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Taking the Bitter with the Sweet - A Preliminary Study of the Short-Term Response of Horses to Various Tastants in Solutions

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Abstract

Horses can distinguish sweet, salty, sour, and bitter tastes, but little is known about their preferences for various tastants. Understanding horse taste preferences can aid in increasing water intake by adding a preferred tastant or by masking an unpleasant taste to encourage administration of medications, for example. The quantity of water intake by horses was examined over five separate trials involving a two-choice preference test between tap water and water containing varying concentrations of sucrose (0-50g/100ml), citric acid (0-2.43mg/100ml), quinine (0-30mg/100ml) or a mix of sucrose (10mg/100ml)/citric acid (1.31mg/100ml) and sucrose (10mg/100ml)/quinine (20mg/100ml). Horses (n = 5) showed a weak preference for sweetened water up to 10mg/100ml ($p < .001$), with a rejection at higher concentrations. Horses rejected all concentrations of both sour (n = 12 horses; $p < .001$) and bitter (n = 6 horses; $p < .001$) solutions. In the mixed tastant trials, sucrose mixed with citric acid was only weakly rejected compared to the sucrose solution alone, which was moderately rejected (n = 5 horses; $p < .001$). Similarly, mixed sucrose/quinine solution intake increased over the quinine solution alone (n = 9 horses; $p < .001$). There was a large variation among individual horses within each trial, with some horses strongly rejecting sucrose solutions and others strongly preferring citric acid solutions. No horse indicated a preference for bitter solution in any trial. Age ($p < .001$), breed ($p < .001$), and exercise ($p = .004$) all influenced total fluid intake in the sour trial, not dependent on treatment ($p = .063$). These preliminary results show that some horses appear to prefer sweet and a preferred tastant can mask a less preferred tastant.

Keywords

Taste perception; preference test; water intake; sour taste

1. Introduction

Taste perception requires the integration of olfactory and gustatory (taste) stimuli to identify five basic tastes: sweet, salty, sour, bitter, and savory (umami). Horses are known to discriminate sweet, salty, sour, and bitter tastes although umami has not been tested [1]. Taste receptors are modified epithelial cells densely packed into groups called taste buds that are found mainly on the tongue [2] with each receptor coding for only one taste. Sweet and bitter tastes involve G-protein receptors [1] while sour and salty tastes activate ion channels and act upon both Na+ and H+ receptors [3,4].

Taste buds advise the animal about the substance in the mouth [5] by sending information to the brain thereby gathering information from the environment to determine what is edible [6]. Sweet and umami tastes signal the energy density of a food source and animals will eat to meet caloric requirements [7]. Bitter and sour are both aversive tastes that inform the animal of spoiled or toxic food to be avoided [8]. Sour taste may be buffered by constituents in an individual's saliva [9]. The desire for salty taste may be affected by the balance between cellular water concentration and intracellular sodium concentration [10]. Over time, animals develop behavioral mechanisms to allow recognition of foods based on nutritional properties and post-ingestive consequences [11].

Although the equine nutrition and pharmaceutical industries are large, little previous work has been done on the taste sensitivities of horses. The most comprehensive work was carried out by Randall *et al.* in 1978 who reported on the discrimination of sweet, salty, sour, and bitter solutions in immature horses. The tested foals preferred a sucrose solution over tap water at concentrations ranging from 1.25 to 10 % sucrose. Above and below this concentration, they were indifferent. For salty, sour, and bitter solutions, the foals showed no preference until a concentration respectively of 0.63% NaCl, 0.16% acetic acid, and 20% quinine. Above those concentrations, all solutions were rejected. In similar experiments, goats and cattle preferred sucrose solutions; however, sheep [12] rejected the sucrose solutions, and chickens [13] and geese [14] were indifferent. Foals [15] appeared slightly more tolerant of higher concentrations across all four tastants than goats, sheep, or cattle [12,16–18]. A more recent study supported Randall *et al.*'s [15] original findings, indicating that the addition of sweet feed to the drinking water of hospitalized horses increased overall fluid intake [19].

