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Is it possible to verify commercially produced eggs as 'free-range' using stable isotope or trace element analysis?

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Introduction

In the wake of numerous scandals within European eggs (Heart of England 2009, German organic egg scandal 2013, Dutch free-range egg scandal 2013, and Swiss quail egg scandal 2016) the need for forensic methods to augment existing traceability has never been greater. Of particular interest are analytical methods that can reliably distinguish between the following egg production methods: organic, free-range, barn, colony cage. There are existing methods to differentiate between 'organic' and 'conventional' (free-range, barn and colony cage) based on stable isotopes (Agroisolab Fig. 1, Rogers, 2009, Rogers et al 2015).

There is also an emerging body of evidence suggesting trace element analysis can be used as a means to differentiate between the various types of conventional eggs (free-range, barn, and cage). In order to evaluate the quality of this evidence, it is necessary to review the current state of knowledge in stable isotope and trace element applications that are being used in research as a platform for the development of commercial tests.

What does 'free-range' mean?

It is important that there are clear definitions for animal husbandry methods. Without these definitions, consumers may be misled, also there is no way to guarantee that a particular form of husbandry is adhered to, or that forensic data from husbandry systems is comparable between countries. With respect to this review, it is important to highlight that many of the countries that have researched forensic methods to verify eggs as 'free-range' or differentiate between husbandry systems have differing definitions of what 'free-range' means. Consequently, one of the most important factors to take into account is whether one country's 'free-range' is equal or relevant to describing free-range egg production in another country. If the definitions are not consistent researchers may end up citing evidence that is not valid, or developing methods that are not applicable to their respective country's animal husbandry and agricultural practices.

One such example is that Brazil has no formal definition of 'free-range' (Murilo Quintiliano (FAI Brazil) 2012), therefore Brazilian 'free-range' egg and chicken producers have been following organic farming practices, which are internationally recognised, to describe their products as 'free-range'. In terms of evaluating data, this means that 'free-range' conditions in papers from Brazil are less similar to 'free-range' in the UK/Europe and are more similar to 'organic' farming practices in the UK/Europe. There are strict requirements in organic farming prohibiting the use of non-organic feeds that stipulate feed ingredients in organic agriculture must be produced in accordance with organic farming methods. However, in European free-range/conventional egg farming, there are no strict requirements separating husbandry systems with different feeds. This may explain the relative success of Brazilian applications to check whether eggs are "free-range" (by their definition) or from other husbandry systems (Barbosa et al 2014).

'Free-range' shell egg production has clear legislative definitions in three regions in the world; European Union member states, Australia, and the USA, (see Appendix. Table 2).

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More confusing is what can be defined as 'free-range' on a scientific paper and what is 'free-range' as a commercial reality. The vast majority of academic papers investigating the difference in trace element signatures between free-range, barn and caged eggs have focused on data from very small production systems more akin to chickens in a back yard (Van Overmeire et al 2006, 2009, Waegeneers et al 2009, Giannenas et al 2009) rather than commercial units ranging from 3000 birds (organic) to multiples of 12000 (Appendix 1. Tab. 2). While eggs from the back-yard production methods are arguably and justifiably 'free-range' it does not guarantee that their data can be used to infer trends on large commercial free-range shell egg farms, which emerging analytical methods aim to regulate.

Key differences exist between small scale 'courtyard' free-range egg production and industrial scale free-range production. These include (but aren't limited to) the type of feed used, access to soil, the likelihood of the birds being outside, and access and availability of forage (insects and worms). These differences are of great significance because stable isotope and trace element methodologies rely on differentiation by what the animals eat to differentiate between husbandry systems.

Presently, within the EU, there is no requirement whatsoever for a farmer to feed free-range, barn and cage chickens different rations, whereas 'courtyard' birds tend to be fed scraps, waste food, and rations produced for small flocks (Van Overmeire et al 2006, 2009, Waegeneers et al 2009, Giannenas et al 2009). Consequently, trends in stable isotope ratios and trace elements as between 'courtyard' and barn or caged eggs should not be inferred without evidence demonstrating there is no significant difference between 'courtyard' feeds and commercial 'free-range' feeds, the feeds must be comparable. It is true that free-range chickens may be given more calorific food than their indoor counterparts, but there is no regulatory requirement mandating this.

