This absence of dependence is in favor of the dietary supplement use.

Trait	Normal diet	Diet + PQQ for 1 - 6 days	Diet + PQQ for 7 days
Meat consumption (n°) Sugar water intake (n°) Linear speed (mm/s) Angular speed (ang.deg./cm)	0.46 0.50 9.6 (8.9 - 10.8) 95 (76 - 119)	0.33 0.69 9.8 (8.7 - 11.1) 116 (99 - 128)	0.29 1.23 9.8 (8.5 - 10.8) 128 (114 - 148)
Trait	**Normal diet**	**Diet + PQQ for 1 - 6 days**	**Diet + PQQ for 7 days**
General activity (n°) Orientation (ang.deg) Escaping ability (n°) Memory (score %)	10.33 30.9 (26.3 - 40.9) after 4': 5 80%	18.90 21.50 (15.7 - 24.1) after 4': 6 85%	26.5 19.6 (15.4 - 25.8) after 4': 6 85%
Dependence: n° of ants sighted on the drug-free *vs* the drug solution →% for these two solutions	Colony A	Colony B	%
	38 versus 47	39 versus 34	48.73 vs 51.26

Table 5: *Ants' adaptation (upper part) and habituation (middle part) to the effect of PQQ; ants' dependence on its consumption (lower part). Details are given in the text. The ants presented no adaption to the impact of PQQ on their meat and sugar intake, nor on their locomotion. They did not habituate themselves to the improved effect of PQQ on their activity, orientation, cognition (escaping ability) and memory. The ants did not acquire dependence on that dietary supplement.*

Time (hours)	Ants' orientation ang.deg.	*vs* t = 0 Statistics *vs* control						Activity mean	Statistics vs t = 0		
		χ^2	df	P	χ^2	df	P		N	T	P
t = 0	19.7 (14.2 - 30.8)	--			16.68	1	< 0.001	23.6	--		
3h	23.1 (16.3 - 37.4)	2.03	1	< 0.20	7.40	1	< 0.01	22.5	5	15	0.031
6h	24.5 (22.1 - 28.1)	13.07	1	< 0.001	4.89	1	< 0.05	21.0	6	21	0.016
9h	25.3 (22.6 - 29.9)	13.07	1	< 0.001	0.65	1	< 0.50	19.1	6	21	0.016
12h	29.1 (22.5 - 40.5)	13.07	1	< 0.001	0.27	1	≤ 0.70	18.8	6	21	0.016
15h	31.0 (19.0 - 42.1)	13.07	1	< 0.001	0		NS	18.1	6	21	0.016
18h								17.4	6	21	0.016
21h								16.1	6	21	0.016
24h								14.8	6	21	0.016
27h								14.0	6	21	0.016
30h								12.1	6	21	0.016
33h								10.3	6	21	0.016
Control	30.9 (26.3 - 40.9)	16.68	1	< 0.001	--			10.3			

Table 6: *Decrease of the effect of PQQ after its consumption was stopped. The table gives the values of the ants' orientation (in ang.deg.) and the activity (in n° of active ants) over time, as well as the results of statistical analysis. The dietary supplement lost its effect regularly, without sudden decrease, in a total of 15h for the orientation and 33h for the activity after weaning. Such a regular, never sudden loss of effect accounted for the absence of dependence. These results are illustrated in Figure 4.*

Decrease of the effects of PQQ after weaning

Numerical and statistical values are reported in Table 6 and are graphically presented in Figure 4. Using the ants' orientation as the trait improved by PQQ, it appeared that this trait was still affected by the dietary supplement 3 hours after its weaning ($P < 0.01$), and still but only slightly 6 hours after weaning ($P < 0.05$). Then, 9 hours after the consumption of PQQ was stopped, the ants' orientation did not statistically differ from the control one ($P < 0.50$). The effect of PQQ went on decreasing, the difference with the control becoming smaller ($P < 0.70$) 12 hours after PQQ consumption was stopped, and null ($\chi^2 = 0$) 15 hours after this stop of consumption. The improved effect of PQQ on the ants' orientation decreased thus regularly, without sudden diminution, and totally vanished in 15 hours. This decrease could best be described thanks to the following linear function:

$E_t = E_i - 0.75\ t$

with E_t = effect (in ang.deg.) at time t, E_i = initial effect, t = time (in hours).

