Effect of aguamiel (agave sap) on hematic biometry in rabbits and its antioxidant activity determination

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Abstract

In this work, we analysed the effect of aguamiel (sap from Agave salmiana) on haematic biometry in rabbits as well as its antioxidant activity with the objective of determining its nutritional value. Nine male Landrace 55-day-old rabbits were sorted into three groups of three rabbits each, which had access to fresh aguamiel, boiled aguamiel and water instead of aguamiel. Commercial rabbit food was supplied ad libitum to each experimental unit. Fresh aguamiel promoted weight increases of about 13% by the seventh week. Haemoglobin counts increased by 4.5% and 9% when rabbits were fed with fresh and boiled aguamiel, respectively. Haematocrit counts increased from 2.6% to 5.3%. Mean corpuscular volume did not show a change. Mean corpuscular haemoglobin significantly increased up to 12% above that previously reported on rabbits. Observed Fe (fixed to transferrin), transferrin and ferritin content increased slightly when fresh aguamiel was included in the diet, as compared to the control. In bone, Fe increased 79.3% (boiled aguamiel) while P increased 23.6% (fresh aguamiel). In antioxidant activity determination, the aguamiel showed 20% with respect to that found in coffee and/or grape juice (commercial beverages with a high antioxidant capability), 226.2 μmol gallic acid equivalent and 35.1 mg ascorbic acid in 250 mL, respectively. These results showed that aguamiel is an innocuous beverage to feed animals, conferring some beneficial properties, and it could be considered as a nutraceutical product.

Introduction

Iron deficiency risk is present in two billion people worldwide, which represents about 30% of the world’s population (Grusak and Dellapenna, 1999; Boccio et al., 2004; Gaitán, 2006). This may be related to the fact that inhibitors for mineral absorption of iron, zinc and calcium are frequently present in food (Dellapenna, 2007). Because Mexican people share the same risk, it is mandatory to look for local affordable alternatives that may enhance mineral availability in the Mexican diet (Rosado, 1998). Fortunately, food and beverages derived from some native plants may play an important role in the prevention of chronic and degenerative diseases (Carvajal et al., 2005; Wilson and Demming, 2007). In addition, there is evidence that micronutrient-fortified beverages (containing iron, iodine, zinc, vitamin A, vitamin C, niacin, riboflavin, folate, vitamin B-12, vitamin B-6 and vitamin E) promote increases both in haemoglobin concentration and ferritin. Furthermore, these beverages reduced the risk of anaemia and iron deficiency anaemia in pregnant women and infants (Makola et al., 2003; Nnám, 2009). Some fruits or vegetables (nopalito-tender cactus, guava, papaya, mango, strawberry, prickly pear and others) grown in Mexico have been considered appropriate owing to their antioxidant properties (Corral-Aguayo et al., 2008). Additionally, in the arid and semiarid lands of Mexico, the pulp of a plant known as maguey manso (Agave salmiana, Gentry) is used as forage, while aguamiel (its sap) is collected in order to produce the alcoholic beverage known as pulque. When the plant is about 7-10 years old, the new unfolded leaves and the stem are removed, leaving a cavity where aguamiel accumulates. Every day during 3-6 months, the cavity is scraped in order to obtain an average total of 900 L of aguamiel. This sap is colourless, transparent, sweet and tasty (Granados, 1991). Besides water, aguamiel contains glucose, sucrose and fructose (26.5%, 8.8% and 32.4%, respectively, w/w, on a dry matter basis), as well as gum, protein, minerals, vitamins and beneficial organisms such as Kluyveromyces marxianus var. bulgaricus (Estrada et al., 2001; Cruz et al., 2006; Ortiz et al., 2008). In addition, in aguamiel inulin reaches 11% (dry matter) while iron and zinc contents are 2.15 mg/100 g and 1.41 mg/100 g, respectively, levels considerably higher than those found in milk (Martínez, 1999; Silos et al., 2007). In the constant and pressing search to be certain that aguamiel is innocuous to animals, this could be a new source for mineral nutrition (according to traditional use by the Mexican population) and represents a nutraceutical product. Thus, we analysed the nutrimental value of aguamiel on rabbit bone and blood, and we determined its antioxidant activity.