Do free-range chickens go outside?

One of the central pieces of reasoning that it might be possible to tell the difference between 'indoor' and 'outdoor' eggs is that birds who can go outdoors, can pick up *something* from the environment, or lose *something* to the environment. This reasoning falls into question if 'outdoor' birds choose not to go outside, or don't have the opportunity to due to health or biosecurity reasons.

Since the 90s (Grigor and Hughes 1993) it has been demonstrated that there are always some 'free-range' birds in commercial houses under EU-style standards that never go outside their house. There are reasons for this, as highlighted in Richards et al (2012), though perhaps a good summary that 'free' in 'free-range' is the freedom of choice of the bird to go outside or stay inside. One way to accurately monitor how many birds range outdoors and how many stay indoors is to use radio frequency identification (RFID) tags attached to the birds to monitor their movements. In a study by Richards et al (2011) four flocks of 1500 free-range hens were used to monitor what percentage of the birds stayed indoors. In each of the four flocks, 10% were tagged with RFIDs (600 birds in total). 8 to 16% of the birds never ranged outside. 75% of the birds that did go outside throughout the trial only went outside on 50% of the days. Richards (et al 2011) also demonstrated that ranging was variable with respect to temperature and weather, which is not a surprising finding given that most animals seek shelter in the rain and cold. In summary, laying hens may either never go outside, or go outside infrequently across the year. This variability logically affects the causality chain posed by researchers suggesting that a test for 'free-range-ness' may work because 'outdoor birds' are picking 'something' up by going outside, or losing 'something' to the environment due to their increased metabolism/immunological stress.

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Principles behind using trace elements or stable isotopes to verify eggs as 'free-range'

Trace elements

Much of the science that claims to differentiate between 'outdoor' (free-range/organic/courtyard) eggs and 'indoor' (cage/barn) eggs is based on the assumption that birds that can go outside will go outside, and therefore will have the opportunity to pick up 'something' by going outside, or will lose 'something' due to the increased metabolism of the chicken from regularly going outdoors. Typically it is cited that free-ranging birds may pick up some trace elements from ranging outdoors [Kucukyilmaz et al 2011, Zhu et al 2015].

In free-range/organic egg testing with trace elements, there are two studies that have attempted to investigate the relationship between husbandry style and trace element signatures in order to establish a causal relationship. These studies were carried out by Kucukyilmaz et al in 2011 and Zhu et al in 2015.

The basis of these types of evaluation is the supposition that if there is more of a particular trace element in an 'outdoor' bird's eggs (organic/free-range) than in an 'indoor' bird's eggs (cage/barn) then the chicken may be taking the trace element in from the environment. Conversely, if an 'outdoor' bird's eggs (organic/free-range) have a lower concentration of a particular trace element than an 'indoor' bird's eggs (cage/barn), then the 'outdoor' chicken may be losing something to the environment by metabolism, or the trace elements it has received are in a less bioavailable form.

Kucukyilmaz et al (2011) performed a highly controlled study to investigate trace element concentrations in eggs from conventional cage chickens and organic chickens. 216 conventional chickens were randomly assigned to six replications of triple-deck battery cages. 100m away, 200 organic chickens were housed in four 'barns' in accordance with international organic standards for laying hens (see appendix, table 2). Both populations were reared from 23 weeks to 70 weeks of age. Though the conventional chickens consumed a conventional diet and the organic chickens consumed an organic diet, analysis of the diets showed near-identical trace element composition. There was no mention of when throughout the life of the chickens the feed sample was taken to be analysed. It is important to note that across the rearing period (23 weeks to 68 weeks) there was no grass available outside, only bare earth. 12 eggs were collected from both conditions at 68 weeks of age and their trace element profiles were compared (Ca, P, Mg, Fe, Zn, and Cu). The results showed some significant differences in potassium concentration and zinc concentration in the edible portion of the egg, and significant differences in the magnesium and zinc content of the shell of the egg between the two conditions (see table below).