Water consumption by horses is one of the key factors in their sporting performance. Endurance riding, for example, is undoubtedly one of the equestrian disciplines where hydration is essential as dehydration can quickly lead to a drop in performance and even more seriously to various pathologies such as colic or myositis [20]. Horses can become rapidly dehydrated due to their hypertonic sweat [20]. Rehydration can be accomplished more efficiently by providing electrolyte solutions rather than by oral pastes, and solutions containing dextrose facilitate sodium absorption and provide energy for ATP production [20]. As it is best to provide electrolytes in water, adding a preferred tastant could increase water intake. Preferred tastants can also be used to increase the palatability of various products. For example, medications often possess a bitter taste, and masking the bitterness with a taste that the horse particularly likes can facilitate administration and avoid rejection of the drug [20].

Improving the palatability of feedstuffs may increase the initiation of consumption and the total quantity consumed [21] but it requires understanding the taste preferences of the species. Horses are known as picky eaters and prefer to forage on a wide variety of feedstuffs [22] despite having little apparent cognition of post-ingestive feedback to avoid certain foods [23]. It could be that horses have individual taste preferences that supersede nutritional intelligence, meaning that they select what to ingest based on their preference rather than whether that substance is good for them. Researchers can measure preferences by presenting animals with a choice between different tastants. Adding a tastant to water instead of feed allows the direct determination of the effect of the tastant rather than the effects of ingestion and nutritional content associated with the food [24]. Taste and smell are inextricably linked, and little research has been done on the reception and sensitivity of horse taste and smell as compared to other mammals (but see [25]).

Animal behaviors in relation to taste are better described as evidence of preference, aversion, or indifference than as a neurobiological response [26]. To determine preference, aversion, and non-discrimination in two-choice preference tests the mean percent of treatment consumed is theoretically assumed to be 50% of total intake for control or non-discriminate tastes. Using a 95% confidence interval, consumption between 40-60% determines non-discrimination. A consumption level below 40% indicates aversion, with < 20% being a strong aversion. Likewise, above 60% is a preferred substance, with >80% being a strongly preferred substance [16]. This method of assuming preference and aversion to different tastants in solution has been widely used [12–18,27,28].

The purpose of this preliminary study was to determine preference or aversion of horses to sweet, sour, and bitter tastes or mixtures of these. It was hypothesized that horses would show a preference for sweet solutions but a rejection of bitter and sour solutions compared to untreated tap water. Based on these assumptions, it was further hypothesized that the provision of a bitter or sour solution mixed with sweet would increase the horse's acceptance.

2. Materials and Methods

2.1. Research Protocol

A total of 37 horses participated in five different trials. Each trial was performed independently with no horse participating in more than one trial. The number and details of horses participating in each trial are indicated below. All of the trials with the exception of the bitter trial were held at the same facility. All horses were housed in individual box stalls bedded with wood shavings and received group turnout daily. All horses were lesson horses participating in weekly beginner and intermediate English riding lessons with the exception of the sour trial. The horses participating in this trial were draft horses and Caspians some of whom were in light training (English riding) and some who were not ridden at all. All horses were fed hay and concentrates according to their needs. All horses had been present in the facility for at least three weeks prior to any testing to acclimate them to the local water.

2.2. General Procedure

Horses were exposed to a two-choice preference test during the time they were in their stalls. The order of treatments was randomized across horses with each horse receiving each treatment for four or five days with at least two days washout in between treatments. Each horse was presented with two identical 19L black water buckets that were scrubbed and rinsed clean of any debris each day prior to filling with water. One bucket contained the treatment solution and the other bucket contained the untreated tap water. Buckets were hung on the front wall of the stall and the position of each bucket was alternated each day to account for any side effects. Buckets were observed multiple times throughout the day and weighed (Matzuo hanging digital fishing scale, Morehead City, NC, USA) and refilled if they contained less than two-thirds the volume to ensure a choice was available at all times. All buckets were weighed and refilled at 9 pm and again at 6 am to make certain free choice water was available throughout the night. Daily values of the volume of tap water and treatment solutions consumed were calculated and expressed as a percent of the total amount of water consumed. Ambient temperature and water temperature were recorded at each weighing time.

2.3. Trial 1: Sweet Tastant

Five horses (1 mare, 4 geldings) of various breeds participated in this trial ranging in age from 8-17 years. Each horse was ridden for 5-10 h/week in light training consisting of intermediate-level English riding lessons. Six different concentrations of sucrose (Lantic and Rogers, Toronto, ON, Canada) were tested (0, 1, 5, 10, 20, 50 g/100mL) based on the results from **[15]**. The trial lasted six weeks with each horse receiving each treatment for five consecutive days with two washout days in between.