Using the impact of PQQ on the ants' activity, it appeared that this impact started to decrease as soon as just after the consumption of the dietary supplement was stopped. Indeed, 3 hours after weaning, the difference with the initial effect was already slightly significant ($P = 0.031$), and became highly significant 6 hours after weaning ($P = 0.16$). The effect of PQQ on the ants' activity went on slowly decreasing (the values are given in Table 6), and finally became null 33 hours after the consumption of PQQ was stopped. After weaning, the improved impact of the dietary supplement decreased thus slowly, without sudden diminution, during about 33 hours, and could be best described by the following linear function:

$E_t = E_i - 0.40\ t$

with E_t = effect (in n° of ants) at time t, E_i = initial effect, t = time (in hours).

Such a slow, regular decrease of the wanted effects of PQQ after weaning accounted for the no development of dependence on its consumption [30].

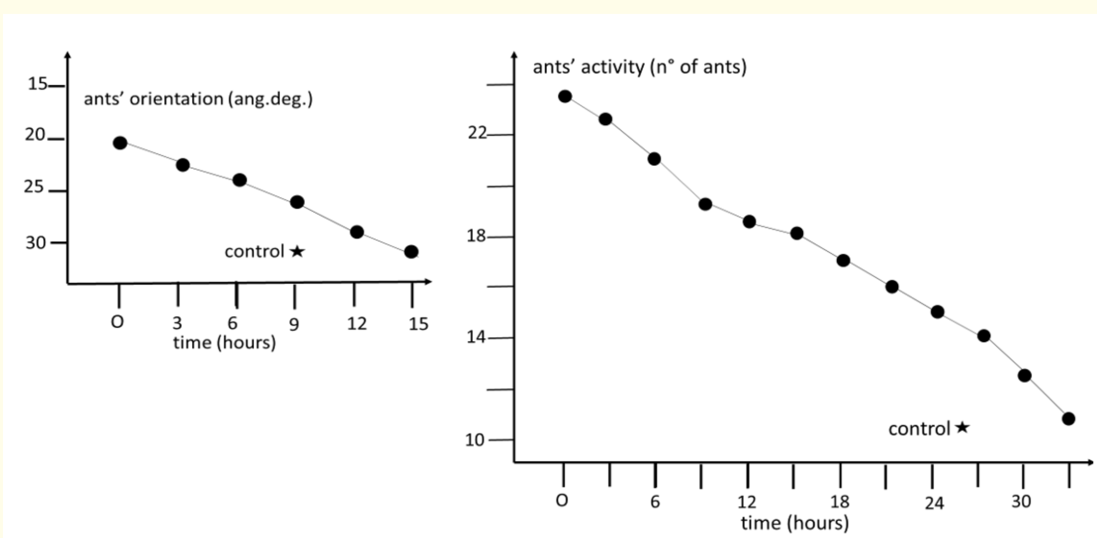

Figure 4: *Decrease of the effect of PQQ on the ants' orientation and activity after its consumption was stopped. These effects decreased slowly, linearly, without sudden diminution, in a total of 15 hours for the orientation and 33 hours for the activity. Numerical and statistical results are given in Table 6, and more information can be found in the text.*