Trace element	**Edible portion of egg** Concentration of trace element in 'outdoor' (organic) bird relative to 'indoor' (cage) bird	**Eggshell** Concentration of trace element in 'outdoor' (organic) bird relative to 'indoor' (cage) bird
Calcium	No significant difference	No significant difference
Phosphorous	LOWER	No significant difference
Magnesium	No significant difference	HIGHER
Iron	No significant difference	No significant difference
Zinc	LOWER	LOWER
Copper	No significant difference	No significant difference

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The study concluded that lower phosphorous and lower zinc concentration in the eggs were explainable by metabolic processes. Kucukyilmaz et al concluded that phosphorous is used in bone tissue and as organic chickens move around more often than caged chickens this caused a greater rate of bone turnover in organic chicken bone. Therefore, phosphorous may have been preferentially used in the bones of the organic chickens as opposed to the turnover in the caged chickens. However, no effort was made in the study to back up this conclusion with data (e.g. analysis of the phosphorous content of the bones of the chickens), and this may warrants investigation.

Kucukyilmaz et al concluded that zinc is used in many metalloenzymes and is used in the immune system (Kidd 2005) therefore, lower zinc in organic (outdoor) hens may be due to the extra environmental/metabolic stresses on the chickens. Another possible explanation for the lower zinc was poor bioavailability of zinc from the organic feed through the gastrointestinal system of the chicken.

Conversely, magnesium concentration was higher in the outdoor/organic chickens than the indoor/caged chickens. Kucukyilmaz et al explained this may have been the product of the chickens consuming grit/soil/small rocks from outdoors which may have been rich in magnesium and calcium. However, no effort was made to measure magnesium or calcium concentration in the grit/soil/small rocks on the site to support this hypothesis. This should be investigated in future studies.

Finally, the sample size used in the Kucukyilmaz study may not have been large enough to apply to bigger units. Only 200 'free-range' birds were used in the study, compared to a commercial unit of 30,000 birds the sample size used is a drop in the ocean. The trends discovered may not represent the true variance of larger free-range units.

The study by Kucukyilmaz et al (2011) was replicated by Zhu et al (2015) with some slight differences. Notably, Zhu et al compared caged hen egg trace element profiles with 'free-range' hen eggs.

In this study the definition of 'free-range' was not overtly clear, nor is the legal definition for 'free-range' in China – however, the conditions the 'free-range' chickens were subjected to were comparable with home-grown/courtyard 'free-range' chickens. These conditions are not directly comparable to conditions on commercial free-range units in the UK/Europe.

108 conventional chickens were housed in an automated hen house and randomly assigned to three replications of triple-deck battery cages. 90 free-range chickens were reared in a yard with self-sown grass and soil across the experimental period (June-July, 1 month). There was no mention of the proximity of the free-range unit to the cage unit other than both were in Daqing, Heilongjiang Province of China (latitude 45^{o}46', longitude 124^{o}19'). It is important to note that the study used different feeds for the conventional and the free-range chickens, despite the lack of a logical or legislative reason to do so. The sample size used in this study was smaller than in the Kucukyilmaz study (2011), therefore the results may have similar limitations in applying to large commercial free-range units.

Analysis of the composition of the dietary rations showed marked differences in selenium, zinc, copper, manganese and cadmium concentrations, therefore both experimental conditions had different trace element inputs. Interestingly, despite the significant difference in cadmium concentration between the two feeds, no significant difference was observed between the eggs from the different husbandry conditions. There was also no mention of when throughout the life of the chickens the feed sample was taken to be analysed, "Egg sample collection commenced 6 weeks

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after feeding on the dietary treatments and lasted for 2 weeks…" (Zhu et al 2015). The yolks of 20 eggs per housing system were analysed for each housing system condition (cage vs. free-range) (Ca, P, Se, Zn, Cu, Mn, Cd, and Pb). Each egg yolk was analysed in triplicate.

Trace element	Yolk of egg Concentration of trace element in 'outdoor' (free-range) bird relative to 'indoor' (cage) bird.
Calcium	LOWER
Phosphorous	No significant difference
Selenium	LOWER
Zinc	LOWER
Copper	LOWER
Manganese	HIGHER
Cadmium	No significant difference
Lead	HIGHER

Interestingly the discussion of results in the Zhu et al (2015) paper begins with a near word-for-word copy of parts of the discussion in the Kucukyilmaz et al (2011) paper. This apparent plagiarism questions the credibility of the paper.