2.4. Trial 2: Sour Tastant

Twelve horses (1 mare, 2 stallions, 9 geldings) participated in this trial ranging in age from 2-18 years. Three of the horses were Caspians and the remainder were draft or draft crosses. Exercise in the form of riding ranged from 0-5h/week of light training consisting of English-style flat work to maintain fitness. Citric acid (Rougier, Mirabel, QC, Canada) was added to the water at 0, 0.49, 1.31, and 2.43 mg/100ml to obtain a pH of 7.6, 5.2, 3.6, and 3.0 respectively. These solutions corresponded to neutral, detectable, weakly sour, and moderately sour **[9]**. The trial lasted four weeks with each horse receiving each treatment for four consecutive days with two days wash out in between. A treatment solution sample was taken each time a new solution was added to the bucket for pH measurements (Fuzion CL-500 pH meter, Fisher Scientific, Mississauga, ON).

2.5. Trial 3: Bitter Tastant

Six Thoroughbred geldings between 3-10 years participated in this trial. All horses were worked under saddle 5-10h/week in light training consisting of English-style riding as determined by their owners. Four different concentrations of quinine monohydrochloride dihydrate (Sigma-Aldrich, Oakville, ON, Canada) were tested at 0, 10, 20, and 30 mg/100ml based on the results from **[15]**. The trial lasted four weeks with each horse receiving each treatment for four consecutive days with three days wash out in between.

2.6. Trial 4: Mixed Sweet plus Bitter Tastants

Nine horses (2 mares, 7 geldings) of various breeds ranging in age from 5-13 years participated in this trial. All horses were worked under saddle between 3-6h/week in light training consisting of intermediate-level English riding lessons. Four treatments were tested based on the results of the previous experiments: tap water, a bitter solution with quinine monohydrochloride dehydrate (20 mg/100ml; Sigma-Aldrich, Oakville, ON, Canada), a sweet solution with sucrose (10g/100ml; Lantic and Rogers, Toronto, ON, Canada), and a mixed solution of quinine monohydrochloride dehydrate and sucrose at a concentration of 20mg/100ml and 10g/100ml respectively. The trial lasted four weeks with each horse receiving each treatment for five consecutive days followed by two washout days in between.

2.7. Trial 5: Mixed Sweet plus Sour Tastants

Five horses (3 mares, 2 geldings) of various breeds ranging in age from 5-13 years participated in this trial. All horses were worked under saddle between 3-6h/week in light training consisting of intermediate-level English riding lessons. Four treatments were tested: tap water, a sour solution with citric acid (1.31 mg/100ml; Rougier, Mirabel, QC, Canada), a sweet solution with sucrose (10g/100ml; Lantic and Rogers, Toronto, ON, Canada), and a mixed solution of citric acid and sucrose at a concentration of 1.31mg/100ml and 10g/100ml respectively. The trial lasted four weeks with each horse receiving each treatment for five consecutive days followed by two washout days in between. A treatment solution sample was taken each time a new solution was added to the bucket for pH measurements (Fuzion CL-500 pH meter, Fisher Scientific, Mississauga, ON).

2.8. Data Analysis

A general linear mixed model with repeated measures was used to analyze the effect of the percent of treatment solution consumed by the horses using SPSS (v28.0.1.1, IBM Statistics, Armonk, NY, USA). Each trial was analyzed separately. The model included week, day, temperature, treatment bucket side and their interactions as fixed factors and horse as the random factor to determine their effects on the amount of treatment water consumed and the total amount of water consumed. Horse age, breed, exercise, and solution pH were included as fixed factors in Trial 2 (sour tastant), and exercise was included as a fixed factor in both mixed tastant trials (Trials 4 and 5). Estimated marginal means determined differences among levels of significant factors. The volume of solutions consumed by the horses is presented in terms of discrimination zones as reported by Randall *et al.* **[15] (Figure 1)**.

3. Results

3.1. Trial 1: Sweet Tastant

Horses displayed a weak preference for sucrose solution at concentrations of 1, 5, and 10 g/100ml and moderate to strong rejection at concentrations of 20 and 50 g/100ml respectively compared to tap water ($F(4,125) = 26.045$, $p < .001$; **Figure 2**).

There was no effect of week ($F(4,125) = 0.611$, $p = .656$), day ($F(4,125) = 1.920$, $p = .111$), the position of the buckets ($F(1,125) = 0.064$, $p = .800$) or the ambient temperature ($F(1,124) = 0.092$, $p = .792$) on the percent consumption of sucrose solution by the horses. Horses did not differ in their individual intake of treatment solutions ($F(4,132) = 0.159$, $p = .959$).

