Quantitation of lithium in eggs

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Background

Previous reports:

- Lithium removal with household water purification devices
- Comparison of Analytical Techniques for Quantitation of Lithium in Food

Large quantities of lithium, such as clinical doses used for treatment of bipolar disorder, are pharmacologically active, and can impact mood, clarity of thought, and body weight.

The effects of lower-than-clinical doses are less well-understood, but these doses may have pharmacological effects as well — an extensive body of research dating back to the 1970s has found relationships between trace levels of lithium in drinking water and public health outcomes like crime rates, suicide rates, and mental hospital admissions.

Tests of food and drink generally find some amount of lithium. But little research exists regarding how much lithium exists in the food supply, and which foods might concentrate lithium. Since lithium is not acutely toxic in the way that heavy metals are, it is rarely included in investigations of trace metals in food. Nor will there be a single answer. Since lithium in water is known to vary widely with geography, lithium content in food would be expected to vary also. For example, crops irrigated with water high in lithium would be expected to have much more lithium than the same crops from regions with low lithium water levels.

In our last study, we tested ten foods using a combination of methods. Of the ten foods we tested, eggs contained the most lithium by fresh weight. Lithium presumably finds its way into the eggs as a result of its inclusion in the chicken's diet, as a trace element in food or drinking water.

A variety of analytical tools are available to quantify lithium, but the most common are Inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma optical mass spectrometry (ICP-MS). The primary practical difference is that ICP-MS is mo re sensitive, able to detect metals like lithium down to lower levels than ICP-OES. However, we chose ICP-OES because our previous study found that ICP-OES is sufficient to quantify lithium in a variety of foods.

Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Today, the most common analytical method for lithium and most other metals is inductively coupled plasma optical emission spectroscopy (ICP-OES). In this technique, the instrument energizes an inert gas to create a high-temperature plasma in its “torch” via the principle of inductive coupling, and this plasma is high enough in energy to ionize any particles entering the torch. When the sample collides with the plasma, it is broken into charged ions, which emit a wavelength specific to each element as they ionize and recombine. The light emitted in this way is measured (the “optical” part) to determine which elements are present and in what quantity.

ICP-OES is very popular for elemental analyses because of its balance of cost, robustness, and reliability. It can handle liquids with high dissolved-solids content and can analyze many elements simultaneously.

Sample preparation

Like most elemental analyses, ICP-OES and ICP-MS can only be used to analyze liquid samples. Liquids can be nebulized and collided with the plasma torch as small droplets; solids or gels cannot. In practice, samples usually enter the ICP instrument as an aqueous solution of nitric acid (HNO3), since the nitrates of most metals are highly soluble and there is minimal risk of precipitates or crystals clogging the nebulizer. However, there are multiple ways to achieve this aqueous solution.

> *Sample prep via acid digestion*
>
> Acid digestion, sometimes referred to as “wet ashing”, dissolves the sample by breaking it down with a mixture of strong acid and oxidizing agent. Nitric acid is popular for this purpose, due to the fact that it is both acid and oxidizer, but aqua regia (mixture of hydrochloric and nitric acids) and hydrogen peroxide are also popular. The goal is simple: To disintegrate all organic matter into small, water soluble compounds and create an aqueous solution suitable for injection. Heat accelerates the process, so heat is applied via convection (e.g. a “hot block” that conducts heat into the samples) or via microwaves. Pressure vessels are sometimes used to enable temperatures higher than the boiling point of the solution. The digested sample can then be diluted as necessary and injected into the ICP instrument.
>
> *Sample prep via ashing*
>
> Ashing, sometimes called “dry ashing” to distinguish it from digestion, uses combustion to break down the sample into water-soluble constituents. Samples are heated in air to high temperatures, usually >400C, where water evaporates and the samples burn. Carbon exits the sample as CO2. The ash left behind is a mixture of compounds, such as oxides and hydroxides, which generally dissolve readily in even mild acid. This dissolved-ash solution can then be injected into the ICP instrument, usually as a nitric-acid solution.