Key words: Agave sap, Rabbits, Antioxidant activity.

Contributions: CLTR carried out most of the laboratory work; CPS, ANC, FGL, FGL reviewed the research project and manuscript critically. LJM performed statistical analyses of experimental data, and contributed to manuscript writing; FGL, JLMHD, HSE collaborated in the laboratory work; HSE conceived the research.

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Materials and methods

Aguamiel extraction
Aguamiel, drained from the wound left in the plant after removing the shoot apex, was obtained from A. salmiana grown in Calvillo, Aguascalientes, México. Aguamiel was collected with the help of agave growers who extracted the sap over 60 days, keeping samples in sterilised jars, maintained at 4°C. Source plants were under commercial exploitation and were selected at random by the agave grower.

Mineral analysis of aguamiel and rabbit food
Iron (Fe), zinc (Zn), magnesium (Mg), calcium (Ca) and phosphorus (P) contents were determined through wet digestion (Blake et al., 1988) in 50% (v/v) nitric acid. The sample was heated in acid until the yellow vapours ceased but before complete dryness; the digest was adjusted to 100 mL with distilled water. Fe, Zn and Mg quantitation was carried out using an atomic absorption spectrophotometer while Ca was analysed through atomic emission spectrometry (both using a 3110 Perkin-Elmer spectrophotometer, Boston, MA, USA) and, finally, P was determined through the inorganic phosphorus colorimetric method (Sumner, 1944) with a Cintra 10e spectrophotometer (GBC Scientific Equipment, Hampshire, IL, USA).

Experimental procedure for feeding the rabbits
Nine male Landrace 55-day-old rabbits were sorted into three groups (labelled as A, B and C), having three rabbits each. Commercial rabbit food (Purina® Granja Familiar) was supplied ad libitum to each experimental unit. Aguamiel, where given, was poured into the tray. Treatments with aguamiel while Ca was analysed through atomic emission spectrometry (both using a 3110 Perkin-Elmer spectrophotometer; Boston, MA, USA) and, finally, P was determined through the inorganic phosphorus colorimetric method (Sumner, 1944) with a Cintra 10e spectrophotometer (GBC Scientific Equipment, Hampshire, IL, USA).

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Results and discussion

Blood analyses
Blood samples were obtained at 0, 30 and 60 days from each rabbit through cardiac puncture and jugular puncture using 5 mL syringes. Samples of 3 mL were used to determine glucose and mineral content, while 2 mL samples were used to perform haematic biometry (haematocrit, haemoglobin content and erythrocyte levels), using Cell Dyn 1700 automated equipment (Abbott Laboratories, Abbott Park, IL, USA). Glucose in serum was determined with the Glucose Oxidase Kit (Stanbio Licon, Boerne, TX, USA) and the UV-visible spectrophotometer (StatFax 3300, Palm City, FL, USA). Minerals such as Mg, P and Ca were determined by the methods xylidyl blue, phospomolybdate and arzenazo III dye assay, respectively (Thermo Electron® Kit, Waltham, MA, USA for the Mg assay; Pointe Scientific® Kit, Canton, MI, USA for the P and Ca assays). Zn was determined by using an atomic absorption spectrophotometer (Perkin Elmer 3110). Finally, Fe determination (Fe saturation, serum Fe, ferritin and transferrin) was done by turbidity measurements in the spectrophotometer (Spinreact® Kit- Lab Center de México SA de CV and Stat Fax 3300).