The total amount of fluid consumed (tap water plus sucrose solution) was not influenced by treatment ($F(4,130) = 1.163$, $p = .330$). However both week ($F(4,135) = 4.135$, $p = .006$) and day ($F(4,135) = 12.875$, $p < .001$) did influence the total amount of fluid consumed, with less total fluid consumed in the second week (average 28.6 ± 10.22 kg/d) compared to the fifth week (average 37.2 ± 11.68 kg/d), and less water consumed on Day 1 (average 23.9 ± 9.42 kg/d) than the other four days (average 34.3 ± 9.16 kg/d).

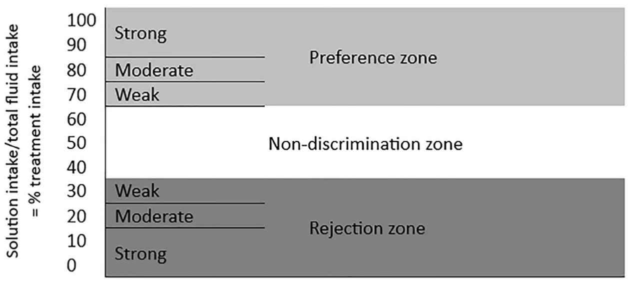

Figure 1: Preference, rejection, and non-discrimination zones in two-choice preference tests where the percent of solution consumed is theoretically 50% of total intake. Using a 95% confidence interval, consumption between 40 and 60% determines non-discrimination. Consumption below 40% indicates rejection (dark grey area) and above 60% is a preference (light grey area). Adapted from Figure 1 in [15].

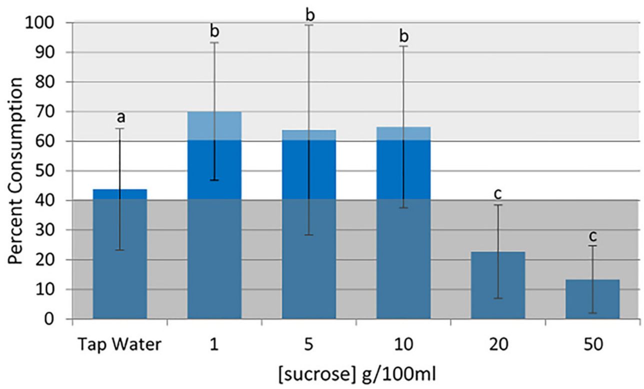

Figure 2: Mean percent (±SD) of water sweetened with sucrose at varying concentrations consumed by horses (n = 5). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) and above 60% is a preference (light grey area) according to [15]. a, b, c differ $p < .001$.

3.2. Trial 2: Sour Tastant

Due to variations in the daily pH of tap water, each treatment had a pH that varied over the course of the trial **(Table 1)** however no treatments overlapped in the pH readings.

Table 1: pH measurements of water with various concentrations of citric acid added in a two-choice preference test to determine taste preferences of horses (n = 12). pH measurements were taken multiple times per day and averaged over the trial.

Treatment (citric acid in water mg/100ml)	Mean pH ± SD	Minimum pH	Maximum pH
0 (Tap Water)	7.55 ± 0.116	7.28	7.89
0.49	5.17 ± 0.34	4.69	6.57
1.31	3.62 ± 0.11	3.38	3.96
2.43	3.02 ± 0.06	2.87	3.13

Horses consumed more tap water than any other treatment ($F(3,130) = 15.114$, $p < .001$; **Figure 3**). Horses displayed a weak rejection of tap water, a moderate rejection of the lowest concentration, and a strong rejection of the two highest concentrations of citric acid.

Horses consumed more citric acid solution during the first (average 30.8%) and second (average 34.8%) weeks compared to the third (average 21.7%) and fourth (average 16.2%) weeks ($F(3,130) = 10.159$, $p < .001$). There was no interaction of treatment by week ($F(9,49) = 1.714$, $p = .111$).

Ambient temperature did influence the percent citric acid solution consumed ($F(1,130) = 11.953$, $p < .001$) with no clear pattern (i.e. higher temperature did not correlate to higher intake). Ambient temperature within the barn ranged from 18-33 °C and water temperature ranged from 11-29 °C over the course of the trial. There was no effect of day ($F(3,133) = .788$, $p = .508$), position of the buckets ($F(1,146) = 3.781$, $p = .054$), horse age ($F(1,133) = 1.095$, $p = .297$), exercise ($F(1,133) = 0.036$, $p = .850$) or breed ($F(1,133) = 0.892$, $p = .347$) on the percent citric acid solution consumption.

