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Effects of Vespa Amino Acid Mixture on Exercise and Cellular Metabolism in Honey Bees

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Abstract

Vespa Amino Acid Mixture (VAAM) is a commercially available pre-workout supplement based on the chemical mixture secreted in saliva of larval Asian giant hornets (*Vespa mandarina*). VAAM is reported to increase aerobic capacity in a variety of animal species, however its mechanism of activity is not clear. To test effects of VAAM on cellular metabolism we exposed adult honey bees (*Apis mellifera*) to diets containing 5% glucose or 5% glucose and 0.3% VAAM (the manufacturer recommended dose). A subset of bees from each group were exercised to exhaustion by a forced swim test and then indicators of cellular metabolism were measured in carcass extracts of exercised and non-exercised bees. Surprisingly, bees treated with VAAM swam for significantly shorter periods of time than control bees (mean swim time 204 seconds vs. 1056 seconds, $p = 0.02$). While remaining ATP in the bee carcasses was not impacted by diet or exercise treatment, carcass isolates from bees fed VAAM had higher levels of free glucose than control bees (mean free glucose 24.1 mg/mL vs. 17.9mg/mL, $p = 0.04$), regardless of whether they had been exercised. These data suggest that VAAM increased mobilization of stored glucose, but it did not seem to increase efficiency of ATP production.

Keywords: Energy Metabolism, Exercise Capacity, Cellular Respiration, Glucose, ATP

1. Introduction

Vespa Amino Acid Mixture (VAAM) is made up of monomer amino acids, which, in combination, increase respiratory capacity of a variety of organisms.[1,3,4,8] These effects have supported use of VAAM as a human exercise supplement.[4,5] While the impacts of VAAM on exercise capacity and metabolic markers have been observed in several animal species, the mechanisms by which VAAM acts are still unknown. Commercially-produced VAAM replicates the saliva of Asian giant hornet larvae (*Vespa mandarina*), which is fed to parents during the period of larval care.[2,7] Adult hornets are incapable of consuming solid foods, so larval hornets produce VAAM to assist the adults to maintain their energy levels.[2] In other species VAAM impacts capacity for exercise and markers of cellular metabolism, suggesting that it alters mechanisms of chemical metabolism common to cellular respiration in animals.

Cellular respiration is a series of reactions that convert the simple sugar glucose and derive a final product of readily available chemical energy, adenosine triphosphate (ATP). Glucose is a simple sugar commonly found in complex carbohydrates and starches – a short term storage form of energy which can be converted into ATP through cellular respiration. ATP is a nucleoside that contains a large amount of available energy which is released when ATP is hydrolyzed. The hydrolysis of ATP allows for the stored energy to be used by organisms in day to day functions such as movement and other cellular functions. Exercise capacity is the point at which exhaustion is met due to low levels of ATP, caused by either insufficient amounts of glucose needed to produce ATP, or the inability of cellular respiration to convert glucose into ATP.

In this experiment, honey bees were given dietary VAAM to determine if it had any effect on exercise capacity, free glucose levels, or ATP levels. Previous experiments have shown that VAAM treatment increases exercise capacity in humans[4], mice[1], rats[8], and houseflies[3]. VAAM treatment also decreases blood lactate, a product of anaerobic cellular respiration, suggesting that VAAM increases the efficiency of ATP production.[1,8] By measuring exercise capacity alongside both glucose and ATP levels we hope to better understand the impacts VAAM treatment has on cellular metabolism.

2. Methods

2.1 Subjects And Dietary Treatments

Honey bees were collected from an artificial slide hive, and were caught and housed in pint size jars with cheese cloth covering. The bees were then divided randomly into two housing groups (N = 12 bees/housing group), one group received a 5% w/v glucose solution and the other received the same glucose solution with 0.3% VAAM-supplement. VAAM was added as Diet VAAM (Meiji Seika Kaisha; Tokyo, Japan), following manufacturer dosage recommendations (0.3% final concentration), so that no additional sugars were present in the VAAM-supplemented diet. The bees had *ad libitum* access to the assigned diet via a soaked cotton ball placed in a dish of liquid for a 24-hour period.

2.2 Evaluation Of Exercise Capacity

After the 24-hour feeding, bees from both diet groups were randomly selected for exercise (N = 6 bees/diet treatment group). Researchers used a forced swim test, in which the bees were placed in water slightly deeper than the bee's legs and forced to swim until exhaustion, which was counted as five consecutive seconds of non-movement. Time to exhaustion was recorded for each bee. After reaching exhaustion the bees were euthanized by freezing to ensure no further cellular activity. Non-exercised bees were also euthanized by freezing.