Zhu et al concluded that the lower zinc concentration in the eggs was explainable by metabolic processes in the same manner Kucukyilmaz et al (2011) explained the causality of the lower zinc concentration in the organic hens. However, Zhu et al made minimal effort to explain the differences in zinc concentration in the eggs in reference to the dietary zinc concentration the chickens received.

According to the compositional analysis of trace elements in the feed between the conventional and free-range chickens, the free-range chickens received lower concentrations of dietary zinc. The only statement regarding this was "The differences in Zn concentrations of eggs did not arise mainly from ingested feed ingredients or additional supplementation of minerals" with no supporting calculations or statistical analysis.

Lower calcium concentration in free-range egg yolks was explained by the calcium being absorbed preferentially by the free-range chicken bones, but again, no supporting evidence was given to demonstrate this effect analytically such as analysing the bones of the free-range and caged chickens.

Lower selenium in free-range egg yolks was explained by the fact "Daqing is a selenium-deficient area" (Xu et al 1986) therefore the grain, grass, soil and grit ingested by the chickens would be short of this element. Again, this informed assumption was not supported by analytical evidence of the soil or grit. Furthermore, Zhu et al (2015) made minimal effort to explain this difference in the context of the results of the dietary compositional analysis where the free-range chickens received ¼ of the dietary selenium the caged chickens received.

Despite the fact that the free-range egg yolks showed significantly lower copper concentration than caged-hen yolks, Zhu et al (2015) failed to comment or provide an explanation for this, perhaps because the free-range chickens received significantly less dietary copper than the conventional chickens according to the dietary compositional analysis.

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Higher concentrations of manganese and lead in the free-range eggs were explained by Mn and Pb being the products of local industry. Again Zhu et al did not provide any direct evidence to support this hypothesis. Nevertheless, other studies have shown the impact of atmospheric deposition of trace elements due to non-ferrous industries (Kloke et al 1984 and De Temmerman et al 2003).

Between the two studies, there are certainly some consistent results, e.g. lower zinc in free-range/organic eggs than in caged hen eggs. This information is potentially useful for further investigation, though further evidence would need to be obtained before causality between birds exercising and lower zinc concentrations can be established. It is also noteworthy that similar results in zinc have been observed in less well-controlled studies (Giannenas et al 2009 – 'courtyard chickens'), though zinc was found to be higher in concentration in the 'free-range'/ 'courtyard' eggs than in the caged eggs seemingly contradicting the conclusions of Zhu and Kucukyilmaz. Nevertheless, as the Giannenas study was in the style of a natural experiment and was not as well controlled as the studies by Zhu and Kucukyilmaz it is difficult to reach any conclusion about why this is so.

It is also worth noting that the 'organic' category eggs in the Giannenas study showed lower Zn concentration than the caged/conventional eggs and the conditions used by Zhu were more similar to organic than free-range. Perhaps zinc actually has more potential of being a useful parameter to differentiate organic from conventional eggs. It is worth noting that even if a causal relationship is established between access to the environment and lower zinc, it still does not mean that the parameter can be utilised to differentiate between 'outdoor' and 'indoor' eggs. The explanation for the lower zinc appears to be metabolic/immunological stress; it is not impossible that there are cases where caged birds may experience metabolic and immunological stresses that cause zinc depletion, meaning that zinc concentration alone might not be a robust parameter for differentiation.

Furthermore, despite the fact that Zhu et al (2015) claim the hens were 'free-range', the conditions the 'free-range' birds were kept in are not entirely analogous to European commercial free-range farms (Hens Directive 2002), which have a significantly greater number birds that have potentially reduced access to grasses due to the stocking density and the impact a large flock has on a field. Finally, it should be noted that both studies place emphasis on 'little stones' or grit, explaining the differences in trace element signatures with little-to-no evidence to back these claims. More work has to be undertaken to investigate this explanation properly before claims of causality can be made. Furthermore, any country attempting to adopt this test at a national level would need to analyse reference samples of eggs from organic/free-range and cage units as well as soil samples from the organic/free-range farms in order to attain causality. To do so without analysing the soil would form the basis of a purely speculative test.