Discussion

PQQ is a dietary supplement recently used for improving humans' activity and cognitive abilities. Nowadays, no information can be found about its potential adverse effects. For this reason and because we previously examined the side effects of Cognizing which contains a small amount of PQQ, we intended to study on ants as models the possible side effects of this PQQ. We found that this dietary supplement had nearly no adverse effect and detained the wanted improving effects on the activity and cognition. Indeed, it increased the ants' activity, orientation ability, cognition, learning and memory. It did not affect the sensory perception nor the social interactions, and did not lead to dependence. The only adverse effects were a decrease of meat consumption, an increase of sugar water consumption, and an increase of the angular speed, with no adaptation to these few side effects. The ants did not habituate to the beneficial effects of the dietary supplement, the effect of which slowly vanished in 33 hours after weaning. No product among the 55 ones we have until now studied presented so few adverse effects and so many wanted ones. Consequently, it is the first time we examined the decrease of the effect of the studied product after weaning using wanted effects instead of an adverse effect.

If we compare these effects of PQQ with those previously found for Cognizing, we can affirm that the side effects observed for the latter dietary supplement were not due to its countenance of a small amount of PQQ, but that the few slight improved effect might result from such a countenance (Table 7).

As promised in the Introduction section, here are a few more information on the PQQ product. This dietary supplement was found to be efficient for treating rats having had a cerebral artery occlusion. Rats which received PQQ after their middle cerebral artery occlusion presented better neurobehavioral scores than the control ones. PQQ may thus be a useful neuroprotectant in stroke therapy [31]. PQQ has also been found to be beneficial for caring of persons suffering from diabetes. Adequate human cells were exposed to glucose or to glucose + PQQ. The latter substance reduced the oxidative damage due to hyperglycemia [32]. Also, used in combination with a small amount of lithium, PQQ could be beneficial to persons suffering from the Alzheimer's disease. Side effects of lithium limit its clinical use. Low dose of lithium + PQQ had more powerful effects than lithium alone in restoring, among others, learning and memory impacted by the illness. A novel therapy could thus consist in using micro doses of lithium together with PQQ, the two substances acting in synergy [33]. As for the implication of PQQ on the mitochondria functioning, the work of He and co-authors revealed that (surprisingly dixit the authors), the effect of this product depends on which kind of biological system it acts. The reactivity of PQQ differed according to its use either on isolated rats' mitochondria or on cells; i.e. it acted as an antioxidant or a pro-oxidant [34]. The clinical study in humans made by Harris and co-authors showed that PQQ improved several biological mechanisms, among others the mitochondria functions. According to the authors, their work was among the first ones liking the effects of PQQ in animals and in humans [35]. As a matter of fact, PQQ is a rather newly used medicinal drug and dietary supplement; all its wanted and side effects are not yet sufficiently studied and even if beneficial effects can valuably be expected, attention should still be paid when using this product.

Traits	Cognizing	PQQ
Meat intake	Decrease	Decrease
Sugar water intake	Decrease	Increase
Activity	Decrease	Increase
Linear speed	Decrease	Increase
Angular speed	Increase	Increase
Orientation	Decrease	Increase
Audacity	Decrease	Increase
Tactile perception	No Effect	No Effect
Brood caring	Decrease	No Effect
Social relationships	Decrease	No Effect
Stress, cognition	Decrease	Slight Increase
Cognition	No Effect	Slight Increase
Learning, memory	No Effect	Increase
Adaptation	None	None
Habituation	None	None
Dependence	Strong One	None
Loss of effects	In 11 Hours	In 33 Hours

Table 7: *Comparison of the effects induced by cognizing and by PQQ, two dietary supplements used for improving humans' cognition. The effects of cognizing are detailed in a previous works [24], those of PQQ are reported in the present paper.*

Conclusion

PQQ, a co-enzyme present among others in the inner membrane of the mitochondria, is a newly used medicinal drug and dietary supplement. Its beneficial effects begin to be rather well known, its adverse ones not yet. In ants, PQQ presented nearly no side effect (e.g. no dependence), but several beneficial ones (e.g. activity, cognition, memory improvement). It is thus valuably promising. However, use of low doses during short time periods is advised until more information on PQQ will be acquired.

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Volume 17 Issue 12 November 2022