2.3 Carcass Isolate Preparation And Analysis

Bee carcasses of all of the four test groups (control diet with exercise, control diet with no exercise, VAAM-treated diet with exercise, VAAM-treated diet with no exercise) were individually weighed and then ground up in a 1% phosphate buffered saline (PBS) solution using a mortar and pestle, and strained through 4 layers of cheesecloth. The volume of PBS used was 2 mL per 0.1 g of carcass mass. The resulting carcass isolates were run through two assays, the first tested free glucose concentration by inducing glucose-dependent color change which was detected through spectrophotometry (Glucose (GO) Assay, Sigma-Aldrich; St. Louis, USA). The second assay tested for ATP concentration by treatment with an enzyme which interacts with ATP to cause luminescence (CellTiter-Glo® 2.0 Assay, Promega; Fitchburg, USA).

2.4 Statistical Analysis

Data were analyzed using JMP Pro 11 software (SAS Institute; Cary, USA). A one-way ANOVA was used to compare swimming endurance duration of control and VAAM-supplemented diet groups. Glucose and ATP concentrations of carcass isolates were compared using two-way ANOVA with diet treatment (control or VAAM-treated) and exercise (with or without) as factors. A Tukey test was used to rank significantly different treatment groups.

3. Results

The effects of VAAM on swimming endurance, glucose, and ATP production were tested in honey bees. The average swimming endurance time of each group of bees is shown in figure 1, with the control bees at an average of 1056 seconds, while VAAM bees swam an average of 204 seconds The VAAM-treated bees swam for a significantly shorter amount of time (t-test, $p = 0.02$). The VAAM-treated groups showed an overall increase in carcass glucose levels regardless of exercise treatment (ANOVA, $p = 0.04$), but not a significant difference between control and VAAM-treated diet groups, as shown in figure 2. VAAM-treated honey bee carcasses contained more free glucose – an average of 24.1 mg glucose/mL isolate as opposed to 17.9 mg glucose/mL isolate (ANOVA, $p = 0.04$). The carcass isolate ATP levels showed no significant difference between the control and VAAM-treated groups, or between exercised and non-exercised groups, as shown in figure 3.

4. Discussion

Surprisingly, the honey bees treated with VAAM swam for a significantly shorter amount of time than bees that did not receive the VAAM treatment, which contradicts the results found in other animal species.[1,3,4,8] It would seem that under these conditions VAAM supplementation is actually counterproductive to exercise endurance. It is possible that honey bees respond differently to the substance than other organisms, but this seems unlikely given that honey bees are evolutionarily more closely related to hornets, the reported natural target of VAAM[7], than any of the other species in which VAAM has been studied, making them more likely to share the same mechanism of its effect. It seems more likely that the duration of exposure prior to exercise interfered with the metabolism of glucose and limited ATP production and exercise capacity of VAAM-supplemented honey bees. The honey bees were allowed to feed for a twenty four hour period, while previous experiments using houseflies used a maximum of fifteen minutes exposure to the VAAM treatment before exercise (Linville, Kaur, and Davis, personal communication, April 28, 2016). This additional treatment time may have allowed for a shift in cellular respiration that has not been observed in previous, relatively short term, experiments. Previous research has not assessed the impacts of VAAM treatment at intervals longer than 180 minutes, with most experiments using a thirty minute period between VAAM consumption and exercise. The effects of VAAM on swimming endurance have been observed to peak at 30 minutes post-exposure and return to basal levels by 120 minutes post-exposure[1], so it is possible that the longer duration of VAAM treatment in the current experiment altered ATP metabolism.

The average free glucose levels of carcass isolates did differ between the diet treatment groups (figure 2), with higher free glucose in VAAM-treated bees. This difference could be due to the fact that VAAM treatment facilitates the release of glucose from stored glycogen without facilitating the transfer of energy from glucose into ATP. The ATP levels of carcass isolates showed no significant impact from diet or exercise treatments (figure 3). During cellular respiration, when more glucose is present reactions should proceed for more ATP to be produced, thus increasing capacity for exercise, but the VAAM treated honey bees failed to show this effect. A potential reason for increased glucose without an increase in ATP may be due to metabolic stress caused by an overexposure to VAAM. Cells under stress can hydrolyze stored glycogen to release glucose[6], so it is possible that VAAM treatment induces stress at a cellular level. The apparent blockade between glucose and ATP in VAAM-treated honey bees suggests metabolic disruption, and supports the hypothesis that VAAM impacts a component of the cellular respiration pathways.