Finally, existing studies have highlighted the variability in trace element signatures over seasons and geographies across various countries and organisms, such as Australian pork (Kreitals 2013, Watling et al 2010), Belgian eggs (Waegeneers et al 2009), Brazilian chicken (Batista et al 2012) suggesting that without continual sampling throughout the year of every farm, or demonstration of low seasonal variability, trace element analysis may not reach its full potential as a tool for geographic verification or detecting whether eggs are 'free-range' or not.

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Stable isotopes

In the UK and Europe, it is typical for egg producers to buy chicken feed in bulk to reduce feed price. Given that producers may have free-range, barn and cage production units, unless otherwise specified, all of the birds they house can be consuming the same feed.

As both stable isotope and trace element technologies are primarily based on what an organism eats, the reasons why there should be a difference between the types of conventional eggs are not completely apparent. The most commonly cited isotope ratios as being helpful for husbandry system differentiation are nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$).

Nitrogen isotope ratios in chicken feed are dictated by the trophic levels of the individual feed ingredients. The nitrogen in the eggs comes originally from either the air (nitrogen fixers such as alfalfa) or from mineral nitrogen (Rogers et al 2015). In organic systems/organic-like systems, the nitrogen can originate from organic fertilisers such as manures, composts and green manures. If there is no requirement to feed the birds different husbandry system different rations and it is cheaper to feed all conventional laying birds the same ration, there should be no difference in their respective nitrogen ratios. Agroisolab (see Fig. 1) and independent researchers (Rogers et al 2015) have found comparable results for the ranges of nitrogen isotope ratios in various types of conventional eggs (free-range, barn, and cage). However, it is known that free-range chickens have a greater metabolic demand on average than 'indoor' birds, this can be addressed by giving free-range birds more calorific feed, or more feed. Regardless, the data from Agroisolab and Rogers et al (2015) encompasses this and shows no significant difference between the conventional husbandry systems by nitrogen stable isotope analysis.

In some of the academic papers discussed in this review, differences in nitrogen isotope ratios between 'cage' and 'free-range' eggs have been attributed, in part, to the birds consuming insects and worms (Rogers 2009). The rationale behind this is because worms and insects have a higher trophic level than the cereals that go into the feed ration, they will have a higher nitrogen ratio, and eggs laid by chickens consuming these organisms will also have a high nitrogen ratio. If this were true in commercial-scale egg production, the difference would be evident from the bigger studies (Agroisolab, Fig. 1, Rogers 2015). Perhaps the absence of this evidence is because there simply aren't enough insects and worms to consistently supply thousands of free-range birds with the extra food they would need in their daily diet to make a difference to nitrogen isotope ratios.

Carbon isotope ratios in eggs describe the portion of C4 (usually maize) and C3 cereals/plants in the hens' ration. Some countries, such as Holland, have shown a weak tendency to prefer maize in conventional eggs (Rogers et al 2015), some show the opposite (UK). Ultimately, the amount of maize in a feed ration is dictated by a human being and has a non-causal relationship with animal husbandry style. In conclusion, carbon isotope ratios have no meaningful use in distinguishing free-range from barn or cage eggs.

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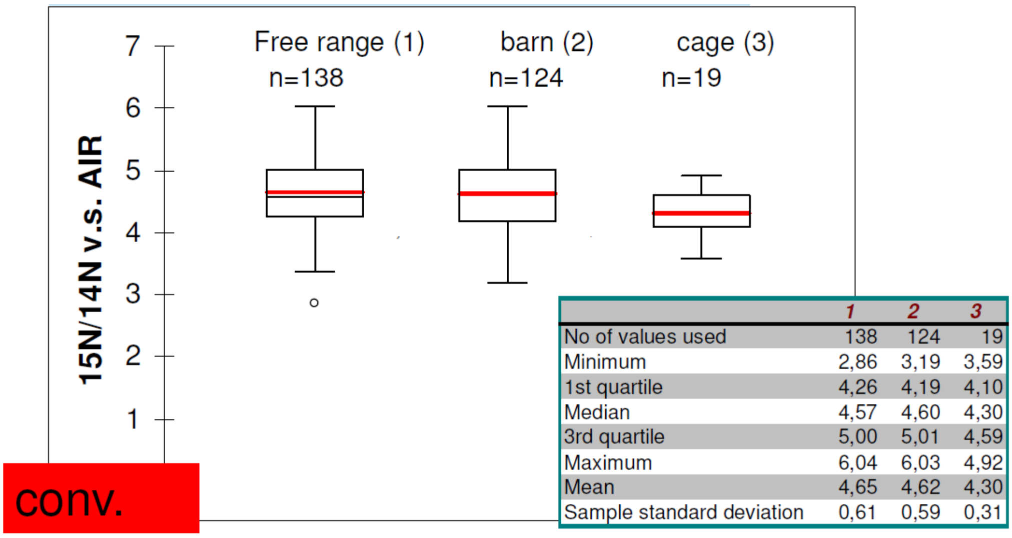