Horses consumed less total fluid (tap water plus treatment solution) in the first (average 11.3 kg/d) and second (average 12.3 kg/d) weeks compared to the third (average 16.1 kg/d) and fourth (average 17.6 kg/d) weeks ($F(3,142) = 26.712$, $p < .001$). The total amount of water consumed was not influenced by treatment ($F(3,142) = 2.488$, $p = .063$). Breed ($F(1,142) = 22.312$, $p < .001$) influenced the total amount of water consumed with draft horses (average 17.4 ± 8.60 kg/d) drinking more than Caspian horses (average 5.2 ± 3.27 kg/d). Horses less than 8 years old (average 15.5 ± 9.25 kg/d) consumed more total water than horses 8 years and older (average 12.7 ± 9.07 kg/d; $F(1,142) = 18.938$, $p < .001$). Horses that worked 3-5 hours per day consumed more (average 18.2 ± 6.87 kg/d) than horses who worked one or less hours per day (average 9.15 ± 6.57 kg/d; $F(1,142) = 8.583$, $p = .004$).

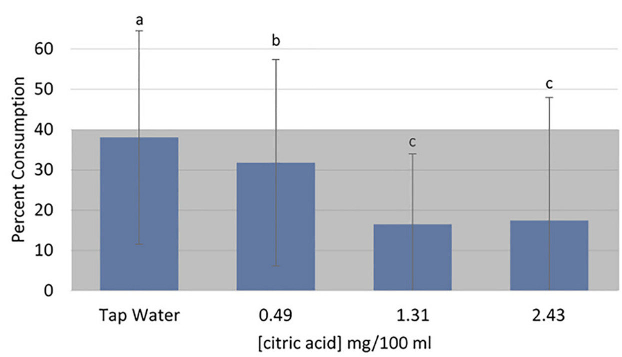

Figure 3: Mean percent (±SD) of water treated with citric acid at varying concentrations consumed by horses (n = 12). The pH of tap water was 7.6, with the pH of the citric acid treatments corresponding to 5.2, 3.6, and 3.0, respectively. Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* [15]. a, b, c differ $p < .001$.

3.3. Trial 3: Bitter Tastant

Horses consumed more tap water and less 10 and 30 mg/100ml quinine solution while the 20 mg/100ml solution was intermediate (F(3,70) = 11.604, p < .001; **Figure 4**). There was a weak rejection of the 20 mg/100ml solution, a moderate rejection of the 10 mg/100ml solution and a strong rejection of the 30 mg/100ml solution.

Horses consumed more quinine solution during week 3 (average 43.6%) compared to week 4 (average 21.1%; F(3,70) = 3.681, p = .016). There was a treatment by week interaction (F(7,72) = 14.903, p < .001). There was no effect of day (F(3,70) = 0.405, p = .750), horse (F(5,70) = .937, p = .463), position of the buckets (F(1,77) = 3.588, p = .328) or temperature (F(1,70) = 0.001, p = .976) on quinine solution consumed. The temperature inside the barn during the trial remained above 0 °C but was not greater than 10 °C.

Treatment had no effect on the total quantity of fluid (tap water plus treatment solution) consumed by the horses (F(3,80) = 1.985, p = .123). There was an effect of week (F(3,80) = 8.679, p < .001) with horses drinking less total water during the first week (average 14.7 ± 4.98 kg/d) compared to the other three weeks (average 20.8 ± 5.69 kg/d).

3.4. Trial 4: Mixed Sweet plus Bitter Tastants

Horses showed a strong rejection of the bitter solution compared to all other treatments (F(3,149) = 30.325, p < .001). The percent intake of tap water, sweet, and mixed solutions were similar **(Figure 5)**.

There was no effect of week (F(3,149) = 2.468, p = .064), day (F(4,149) = 0.673, p = .612), horse (F(8,149) = 1.047, p = .404), exercise (F(1,149) = 1.018, p = .315), position of the buckets (F(1,149) = 2.230, p = .137) or ambient temperature (F(1,149) = 1.489, p = .224) on the percent of treatment solution consumed by the horses. The temperature inside the barn during the trial remained above 3 °C and was not greater than 15 °C.