Most food studies use acid digestion due to the higher throughput. Many samples can be digested at once, and the technique lends itself to economies of scale. However, we previously found that acid digestion appears to drastically underestimate lithium levels compared to dry ashing, so we used dry ashing to prepare all food samples in this study.

Eggsperimental Methods

We were interested in investigating lithium levels in a variety of eggs: Different brands, and from different geographies around the United States. The primary question to be answered was, might eggs be a significant dietary source of lithium? If so, how do lithium levels vary based on egg type or place of origin?

Egg selection and preparation

Eggs were selected from a variety of brands and locations. Seven different brands were purchased in Colorado (Boulder) intended to provide a cross section of different types: White and brown, conventional and organic. Eggs were also shipped from Ohio, New York, and Washington D.C., to be prepared and analyzed in the same way. Table 1 summarizes the eggs used in the study.

Table 1.

Egg ID	Purchase/Arrival Date	Location	Brand	Color	# of eggs in sample
E01	29-Oct-23	Colorado	Trader Joe's	White	4
E02	3-Nov-23	Colorado	Kroger Grade AA, batch 1	White	4
E03	3-Nov-23	Colorado	Kroger Grade AA, batch 1	White	4
E04	3-Nov-23	Colorado	Kroger Grade AA, batch 3	White	1
E05	3-Nov-23	Colorado	Kroger Grade AA, batch 4	White	1
E06	3-Nov-23	Colorado	Kroger Grade AA, batch 5	White	1
E07	3-Nov-23	Colorado	Simple Truth AA, batch 1	Brown	4
E10	3-Nov-23	Colorado	Simple Truth AA, batch 2	Brown	4
E08	3-Nov-23	Colorado	Organic Valley, batch 1	Brown	4
E09	3-Nov-23	Colorado	Organic Valley, batch 2	Brown	4
E11	3-Nov-23	Colorado	Vital Farms, batch 1	Brown	4
E12	3-Nov-23	Colorado	Vital Farms, batch 2	Brown	4
E13	3-Nov-23	Colorado	Whole Foods, batch 1	Brown	4
E14	3-Nov-23	Colorado	Whole Foods, batch 2	Brown	4
E15	3-Nov-23	Colorado	CostCo, batch 1	Brown	4
E16	3-Nov-23	Colorado	CostCo, batch 2	Brown	4
E17	15-Nov-23	NYC	Alderfer, batch 1	Brown	4

E18	15-Nov-23	NYC	Alderfer, batch 2	Brown	4
E19	15-Nov-23	NYC	Land-O-Lakes, batch 1	Brown	4
E20	15-Nov-23	NYC	Land-O-Lakes, batch 2	Brown	4
E21	23-Nov-23	Ohio	Eggland's Best, batch 1	White	4
E22	23-Nov-23	Ohio	Eggland's Best, batch 2	White	4
E23	8-Dec-23	Washington, DC	Whole Foods ("365"), batch 1	White	4
E24	8-Dec-23	Washington, DC	Whole Foods ("365"), batch 2	White	4

Due to an oversight, we included only one batch of Trader Joe's eggs. Whole Foods eggs from Colorado were labeled just "Whole Foods", while Whole Foods eggs from DC were labeled "365" (Whole Foods' store brand).

The eggs were prepared for analysis using the following procedure:

Eggs from a sample set were combined in a clean jar, washed and triple-rinsed with distilled water. They were homogenized/blended for 1 minute with a stick blender to obtain a smooth, merengue-like texture. The blended mixture was then transferred to drying dishes to be dried in a consumer-grade food dehydrating oven.

Drying was conducted at a temperature of 60 °C (140 °F). Eggs from only one source were dried at a time to minimize risk of cross-contamination. The dehydrator passes a gentle current of warm air up through racks of trays to evaporate moisture. Samples were dried until constant weight (within 0.1g, typically overnight). Weight was recorded before and after drying to relate "dry lithium content" to "as-purchased lithium content". Sample masses were measured with a 4-place (0.0001g) analytical balance (Sartorius CP224S), calibrated daily. All eggs had a similar moisture content, having about 23%-26% dry matter by mass.