Bone analysis for mineral content
At the end of the experiment (60 days), two rabbits were selected at random from each group and sacrificed. Femurs were dissected out, washed, dried and exposed to n-hexane Soxhlet extraction during 6 h before wet digestion with 50% nitric acid (v/v), as described previously. Minerals were determined according to methods as described for the mineral analysis for aguamiel and rabbit food.

Table 1. Blood analyses performed at 0, 30 and 60 days in rabbits after diets were supplemented with fresh or boiled aguamiel (Agave sap) or water.

| Treatment | Day | Haemoglobin, mg/dL | Haematocrit, % | Mean corpuscular volume, fl | Mean corpuscular haemoglobin, pg | Mean corpuscular haemoglobin concentration, % |
|-----------|-----|---------------------|----------------|-----------------------------|---------------------------------|-----------------------------------------------|
| Control   | 0   | 12.40±0.5           | 41.73±1.2      | 66.60±3.4                   | 19.93±1.3                       | 29.87±0.6                                     |
| (no aguamiel) | 30  | 13.00±0.3           | 42.13±1.2      | 67.06±2.6                   | 20.65±1.0                       | 30.78±0.3                                     |
|           | 60  | 14.73±1.0           | 44.63±3.8      | 68.23±1.7                   | 25.67±4.8                       | 33.87±1.5                                     |
| Fresh     | 0   | 11.40±1.4           | 38.77±5.3      | 64.67±1.9                   | 19.06±0.7                       | 27.77±2.9                                     |
| (aguamiel) | 30  | 11.30±1.2           | 41.27±3.4      | 70.74±6.7                   | 20.45±1.9                       | 30.91±0.3                                     |
|           | 60  | 15.40±1.4           | 45.83±3.7      | 70.77±2.5                   | 28.97±3.8                       | 34.70±1.2                                     |
| Boiled    | 0   | 10.97±2.0           | 36.70±7.5      | 65.00±2.1                   | 19.63±0.6                       | 29.97±1.0                                     |
| (aguamiel) | 30  | 11.20±1.6           | 37.10±6.3      | 71.24±5.9                   | 21.53±1.0                       | 30.28±0.9                                     |
|           | 60  | 16.10±1.4           | 47.03±1.6      | 70.73±5.0                   | 28.30±5.0                       | 34.47±1.6                                     |
| Reference | 0   | 10-17.4             | 33-50          | 57.80-65.5                  | 17.1-23.5                       | 29-37                                          |

Values shown are means ± standard deviation.
times lower than previously reported (Silos et al., 2007). On the other hand, Ca content was twice that reported by the same paper, while P content remained about the same. Based on results, the previously mentioned report (Silos et al., 2007) recommended an aguamiel intake of a half-litre, in order for a person to obtain approximately 60% of the daily requirement of Fe and Zn. Comparing the aguamiel mineral levels with those in the rabbit food (Fe, 3.69; Zn, 8.81; Mg, 17.88; Ca, 7.96; P, 0.0 mg/25 g), supplemented during the experiment, it was noted that aguamiel (when added to the rabbit’s diet) was a good source of P and Ca.

Rabbit weight gain

At the beginning of the experiment, the rabbits used as controls weighed 1130±52.9 g, and the rabbits given boiled aguamiel weighed 1153.3±103.73 g and rabbits given fresh aguamiel weighed 965±126.3 g, respectively. Weight gain was similar to that in rabbits that drank fresh aguamiel (up to 16% at 47 d) as compared to those that drank boiled aguamiel and the controls (data not shown). Additionally, fresh aguamiel was more palatable, promoting a more healthy appearance (personal observations). At the end of the experiment, rabbits drinking fresh aguamiel lost some weight, probably owing to some addiction to aguamiel, thus reducing normal food consumption. After 51 d, boiled aguamiel promoted about 8% weight gain as compared to the control. Even though the rabbits that drank aguamiel had reduced food consumption, the rabbits that drank aguamiel had increased about 12% above the highest value (23.5 pg) reported (Birchard, 1996).