Horses drank less total fluid (tap water plus treatment solution) when provided with bitter treatment (average 31.9 ± 8.94 kg/d) compared to the sweet treatment (average 36.7 ± 11.63 kg/d; F(3,165) = 4.922, p = .003).

3.5. Trial 5: Mixed Sweet plus Sour Tastants

Horses consumed more tap water compared with all other treatments. The quantity consumed for sweet, sour, and mixed solutions was similar (F(3,63) = 11.410, p < .001; **Figure 6**) with sweet water being moderately rejected and the sour and mixed solutions weakly rejected.

Horses consumed more treatment water during week 2 (45.4%) compared to week 1 (28.4%) with the other weeks not differing (F(3,63) = 7.970, p < .001). There was a treatment by week interaction (F(7,64) = 2.932, p = .01). There was no effect of day (F(3,63) = 1.350, p = .266), horse (F(4,60) = .831, p = .511), position of the buckets (F(1,63) = 1.373, p = .246), exercise (F(1,63) = 1.467, p = .230), treatment pH (F(1,63) = 0.008, p = .930) or temperature (F(1,63) = 2.597, p = .112) on treatment water consumed. The temperature inside the barn during the trial remained above 5 °C but was not greater than 16 °C.

The total amount of fluid consumed (tap water plus treatment solution) was not influenced by treatment (F(3,56) = 2.303, p = .087).

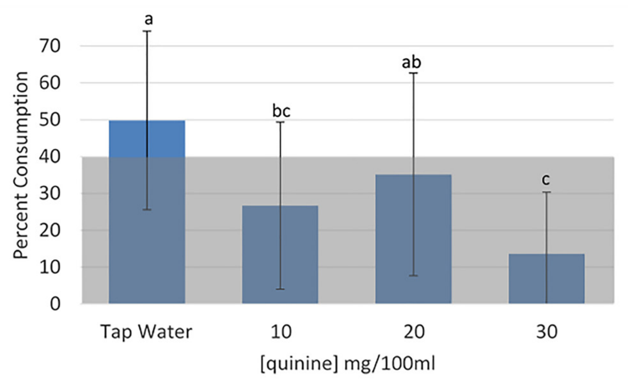

Figure 4: Mean percent (±SD) of water treated with quinine at 0 (tap water), 10, 20, and 30 mg/100ml consumed by horses (n = 6). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* **[15]**. a, b differ p < .001.

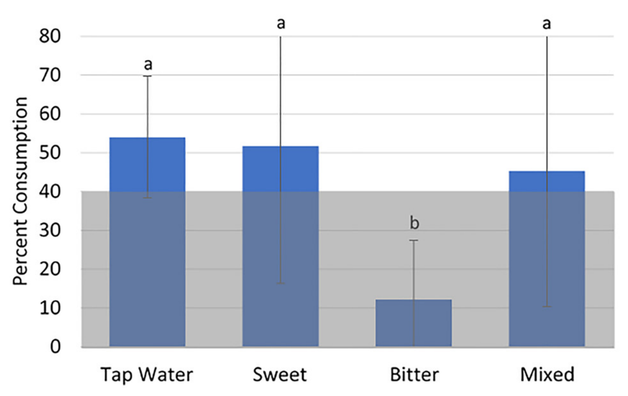

Figure 5: Mean percent (±SD) of water consumed by horses (n = 9) consisting of tap water, sweet solution (sucrose concentration of 10g/100ml), bitter solution (quinine concentration of 20mg/100ml), and mixed solution (containing both sucrose (10 g/100ml) and quinine (10 mg/100ml)). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* **[15]**. a, b differ p < .001.

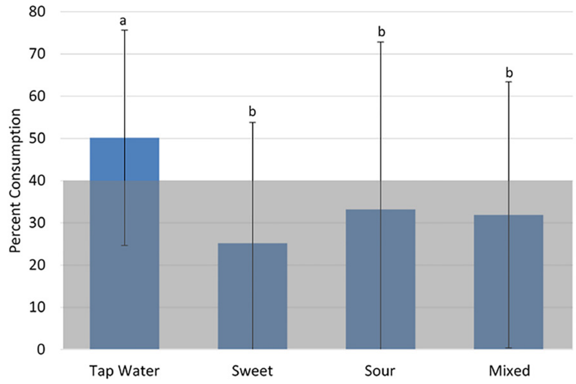

Figure 6: Mean percent (±SD) of water consumed by horses (n = 5) consisting of tap water, sweet solution (sucrose concentration of 10g/100ml), sour solution (citric acid concentration of 1.31 mg/100ml), and mixed solution (containing both sucrose (10 g/100ml) and citric acid (1.31 mg/100ml)). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* [15]. a, b differ $p < .001$.