Once dry, samples were crumbled up to a fine powder and mixed/stirred before being weighed into airtight 50mL polypropylene tubes for further processing.

Analytical chemistry
Samples were ashed and then analyzed by ICP-OES. Each analysis was performed in triplicate.

Ashing was conducted using the following procedure: Samples (approximately 0.1g) were placed in crucibles, which were placed in a muffle furnace (air atmosphere) at 200°C for 1hr, increased to 300°C for 1 more hour, then increased to 500°C for 2 hours. Muffle furnace was shut off and crucibles were left to cool overnight in furnace. After this ashing was complete, 200 uL of concentrated HNO_3 was added to the crucible and swirled with the ash. After the ash dissolved, this solution was pipetted to an empty centrifuge tube for dilution and analysis.

Nitric acid/hydrogen peroxide digestion was conducted using the following procedure: Samples (approximately 0.1g) were placed in pre-weighed fluropolymer (PFA) digestion vessels. 1.25mL concentrated HNO_3 was added to the vessel, and it was allowed to digest for 16 hours at room temperature. The vessels were then inserted into a heating block and heated at 95C for two hours. After cooling to room temperature, an additional 0.5mL of 30wt% H_2O_2 was added to each vessel. H_2O_2 was allowed to react at room temperature for 15 mins or until effervescing stopped, whichever took longer. Samples were then heated at 95°C for 1 additional hour. Vessels were cooled to room temperature prior to weighing to account for weight change. Digestates were then pipetted to an empty centrifuge tube for dilution and analysis.

The nitric acid/hydrogen peroxide digestions and dry-ashing both resulted in clear solutions, with at most a few flecks of undissolved matter. These digests were then advanced to ICP-OES. However, it should be noted that the ICP-OES was run on separate digestions/ashing preps. (That is, they were replicates of the full sample-prep process, not just the ICP analysis).

ICP-OES was performed on a Perkin Elmer 8300 ICP-OES. Analyses included calibration curves for lithium and sodium and blanks (18 MΩ deionized water), but not internal standards. Sample order was randomized to minimize risk of systematic errors due to sample carryover. Lithium and sodium were analyzed simultaneously on each injection. Sodium was analyzed side by side because it is chemically similar to lithium, and naturally abundant in most foods; this makes it a useful point of comparison.

Results

Raw data can be found here and the analysis script is here.

Results are summarized in Table 2. Note that all results are given as **micrograms of Li+ ion per gram of moist (as-purchased) food**. (This is equivalent to mg/kg or ppm). The foods were necessarily dried as part of the sample preparation process, and the mass fraction of solid matter is noted as well. The eggs all had a dry-matter content of around 25% (the rest being water), so there is approximately a 4x difference between a just-cracked egg and a dried egg in lithium concentration.

Table 2.

Egg ID	Replicate	Mass frac solids	Li+, µg/g of moist food	Na+, µg/g of moist food
E01	1	0.254	1.1766186	1287.43375
E01	2	0.254	1.45048672	1324.45666
E01	3	0.254	0.43869616	1145.42806
E02	1	0.251	0.35148262	1503.59245
E02	2	0.251	4.67471889	1094.61731
E02	3	0.251	0.39416266	1337.8935
E03	1	0.256	0.52670814	962.39294
E03	2	0.256	0.90000615	1241.59939
E03	3	0.256	5.99066593	963.671357

