Effects of Taurine on Eusociality of Ants

Ha Won Kim and Dong Hee Lee

Abstract The effects of taurine have been characterized primarily in mammals, and insects are not generally used to study taurine. In this study, ants were used to examine the effect of taurine on eusociality. Ants are the principal models for studying eusociality and superorganisms. Japanese carpenter ants (Camponotus japonicus) were fed a taurine-supplemented diet and tested using ant eusocial indexes. Ant farm structures were constructed using transparent PET bottles containing autoclaved soil. Three categories of vital index were used to study the effect of taurine on group activity: creation of formicaries (residence chambers), cooperative defense efforts, and population density (or group size and composition). Control, low-, and high-taurine diets were prepared using three different levels of taurine in sucrose powder: 0, 5, and 20% (g/g), respectively. The cooperative defense efforts against exogenous queen ants were recorded daily. The high-taurine group took less time to complete their defense formation than the other groups. At least 16% more formicaries (chambers) were observed in the taurine-fed groups than in the control. There were evident differences between control and taurine-fed groups in the total numbers of ants and eggs. The taurine-fed group sustained higher total numbers of ants, excluding the queen. Taurine-fed groups showed a significant increase both in the number of workers and eggs. When fed with taurine, ants responded positively on the eusocial vitality indexes. These results show that taurine exerts a positive effect on the eusociality of ants at the level of the superorganism.

Keywords Ants · Eusociality · Eusocial vital index · Superorganism · Taurine

Abbreviations

CDI	Cooperative Defense Index
PET	polyethylene terephthalate

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1 Introduction

Taurine, 2-aminoethanesulfonic acid, comprises sulfonic acid and amino functions that branch from each carbon backbone. In mammals, taurine protects the brain from various impairments, lessens withdrawal syndromes, and helps to augment visual function. Taurine is a vital nutritional constituent, especially during the infant stage (Whittle et al. 2007). Taurine plays an important role in muscle maturation during the developmental period, and is indispensable for cardiovascular function, development and function of skeletal muscle, the retina, and central nervous system (Schaffer et al. 2000). Taurine has many essential biological roles in antioxidation, osmoregulation, and membrane stabilization, and modulation of calcium signaling (Solis et al. 1988).

To date, the effects of taurine have been studied primarily in mammals. There is no report of its effects in insects. Taurine is widely found in animal organs and accounts for 0.1% of total body weight of humans (Huxtable 1992). Taurine is also abundant in ants (Hymenoptera: Formicidae) and can account for up to 6.5 mg/g of dry matter (McCusker et al. 2014). This is surprising, since taurine content does not exceed 1.0 mg/ml in most insects (Spitze et al. 2003). The taurine content in ants even exceeds that of many oceanic fishes and mollusks (Table 1). Despite its extremely high content in ants, the actual function of taurine in ants is unknown.

Eusociality refers to a highly advanced social structure between group members (Crespi and Yanega 1995). Eusociality evolved after recurrent modifications of social behaviors in insects and other hexapods more than three million years ago (Wilson and Nowak 2014; Wilson and Holldobler 2005). Most ants show a form of eusociality, but it is rare in other Hymenoptera, such as bees and wasps, and vertebrates, such as mammals (Linksvayer and Wade 2005; Nowak et al. 2010). One of the characteristic features of eusociality is a division of labor between reproductive and non-reproductive members (Purcell et al. 2014). A eusocial colony has a distinctive hierarchy, maintained under a strict caste system (de Wilde and Beetsma 1982). Queens and reproductive males are the sole reproducers, while most offspring assume the roles of workers or soldiers who gather food, protect the colony, or raise the offspring produced by the queen (Rehan and Toth 2015). Soldiers and workers are interdependent on the overall growth, solidarity, and proliferation of the colony for their survival. Eusocial ants defend their colonies by subduing invaders, and alert nest mates to rich food sources. Eusocial ants have a high level of interaction

Table 1 Taurine contents in selected organisms

Kingdom	Species	Source of sample	Dried Matter (mg/g)
Planta	Purple kale (*Brassica oleracea*)	Leaf	0.02[a]
Fungi	Yeast (*Saccharomyces cerevisiae*)	Whole	0.00[a]
Animalia	Cow (*Bos taurus*)	Heart	3.64[b]
	Ant (*Pogonomyrmex occidentalis*)	Whole	6.42[a]
	Tuna (*Thunnus thynnus*)	Red meat	8.69[b]

[a]McCusker et al. (2014); [b]Spitze et al. (2003)

among peers and are highly collaborative with other members to ensure the success of their community (Wilson and Nowak 2014; Crozier and Pamilo 1996). However, individual ants within a eusocial community may also experience a high level of pressure and stress, which might be relieved by taurine.