4. Discussion

In a two-choice preference test, horses showed distinct preferences for various tastants provided to them in solution indicating that they are able to discern sweet, sour, and bitter tastes. Similar to previous research [15] the results of our various trials show that horses, when given a choice between tap water and sweet solution, displayed a weak preference for sweet, and when given a choice between tap water and sour or bitter solutions, showed a rejection of sour and bitter tastants. We also showed that a less preferred taste can be masked by a more preferred taste which is important when having to administer certain substances like medications.

These results should be interpreted with caution since all trials in this study were carried out independently and involved small numbers of horses. Only a sufficient number of horses participated in the sour trial to be able to analyze age, breed, and exercise factors on total water intake. Additionally, preference tests can only measure an animal's preference of one choice in comparison to another, which may not be indicative of the animal's overall preference [29]. One drawback of this forced choice test is that the consumer does not have the ability to state "no preference." Thus while it is assumed that a 50% consumption of one option indicates no preference, it can just as easily mean that half of the consumers preferred option A and the other half preferred option B [30]. However, our results are not dissimilar to other published studies.

The addition of sweet to various feedstuffs made available to horses is not uncommon. Perhaps it is because humans find sweet flavors so attractive [31] that perpetuates the idea that non-human animals should also prefer sweet. Indeed, sweeteners are routinely added to piglet diets to encourage feed intake upon weaning [32]. Limited research in this area shows that horses do have some preference for sweet [15,19,33] but perhaps this is overrated. Our results in the sweet trial, similar to [15], show only a weak preference for sweet when tested alone, and in our mixed tastant trials, horses showed no preference or even a moderate rejection of sweet solutions. Likewise, when testing taste additives to feedstuffs, results from other studies showed horses preferred salt and sour apple pellets over sweet apple or sugar beet pellets [34] and preferred higher protein content versus sweetener [25].

Our results showed all concentrations of sour solutions provided to the horses were rejected whereas Randall *et al.* [15] reported no discrimination of sour solutions in weanlings until a pH of 3.1. This difference could be due to the fact that Randall *et al.* [15] used acetic acid in their solutions while we used citric acid. Although response to sour taste is pH dependent, the pH of a solution is not necessarily proportional to the magnitude of sourness [35]. Citric acid ($C_3H_5O(COOH)_3$) has a higher pH and greater solubility than acetic acid (CH_3COOH) and is the most widely used organic acid in the food industry due to its appealing effects on taste [36].

Horses in our study showed a rejection of all bitter solutions whereas Randall *et al.* [15] showed no discrimination at the lower concentrations of bitter. This could be a result of acclimatization since the concentration of bitter solute was doubled every other day in Randall *et al.*'s [15] protocol whereas in our study the horses were presented with the randomized concentration of bitter solution; some horses received a higher concentration for the first week followed by a lower concentration and vice versa. Rejection could also have been a result of neophobia, although we observed no change in water intake over the four days of the bitter trial. Other researchers have demonstrated neophobia to foodstuffs resolving after two days [25].

Bitter substances often contain alkaloids which can be toxic when ingested, thus the ability to perceive bitter taste is essential to avoid poisoning [37]. This preservation strategy becomes problematic when it is necessary to administer, for example, medications [21]. Understanding taste preferences can aid in encouraging ingestion of required feedstuffs or solutions. Despite horses rejecting bitter solutions, our results in the mixed trial showed that when the bitter taste was masked with a sweet taste, horses no longer rejected the solution. Although our results with the mixed sweet and sour tastants were unexpected, the same outcome was achieved – the addition of a more preferred tastant to a less preferred tastant increased the tolerance for the less preferred tastant.