E04	1	0.255	1.43527911	1172.22974
E04	2	0.255	0.51077548	1176.31594
E04	3	0.255	1.15946035	1215.90104
E05	1	0.262	5.05983405	1166.38351
E05	2	0.262	4.11603081	1346.75479
E05	3	0.262	5.61038594	1140.95325
E06	1	0.234	0.62415848	1284.31712
E06	2	0.234	2.33299687	1223.7714
E06	3	0.234	0.58441805	1163.45946
E07	1	0.25	1.15854276	1316.43487
E07	2	0.25	2.22950669	1028.42564
E07	3	0.25	1.34120717	1035.9324
E08	1	0.249	0.36159229	1177.04524
E08	2	0.249	1.04737076	1244.37622
E08	3	0.249	15.072164	1132.90604
E09	1	0.252	BLOQ	BLOQ
E09	2	0.252	0.31700887	1257.21692
E09	3	0.252	0.67930472	1096.19655
E10	1	0.245	1.47253734	1182.6929
E10	2	0.245	BLOQ	7.55902499
E10	3	0.245	0.77553633	1309.82196
E11	1	0.255	BLOQ	1.52657951
E11	2	0.255	0.85519086	959.345448
E11	3	0.255	2.75448041	1094.6443
E12	1	0.258	2.75527276	1118.58924
E12	2	0.258	0.91670757	1576.94303
E12	3	0.258	2.93552425	1337.72355
E13	1	0.249	1.00249313	859.208753
E13	2	0.249	0.81592493	898.263696
E13	3	0.249	0.42786307	948.512732
E14	1	0.247	2.10192959	875.144567
E14	2	0.247	1.91893807	834.095119
E14	3	0.247	2.12171246	866.736849
E15	1	0.256	3.47604769	928.564799
E15	2	0.256	0.85878825	1075.78564
E15	3	0.256	10.4281431	865.68923
E16	1	0.253	1.10644913	1096.09196

E16	2	0.253	7.37632752	900.821367
E16	3	0.253	3.41028841	807.859431
E17	1	0.259	5.02170359	916.849181
E17	2	0.259	0.51252439	649.197557
E17	3	0.259	1.23989486	993.210138
E18	1	0.263	0.90579327	881.568565
E18	2	0.263	0.63194879	781.510006
E18	3	0.263	2.21972013	932.651092
E19	1	0.242	2.7385652	1048.89471
E19	2	0.242	9.03968867	942.50266
E19	3	0.242	0.72220569	1059.07344
E20	1	0.254	0.91194006	832.939123
E20	2	0.254	7.41745117	909.399836
E20	3	0.254	1.66892651	868.248223
E21	1	0.265	1.57070073	684.793678
E21	2	0.265	3.71449496	710.795142
E21	3	0.265	15.388622	940.032545
E22	1	0.256	1.19907327	808.605818
E22	2	0.256	1.06328078	639.761954
E22	3	0.256	0.42531231	801.431876
E23	1	0.252	2.77143028	855.112214
E23	2	0.252	1.4612996	876.275864
E23	3	0.252	0.30737681	902.730425
E24	1	0.261	2.47384358	859.70636
E24	2	0.261	1.78158535	883.478246
E24	3	0.261	0.53290823	722.299631

All results are given as micrograms of Li+ ion per gram of moist (as-purchased) food (µg/g).

Nearly all egg samples contained detectable levels of lithium, and around 60% of samples contained more than 1 mg/kg lithium (fresh weight). Lithium results for the main batches are visualized in Figure 1, and sodium results in Figure 2.

These results match our earlier findings and confirm that ICP-OES is more than sensitive enough for the analysis of lithium levels in most foods. Readings that appear as BLOQ indicate “below the limit of quantification”, which on a fresh basis works out to about "less than about 0.04 µg/g", a very small amount.

The samples E09-1, E10-2, and E11-1 all showed lithium levels below the level of quantification. However, these three samples also showed sodium readings that were drastically lower than all other sodium results — below the limit of quantification, 7.6 µg/g, and 1.5 µg/g respectively. This suggests that something may have gone wrong with the analysis of these samples, such that both the lithium and the sodium results showed falsely low readings. If this is the case, and we exclude these three samples, then *all* egg samples contained detectable levels of lithium.

Figure 1.