Blood analyses

Aguamiel promoted some increases in the results of blood testing (Table 1), such as haemoglobin content (HGB) (4.5% with fresh aguamiel and 9% with boiled aguamiel) after 60 days, as compared to the control (P=0.0005). Haematocrit content (HCT) increased slightly: 2.6% and 5.3% for fresh and boiled aguamiel, respectively. No significant changes were observed in mean corpuscular volume (MCV) (Birchard, 1996). Mean corpuscular haemoglobin (MCH) in rabbits fed with aguamiel increased about 12% above the highest value (23.5 pg) reported (Birchard, 1996). Finally, the mean corpuscular haemoglobin content (MCHC) was positively affected by both boiled and fresh aguamiel intake during the experiment (P=0.001). The anova and covariance tests for mean comparisons were performed first for all of the variables, but as no significant difference was found for any of these analyses, we used tendency analysis for

| Measurements | Control | Boiled Aguamiel | Fresh Aguamiel | Significance |
|--------------|---------|----------------|----------------|-------------|
| Transferin-fixed iron | 307±40.7 | 239±41.4 | nd | nd |
| Total transferin | 315±24.7 | 276±54.3 | nd | nd |
| Ferritin | 154±8.5 | 155±26.8 | nd | nd |
| Iron | 141±11.5 | 154±31.4 | 22.1±0.0 | 20.6±1.9 |
| Magnesium | 8.9±2.0 | 8.6±3.7 | 68.3±5.7 | 71.4±4.8 |
| Phosphorus | 4.2±1.2 | 4.7±2.5 | 48.0±3.0 | 42.7±1.0 |
| Calcium | 17.5±3.0 | 15.2±2.7 | 601.2±297.8 | 601.2±297.8 |

Table 2. Analyses of linear regression for haemoglobin, haematocrit and mean corpuscular haemoglobin response in rabbits during two months after diets were supplemented with water, boiled aguamiel or fresh aguamiel.

| Statistics | A | B | C | A | B | C |
|-----------|---|---|---|---|---|---|
| r² | 0.82103 | 0.78789 | 0.76726 | 0.50103 | 0.633244 | 0.631836 |
| Adjusted r² | 0.674095 | 0.629118 | 0.588742 | 0.251004 | 0.405355 | 0.399217 |
| F | 14.479** | 11.451* | 10.021* | 4.3462 ns | 4.7358 ns | 4.6355 ns |
| OSL | 0.0066 | 0.0117 | 0.0158 | 0.16945 | 0.0660 | 0.06794 |
| Typical error | 0.731720 | 1.453661 | 1.996150 | 2.327250 | 3.983448 | 5.988309 |

Table 4. Antioxidant capability, phenol and vitamin C content in aguamiel.

| Measure | Control | Aguamiel | Fresh Aguamiel | Boiled Aguamiel |
|---------|---------|----------|---------------|-----------------|
| Trolox equivalent antioxidant capacity | 6.8 | 27.42 | 7.1 | 226.2 |
| Gallic acid equivalent | 9.6 | 141.8 | 11 | 113 |
| Ascorbic acid | 3.7 | 77.9 | 11.2 | 128.9 |
| Coffee (Nescafé) | 42.5 | 157.48 | 156.1 | 320.5 |
| Grape juice | 38.9 | 611.12 | 29.3 | 112.4 |

Table 3. Iron fixation, transferrin, ferritin and mineral content in serum and bone from rabbits, diets supplemented with aguamiel (Agave sap) during 60 days.

| Measure | Control | Aguamiel | Fresh Aguamiel | Boiled Aguamiel |
|---------|---------|----------|---------------|-----------------|
| Ferritin | 127±26.3 | 154±8.5 | 135±52.6 | nd |
| Iron | 120±17.9 | 138±11.5 | 114±36.1 | 22.1±0.0 |
| Magnesium | 2.9±0.4 | 2.5±0.7 | 2.6±0.6 | 68.3±5.7 |
| Phosphorus | 8.3±3.5 | 7.4±0.5 | 7.7±2.2 | 547±1039 |
| Calcium | 13.5±3.3 | 17.5±2.9 | 15.2±2.7 | 601.2±297.8 |

Figure 1. Haemoglobin response to aguamiel supplementation in rabbit diets.
HGB, HCT and MCH over the time, with the three treatments. The results of the three treatments for both HGB and MCH showed an increase over the two months of the experiment. This tendency was found to be either significant or highly significant. On the other hand, HCT was not significantly different (Table 2; Figures 1, 2 and 3).