	1	2	3
No of values used	138	124	19
Minimum	2,86	3,19	3,59
1st quartile	4,26	4,19	4,10
Median	4,57	4,60	4,30
3rd quartile	5,00	5,01	4,59
Maximum	6,04	6,03	4,92
Mean	4,65	4,62	4,30
Sample standard deviation	0,61	0,59	0,31

Fig.1 Agroisolab - nitrogen isotope ratios in free-range, barn, and cage eggs from European countries

Conclusions

Based on currently available evidence, there is no clearly established causal relationship between any particular trace element signature, stable isotope signature, and the ‘free-range’ status of an egg. All currently published data hints there may be some link, but to date, all conclusions are based on informed speculation and not on actual data. Hopefully, this will be improved through subsequent research, as the need for real functioning tools to authenticate husbandry systems in eggs is substantial. Much of the research cited in this paper suggests that there are measurable differences in trace element or stable isotope signatures as between chickens ‘picking something up’ when outside, compared to housed chickens, who do not. But none of the papers cite good evidence to substantiate this hypothesis; it is possible that no such evidence will be found, especially in commercial, multi-thousand flocks, where actual roaming outside is either limited, due to flock size, or irrelevant for the significant proportion of birds, who may not go outside at all (Richards et al 2012). Furthermore, commercial free-range and conventional egg-producing flocks are not fed significantly different rations, other than to satisfy the calorific demands of free-range birds. There are no legislative requirements for altered rations as between caged/barn and free-range production systems.

One of the reasons academic papers may be showing differences in stable isotope and trace element profiles of eggs from different husbandry systems is that they deliberately used different feeds which, of course, will generate measurable differences at analysis. Further studies are needed where indoor and outdoor hens are fed the same ration on the same site to control for this. However, as a portion of free-ranging birds on commercial units never go outside or go outside sporadically, there is no logical reason to believe environmental factors may affect them, meaning that their ‘free-range’ eggs may be indistinguishable from other types of conventional eggs by currently available analytical methods.

Currently, the only trace element parameter that shows promise to distinguish ‘outdoor’ eggs (free-range/organic) from indoor (barn/cage) is zinc concentration. However, more work must be done to investigate this and establish causal reasons. Furthermore, this parameter needs to be checked across the industry to ascertain the degree of overlap between it and the various husbandry styles.

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If a test is developed to distinguish free-range eggs from other types of conventional eggs it needs to establish causal reasons why it works that are factually sound and are applicable to commercial free-range egg production, rather than small-scale ‘hobbyist’/courtyard free-range production. If not, it is entirely possible that despite statistics showing separation between ‘free-range’ and caged eggs, the variance might actually relate to some other attribute (e.g. geographic origin) that can classify the husbandry system of eggs by coincidence. Developers of forensic methods must be careful to control for these errors, as developing statistical models may initially show promise, but if used improperly they may lead to potentially damaging and incorrect conclusions. Those who wish to adopt forensic methods in their due diligence testing must also do so in the knowledge of the limitations of the test’s validity.

In-short, there is a need for more rigorous experimental protocols; ones where flock sizes are commercially representative. Most importantly with strong control elements of feed type, building type, and adherence to usual commercial practice.

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Appendix.