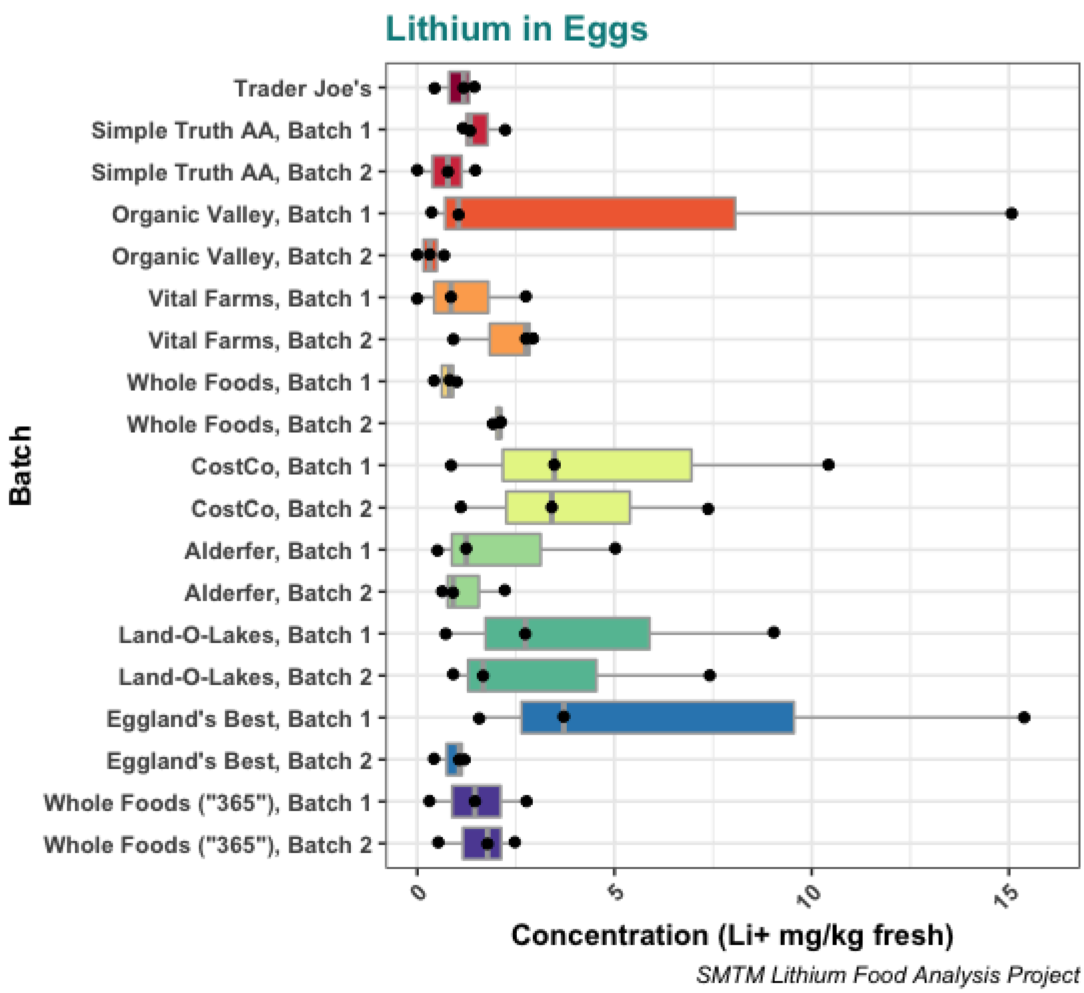