This study used Japanese carpenter ants (Camponotus japonicas) to examine the effect of taurine on eusociality. Ants were fed with taurine-supplemented diets and tested according to eusocial indexes. The aim was to analyze the potential function of taurine in eusociality. The effect of taurine as a eusocial enhancer in ants was measured using three indexes: residence number, cooperative defense index, and population density. The three indexes are summarized in a composite diagram.

2 Methods

2.1 Maintenance of Ant Farms

Stocks of carpenter ants (Camponotus japonicas) were purchased from Biobiba Ants (Daegu, Korea) and maintained in transparent PET bottles containing autoclaved soil. Ant farm structures were created by inserting a 500-ml PET bottle into a 2000-ml bottle. The larger bottle was cut open to accommodate the smaller one then sealed using transparent tape and the space between the two bottles was filled with autoclaved soil.

Ventilation holes were made by piercing the outer (2000 ml) bottle with a probe smaller than ant body size. Water was supplied through the ventilation holes using a squeezable bottle twice a week. The ant farms were wrapped in aluminum foil and kept in the dark. Ants were regularly fed with sucrose. Three groups of ants were fed a taurine-free or taurine-supplemented diet. Control, low-, and high-taurine diets were created using taurine and sucrose at 0, 5, and 20% (g/g), respectively. The effects of taurine were tested using a combination of ant eusocial indexes. Three indexes of social strength were used to study the effect of taurine on group activity: number of formicaries (chambers) formed, cooperative defense efforts, and population density (or group size and composition).

2.2 Observation of Newly Formed Formicaries

Ant colonies were initiated by implanting 1 queen and 10 workers into the ant farms. The farms were maintained for 30 days and fed with sucrose or a mixture of sucrose and taurine in the concentrations described above. The number of formicaries was recorded daily and newly formed formicaries were circled with a marking pen. The cumulative number of newly made formicaries was calculated every 3 days.

2.3 Assessment of Cooperative Defense Efforts

The level of cooperative defense effort was assessed by recording the time taken to complete the defense formation against a foreign queen. To initiate a defensive response, a foreign queen was carefully added to an established ant farm using a pair of forceps. When soldiers or workers responded to the extraneous queen, the time taken for four workers to pull each leg of the foreign queen was measured. The time taken in hours was converted into reciprocals to obtain the cooperative defense index (CDI). The CDI was calculated as follows: CDI = 1/(hours taken until completion of defense formation).

2.4 Comparison of Population Density

After 30 days, the number of ants and eggs in each group, and the composition of each group were measured. To count the number of ants in each class, the whole ant farm was emptied into a container and the soil was carefully removed. The number of ants was compared between taurine-free and taurine-supplemented groups.

2.5 Composite Strength Index

Composite diagrams for the three indexes of eusocial strength were prepared using a three-dimensional chart by integrating the social data from the three experiments: number of formicaries formed, CDI, and total numbers of ants and eggs. The performance of each group was categorically quantitated using a control performance of 100.

3 Results

Three eusocial strength indexes were measured to examine the effect of taurine on group activity: number of formicaries formed, CDI, and population density (group size and composition).

3.1 Taurine Promoted Construction of Formicaries

Ants began to assemble formicaries 3 days after they were introduced into the ant farms. The number of formicaries in the three different taurine concentrations in sucrose was counted every 3 days. Figure 1 shows the cumulative number of

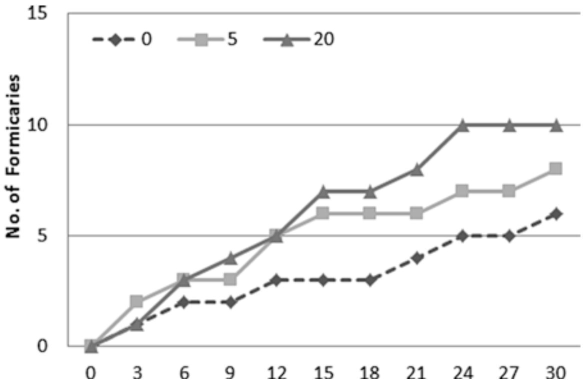

Fig. 1 Formicary Construction Comparison. After 3 days with three different taurine diets: control, low and high [0, 5, and 20% (g/g)], respectively, construction of formicary chambers was monitored. The number of formicary was counted every 3 days up to 30 days since the deployment of ants into the chamber culture bottles. The numbers represent those which were observed on the day of counting. The *x-axis* refers to days after transplantation and *y-axis*, # of formicaries

formicaries up to 30 days post-implantation of individuals into the ant farms. The number of formicaries increased as the taurine content in the ant diet increased. This indicated that taurine prompted ants to build more formicaries and/or that more dynamic activity, stimulated by taurine, resulted in a higher number of formicaries.