Individual taste preferences were evident in all our trials as indicated by the large standard deviations in all the graphs. While no treatment was completely rejected in any trial, certain horses demonstrated specific likes or dislikes to the various tastants. In the sweet trial, there was a high variation among the horses ranging from strong preference to strong rejection. In the sour trial, some horses showed a strong preference for sour solutions. However, no horse showed a preference for any bitter solution in the bitter trial. High variability was noted previously both among and within individual horses in a two-choice preference test for water treated with increasing salt concentrations [27]. Individual horse variation led to inconclusive results when Murphy *et al.* [38] tested a variety of flavored solutions in Thoroughbred horses. Similarly, van den Berg *et al.* [39] reported a large individual variation in the acceptance of novel foodstuffs. Our results also showed a variation in solution intake across days or weeks of the trials which could be influenced by many independent factors that could not be controlled such as ambient temperature, humidity, forage type, salt availability, breed, age, and exercise. Future research should endeavor to use a more controlled methodology with larger numbers of animals who participate in testing all the treatments.

Ambient temperature did affect intake of sour solution in our study but no clear pattern emerged. This trial was the only trial conducted during the summer months when the ambient temperature was generally quite hot. Previous studies reported ponies drinking more when warm water was provided during the winter months compared to cold water **[40]** but during the hot summer months, ponies did not alter their water intake regardless of whether they were provided with cold or warm water **[41]**.

An important aspect of understanding taste preferences in horses is in regard to monitoring water intake. Horses who travel to different locations may reject a local water source but the addition of a tastant to the water could override that. Mars *et al*. **[42]** found that mares drank more apple-flavored water at an unfamiliar location compared to clover-flavored water. Water intake is highly important in horses competing in high-performance disciplines such as three-day eventing who can quickly lose electrolytes and experience dehydration through exertion **[20,43]**. Both problems can be ameliorated by offering electrolytes in solution for the horse to ingest, and the volume of voluntary intake can be increased with the addition of a preferred taste **[20,43]**.

The total amount of fluid ingested daily by the horses across all our trials was not affected by treatment with the exception of the bitter tastant during the mixed trial. In this instance, horses drank less total water when presented with bitter solution compared to the sweet or mixed solutions. Rats were also reported to diminish daily fluid intake when presented with quinine-adulterated water **[44]**. Horses are noted to have a higher sensitivity to bitter **[2]** which may aid them in avoiding toxic plants such as artemisia **[45]**. The presence of the bitter tastant in the water in our trial may have resulted in a learned response to avoid water as much as possible **[2]**.

Age, breed, and exercise all affected the total amount of fluid ingested by the horses in our sour trial. Older horses ingested less total fluid than younger horses which has previously been noted **[40]**. The breed effect is not surprising as Caspian Horses, who weigh an average of 270kg, are significantly smaller than draft horses, with an average weight of 800kg (**https://equi-analytical.com/resources/typical-body-weights/),** thus fluid intake would be significantly less for the Caspian horses. Breed differences have also been noted in taste preferences, with cold-blooded horses preferring salty feeds and Arabians (hot-blooded) preferring sour **[34]**. However, our results presented here did not show any breed effect in the consumption of sour solutions. This may indicate that aversion to sour is a factor of evolution, as suggested by Kyriazakis **[11]**. Horses in our sour trial who received more daily exercise ingested more total fluid than those who were idle. This would be expected as moderate exercise in temperate weather can result in a water loss of 25ml/m^2/min **[43]** which would need to be regained through drinking.

It should be stressed that these trials were all short-term, where horses were exposed to various tastants for only four or five consecutive days. It could be that longer-term exposure would lead to recognition of post-ingestive consequences **[11]** or an adaptation to the presence of a particular tastant, resulting in more obvious preferences. Nevertheless, these results shed some light on the taste preference of horses for sweet, sour, bitter, and mixed solutions.

5. Conclusions

It is known that horses can distinguish between sweet, salty, sour, and bitter. The results of this study show that some horses have only a weak preference for sweet solutions and some reject sour and bitter solutions. Importantly, a less preferred taste can be masked by a more preferred taste to increase consumption. This is helpful when it is necessary to administer unpalatable substances to horses such as medications. This information adds to the scarce research on this topic to date.

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Authors' Contributions

K.M., M.V., J.D, J.C., and E.D. conceived the idea and planned the study. M.V., J.D, J.C., E.D., and L.F. collected and curated the data. K.M., M.V., J.D, J.C., E.D., and L.F. analyzed the data and contributed to the interpretation of the results. K.M. took the lead in writing the manuscript and M.V., J.D, J.C., E.D., and L.F contributed to the final version. K.M. supervised the project.

Data Availability

Data supporting the findings of the study can be supplied upon request to the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

All procedures were approved by the Institutional Animal Care Committee in concordance with the Canadian Council for Animal Care guidelines for the use of animals in research.

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