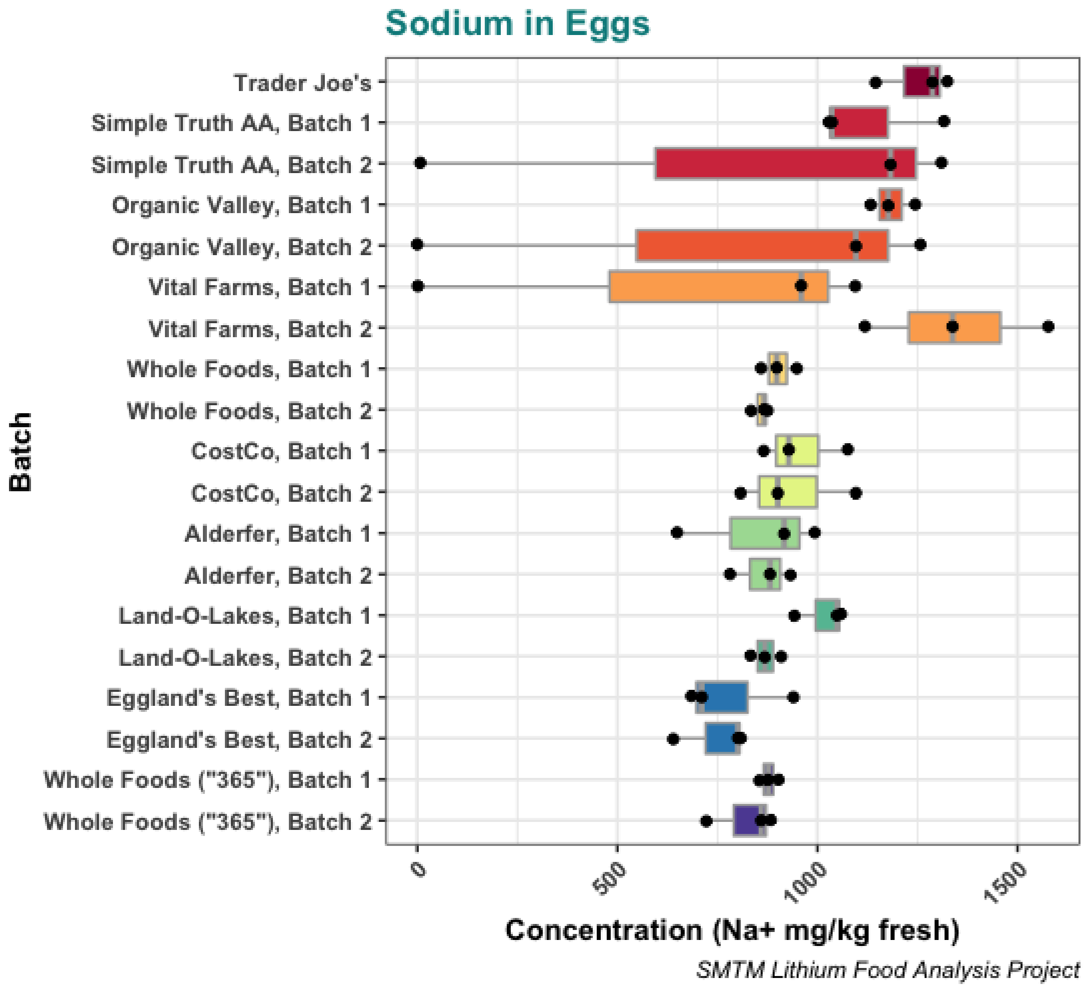