3.2 Cooperative Defense Index (CDI) Was Enhanced under Treatment with Taurine

Defensive responses were observed after a foreign queen was introduced. The defensive response began as early as 10 minutes after the implantation of a foreign queen. The cooperative defense index (CDI) was calculated using the reciprocal of the time taken to complete the defensive response, which was several ants holding each leg of the foreign queen. We compared the time taken for the defensive formation between taurine-free and taurine-supplemented groups. There was a significant difference in the time taken to complete the defense formation between the two groups. Taurine-supplemented groups exhibited more rapid response at all taurine concentrations than the control. Accordingly, the taurine-supplemented groups had a higher CDI (Fig. 2). That is, taurine-supplemented groups showed higher levels of readiness against the introduced foreign queen ant. Considering that successful defensive efforts are vital to maintaining a community, taurine may play a significant role in the protection of a eusocial community.

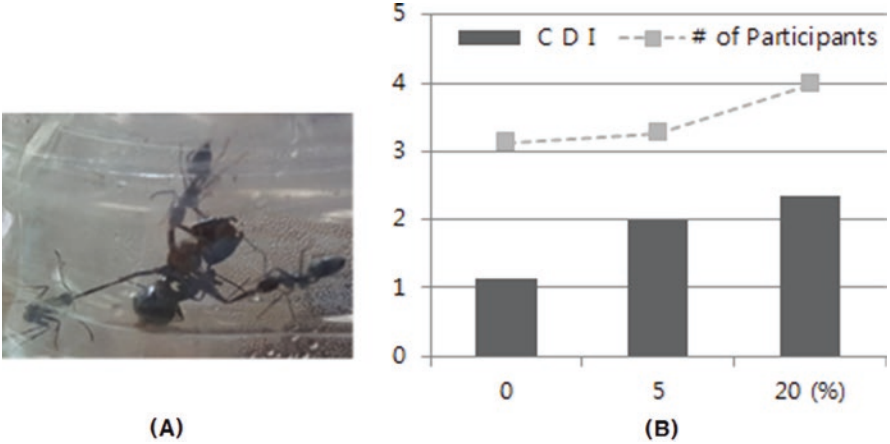

Fig. 2 Cooperative defense (CD) effort. Defensive response was observed immediately after the introduction of a foreign queen. The level of cooperative defense effort was quantitated based on the intensity of readiness against the introduced foreign queen ant. (**a**) Typical image of CD (**b**) CDI and number of participants in the CD at the three different taurine concentrations. CDI = 1/[F] (*F* hours taken for completion of the defense form against a foreign queen)

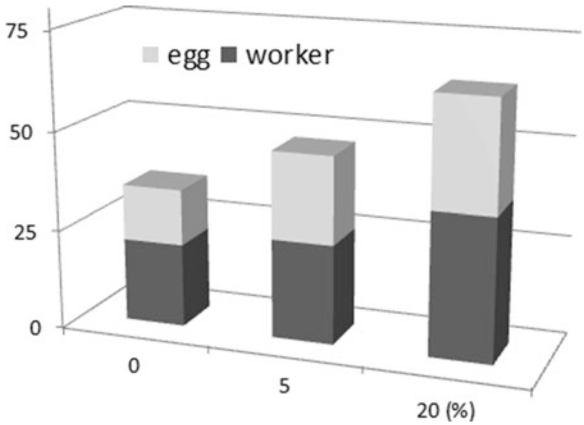

Fig. 3 Population size and composition. Ant farms were maintained for 30 days by feeding taurine-free or taurine-supplemented diet. At the termination of the experiment, the ant group was evaluated according to the total number and colony composition of worker and egg except for the queen

3.3 Taurine Increased the Size of Ant Community

Ant colonies were initiated using 1 queen and 10 workers and sustained for 30 days. After 30 days, population density and composition was compared among the three taurine-fed groups. There was a significant difference between the groups. Figure 3 shows the population size and composition of each group after 30 days. The high-taurine group had more workers and eggs than did the taurine-free group. There was at least a 40% difference between the taurine-free and the low-taurine group. There was also an obvious difference between high- and low-taurine groups, suggesting the effect was dependent on the concentration of taurine. There was a significant