Cellular respiration is a key part of an organism's daily function because it is how energy is transformed into a form cells can use. Energy is not only important for movement of organism, but also for internal function of cellular processes. VAAM treatment showed no effect on ATP levels in honey bees, but, under these conditions, it seems to have altered glucose metabolism leading to lower capacity for producing and exerting energy. A further test of this effect would be to vary the exposure duration of VAAM treatment for the organism, because VAAM-supplementation for 24 hours may be causing metabolic stress. This stress could be the cause of the shorter swim test results and high glucose levels in the VAAM-supplemented honey bees, and warrants further investigation.

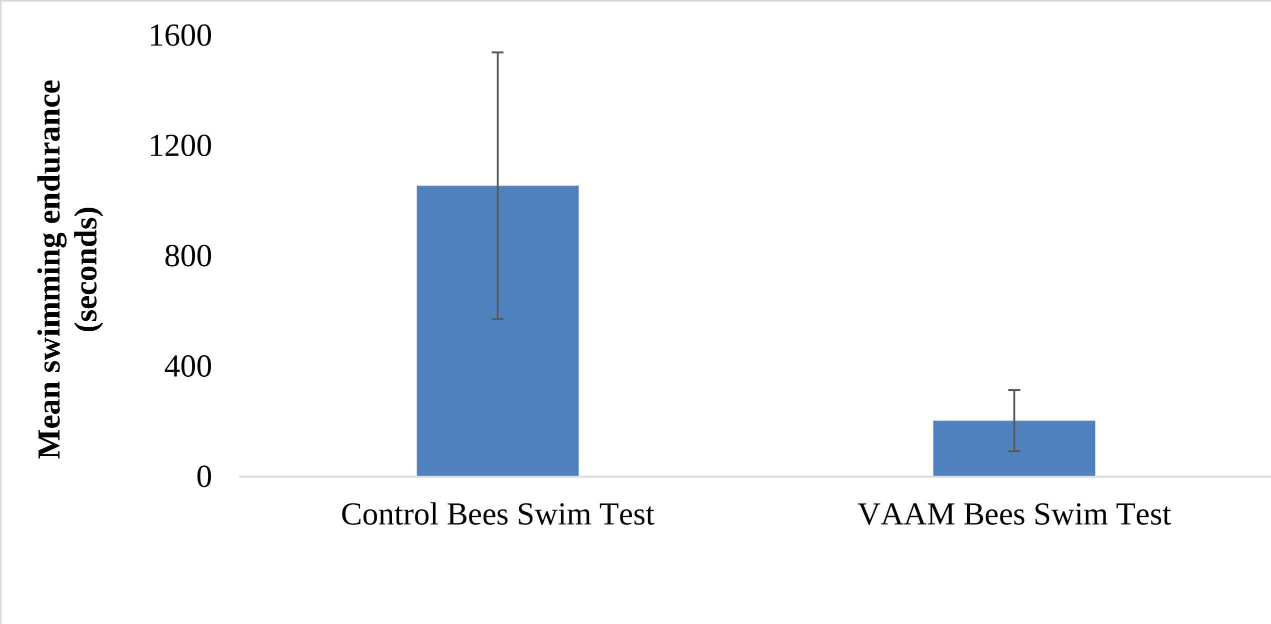

Figure 1. Swimming endurance of control and VAAM-supplemented bees based on time until exhaustion (five seconds of no motion) when placed in water (N = 6 per treatment, mean +/- standard error)