Figure 2.



In both lithium and sodium analysis, there is considerable variance between replicates. Each batch was analyzed in triplicate, and outliers within batches are striking.

Variance
Anticipating this result, we intentionally included a variance test.

Batches E02 - E06 all came from the same carton, but E02 and E03 involved blending 4 eggs together for analysis and E04/E05/E06 involved taking a single egg and mixing/sampling just that single egg.

Single-egg analyses do have lower variance than the 4-egg batches (Figure 3 and Figure 4). This suggests that there really is egg-to-egg variation, which likely accounts for the variation between replicates in the other 4-egg batches.

Figure 3.

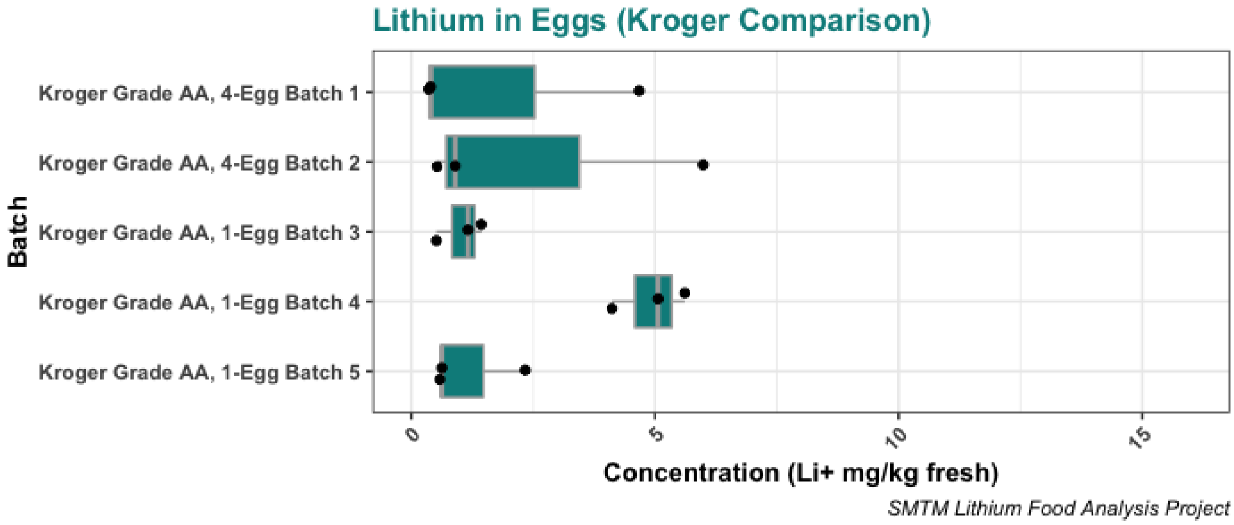
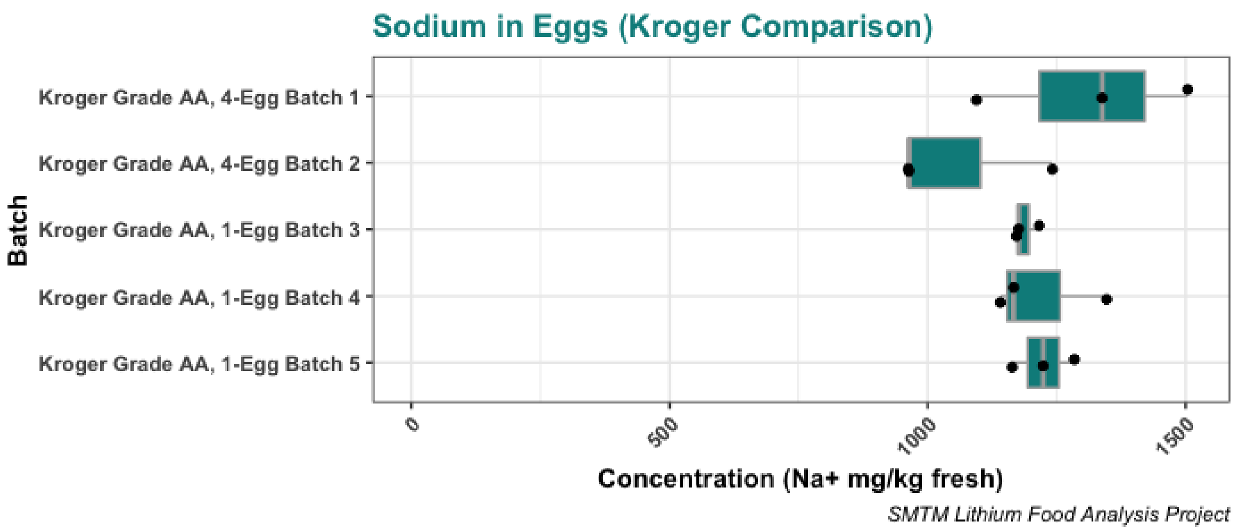


Figure 4.



Possible next steps

An alternative analysis, such as atomic absorption spectroscopy (AAS), may also be helpful to confirm these results using an orthogonal method, especially because previous results found differences in quantitation between different analytical techniques. AAS is less sensitive than ICP-OES, so food samples must be relatively high in lithium to bring them into the quantitative range. Fortunately, some of the egg samples in this study appear to contain such concentrations, with lithium levels in the dry weight as high as 60 µg/g.

Studying a larger variety of foods can shed light on the overall levels of lithium in the American food supply. Eggs are likely not the only food that contains appreciable levels of lithium, and there may be some foods that contain even more.