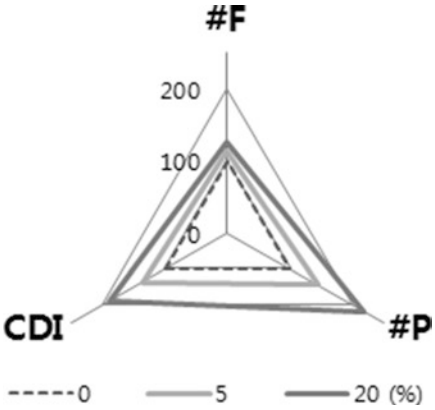

Fig. 4 Integration of the three eusocial indexes. Three indexes in this study were standardized to assess a specific effect of the taurine on the eusociality. *#F* # of formicaries, *#P* population size encompassing workers and eggs, *CDI* cooperative defense index

difference in egg number between taurine-free and taurine-supplemented groups. There was no significant difference between high- and low-taurine groups in the percentage of eggs.

3.4 Composite Eusocial Pattern Indicates the Size of Ant Community Is the most Affected by Taurine

The effects of taurine on eusocial indexes were evaluated using three-dimensional analysis. Three categories of eusocial performance were combined and expressed as a three-dimensional pattern of eusocial strength. The performance of each group was categorically quantitated using a performance control of 100. The most affected aspect was the population size and composition (Fig. 4). The high-taurine group showed the highest numbers of ants and eggs.

4 Discussion

Taurine consistently enabled ant members to enhance their eusocial performance. In the taurine-supplemented groups, ants built more formicaries in a dose-dependent manner. Taurine also enabled ants to respond quickly to a foreign queen and reduce the time taken to complete cooperative defense formations. Taurine augmented the fecundity of the queen ant and the care of offspring by workers. Undoubtedly the positive effects of taurine on all three indexes of group activity are important in sustaining a eusocial community.

Ants have an unusual amount of taurine compared to that in other insects. Ants are typically eusocial insects, and are known as true social insects with complex and diverse biological traits (Thorne and Traniello 2003). The taurine content in ants is comparable to that in the heart of cow (Bos taurus) and the muscle of tuna (Spitze et al. 2003). The basis for the unusually high taurine content in ants is unknown. The link between taurine and major features of ant sociobiology and physiology has not yet been elucidated; however, taurine may play a major functional role in ants that are members of a eusocial community.

Our results for population size and composition were unexpected. Since the queen is the only reproductive member of the community, her fecundity and capacity to care for the offspring reflect the overall viability of the ant community. The increased number of ants is a strong indicator of the total capacity for reproduction. Taurine may have exerted positive effects both on the queen and workers in producing and caring for offspring despite limited resources in the ant farms.

Variation in taurine content between insect species can explain the prevalence of eusociality in ants, which have very low taurine content. Taurine might have supported specific behaviors during the evolution of eusociality in ants. For example, taurine may have reduced stress from mental and physical exertion in obedience to the queen. In addition, ants, especially workers, perform their assigned roles incessantly. This would be impossible without taurine, which is a powerful antioxidant. Our results imply that ants may utilize taurine to reduce stress from being a member of a eusocial community. Ants were highly responsive to taurine supplements under reduced pressure. Ants with an additional supply of taurine built more formicaries. Also, ant fecundity and population size increased in response to taurine in a dose-dependent manner. Worker ants responded to the invasion of a foreign queen spent in a shorter amount of time when their food was supplemented with taurine.

Eusociality is a highly sophisticated system and a relatively recent development in this group of insects (Hunt 2012; Ratnieks and Helantera 2009). The evolution of eusociality involves an increase in complexity (Wilson and Holldobler 2005). The continual interaction between biological organization and natural selection results in a unique system requiring compulsory collaboration between individuals (Billen 1992). Although the eusocial system is a very efficient system for the survival of a group, it can be very stressful for individual members who must sacrifice their individual reproduction, and commit to the needs of the colony (Robinson 1992). Most members of an ant colony must provide continual labor without any direct individual advantage (Norman and Hughes 2016). Under these circumstances, the unusual amount of taurine in Formicidae may serve to relieve the stress in members of eusocial communities.

To our knowledge, this is the first report of the potential effect of taurine on eusocial organisms characterized using quantitative indexes of eusociality. The results strongly indicate that taurine plays an important role in maintaining ant communities as a eusocial superorganism. Taurine is one the essential biomolecules that sustains eusociality in insects and other higher organisms. Future study is necessary to determine whether taurine helps eusocial organisms increase their eusocial strength.

5 Conclusion

Taurine consistently augmented the level of eusociality in groups of ants fed diets supplemented with taurine. Ants built formicaries in response to taurine in a dose-dependent manner. Taurine also enabled ants to reduce the time required for cooperative defense. Taurine boosted the productivity of queen ants and the care of offspring by workers. This study increases our understanding of eusociality and the contribution of taurine to maintaining eusociality.

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