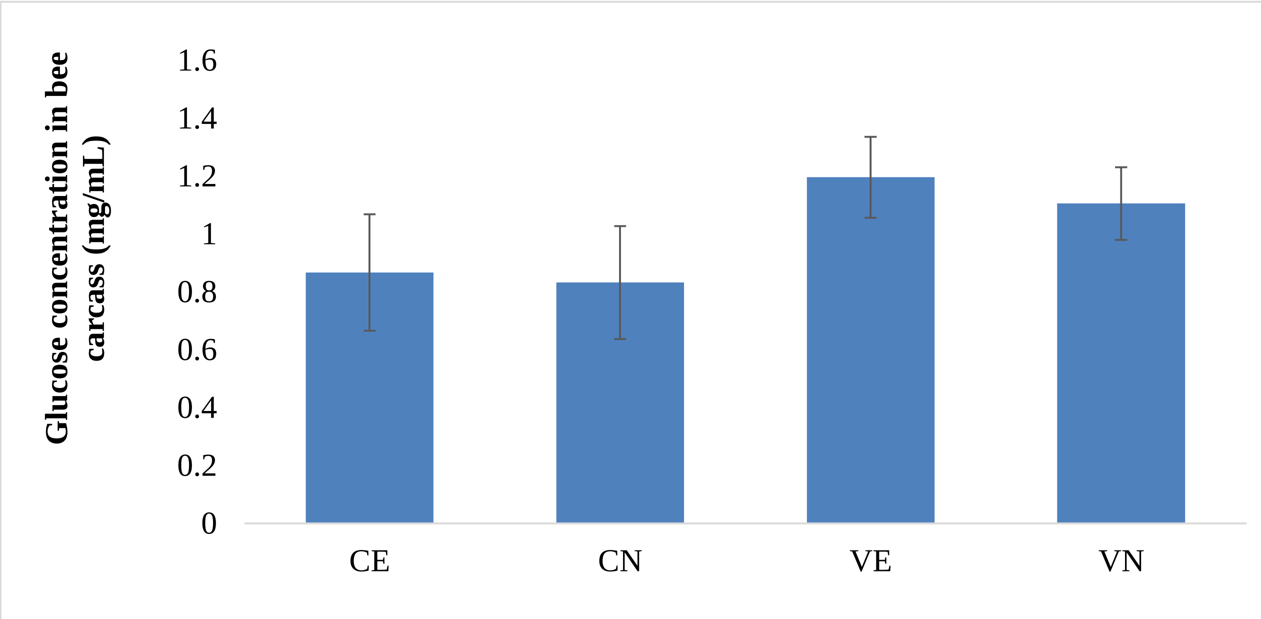

Figure 2. Glucose concentration in bee whole-carcass isolates following dietary and exercise treatments based on a colorimetric assay using glucose oxidase. (N = 6 per treatment group, mean +/- standard error, CE = control diet with exercise, CN = control diet with no exercise, VE = VAAM-supplemented diet with exercise, VN = VAAM-supplemented diet with no exercise)

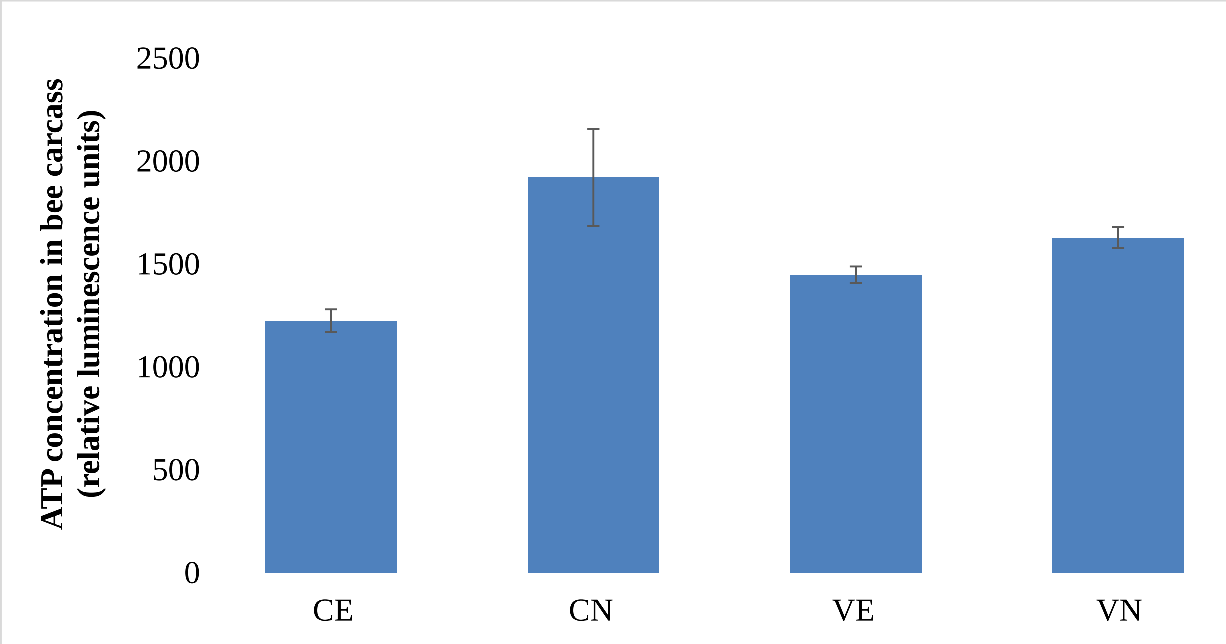

Figure 3. ATP concentration in bee whole-carcass isolates based on luminescence in a luciferase assay. (N = 6 per treatment group, mean +/- standard error, CE = control diet with exercise, CN = control diet with no exercise, VE = VAAM-supplemented diet with exercise, VN = VAAM-supplemented diet with no exercise)

5. References

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