**Glucose and mineral tests on serum and bone**

Glucose was found within the range previously reported (75-155 mg/dL) in rabbits (Birchard, 1996), during the time that boiled or fresh aguamiel was supplied to them (109.8±7.9 mg/dL to 140.0±7.9 mg/dL, respectively). Although no significant difference was found in Fe content in serum, slight increases were observed on the transferring-fixed Fe, total transferrin and ferritin levels (11.23%, 7.14% and 21.26%, respectively), when fresh aguamiel was included in the diet, compared to the control. On the other hand, boiled aguamiel promoted the opposite effect, suggesting that high temperature lowers Fe availability, as reported previously for boiled legumes: soybeans and peas (Hoppler et al., 2008). Ca and Zn in serum increased slightly when the rabbit’s diet included either type of aguamiel, Fe only showed small increases for the fresh aguamiel treatment, and Mg and P decreased slightly for both aguamiel treatments. According to previous experiences in humans, the use of fortified beverages with iron, iodine, zinc and vitamins promoted increases in haemoglobin and ferritin contents, reducing the risk of anaemia in pregnant women (Makola et al., 2003) and infants fed with Moringa oleifera leaf (Nnam, 2009). In this experiment, the chemical analysis of rabbit bone showed about 79.3% increase in Fe content, owing to intake of boiled aguamiel, as compared to the control. Fresh aguamiel increased P levels up to 23.6% but, at the same time, decreased the Ca content about 13.8% (Table 3). As regards mineral content in the rabbit’s bone, the highest F values were for Fe and Zn. Nevertheless, the lack of significance for all of the covariables prevented further comparisons of means.

**Vitamin C, acidity and antioxidant capability of aguamiel**

Aguamiel compared to commercial beverages such as coffee or grape juice showed low antioxidant capability (about 20%) but, nevertheless, it is double that of pulque and is three and a half as much as that of orange juice mixed with nopal extract (Table 4). A similar trend was found for phenolic compounds, where aguamiel was almost double that of orange juice mixed with nopal extract. The latter comparison is in agreement with a report that emphasises the antioxidant capability of aguamiel over papaya, nopal, avocado, mango and prickly pear (Corral-Aguayo et al., 2008). Finally, the ascorbic acid content in aguamiel was approximately 0.8 g/dry base.

**Conclusions**

It seems that aguamiel could be used as a food supplement for rabbits and other mammals including man, in order to gain weight and to correct malnourishment according to Mexican popular belief. Because aguamiel supplies enough Fe and Zn (Silos, et al., 2007) in the diet, our study reinforces the point of...
view that fresh aguamiel would correct iron deficiencies. In addition, aguamiel showed adequate antioxidant activity compared to coffee or grape juice (commercial beverages showed low antioxidant capability). Therefore, aguamiel is a natural product that is considered a good supplement or nutraceutical complement, which could be adopted by health programmes of developing countries to lower iron and zinc deficiencies (Rosado et al., 1992; Muñoz, et al., 2000; Hambidge and Krebs, 2007). Additionally, statistical analyses of our results enabled us to identify a beneficial effect of aguamiel for rabbits. However, this study is also important because it is the first report about the effect of aguamiel on the animal system, with no adverse effects found in the rabbits. Despite this, more studies are necessary to understand this beneficial effect and to identify the possible biochemical agent in aguamiel.

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