Table 1: Cross-comparison of trace element profile differences between 'outdoor' (Free-range/Courtyard/Organic) and 'indoor' (Cage/Barn) eggs from currently published works

Study	**Kucukyilmaz et al (2011)**	**Kucukyilmaz et al (2011)**	**Zhu et al (2015)**	**Giannenas et al (2009)**	**Giannenas et al (2009)**	**Giannenas et al (2009)**	**Giannenas et al (2009)**
Trace element	**Edible portion of egg** Concentration of trace element in 'outdoor' (organic) bird relative to 'indoor' (cage) bird	**Eggshell** Concentration of trace element in 'outdoor' (organic) bird relative to 'indoor' (cage) bird	**Yolk of Egg** Concentration of trace element in 'outdoor' (free-range) bird relative to 'indoor' (cage) bird.	**Yolk of Egg** Concentration of trace element in 'outdoor' (courtyard/free-range) bird relative to 'indoor' (cage) bird.	**Albumin of Egg** Concentration of trace element in 'outdoor' (courtyard/free-range) bird relative to 'indoor' (cage) bird.	**Yolk of Egg** Concentration of trace element in 'outdoor' (ORGANIC) bird relative to 'indoor' (cage) bird.	**Albumin of Egg** Concentration of trace element in 'outdoor' (ORGANIC) bird relative to 'indoor' (cage) bird.
Calcium	No significant difference	No significant difference	**LOWER**	*N/A*	*N/A*	*N/A*	*N/A*
Phosphorous	**LOWER**	No significant difference	No significant difference	*N/A*	*N/A*	*N/A*	*N/A*
Selenium	*N/A*	*N/A*	**LOWER**	**LOWER**	No significant difference	**HIGHER**	No significant difference
Zinc	**LOWER**	**LOWER**	**LOWER**	**HIGHER**	**HIGHER**	**LOWER**	No significant difference
Copper	No significant difference	No significant difference	**LOWER**	No significant difference	No significant difference	No significant difference	No significant difference
Manganese	*N/A*	*N/A*	**HIGHER**	**LOWER**	No significant difference	No significant difference	No significant difference

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Study	Kucukyilmaz et al (2011)	Kucukyilmaz et al (2011)	Zhu et al (2015)	Giannenas et al (2009)	Giannenas et al (2009)	Giannenas et al (2009)	Giannenas et al (2009)
Trace element	**Edible portion of egg** Concentration of trace element in 'outdoor' (organic) bird relative to 'indoor' (cage) bird	**Eggshell** Concentration of trace element in 'outdoor' (organic) bird relative to 'indoor' (cage) bird	**Yolk of Egg** Concentration of trace element in 'outdoor' (free-range) bird relative to 'indoor' (cage) bird.	**Yolk of Egg** Concentration of trace element in 'outdoor' (courtyard/free-range) bird relative to 'indoor' (cage) bird.	**Albumin of Egg** Concentration of trace element in 'outdoor' (courtyard/free-range) bird relative to 'indoor' (cage) bird.	**Yolk of Egg** Concentration of trace element in 'outdoor' (ORGANIC) bird relative to 'indoor' (cage) bird.	**Albumin of Egg** Concentration of trace element in 'outdoor' (ORGANIC) bird relative to 'indoor' (cage) bird.
Cadmium	*N/A*	*N/A*	No significant difference	No significant difference	No significant difference	No significant difference	No significant difference
Lead	*N/A*	*N/A*	**HIGHER**	*N/A*	*N/A*	*N/A*	*N/A*
Iron	No significant difference	No significant difference	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Magnesium	No significant difference	**HIGHER**	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Vanadium	*N/A*	*N/A*	*N/A*	No significant difference	No significant difference	No significant difference	No significant difference
Chromium	*N/A*	*N/A*	*N/A*	**HIGHER**	**HIGHER**	**HIGHER**	No significant difference
Nickel	*N/A*	*N/A*	*N/A*	No significant difference	No significant difference	No significant difference	No significant difference
Thallium	*N/A*	*N/A*	*N/A*	No significant difference	No significant difference	No significant difference	No significant difference
Arsenic	*N/A*	*N/A*	*N/A*	No significant difference	No significant difference	No significant difference	No significant difference

Table 2. Comparison of regulations surrounding husbandry systems in Australia, EU, and USA.

Country	Farming method	Regulatory body	Reference	Minimal acceptable stocking densities for birds inside the shed/building (birds/m2)	Feeding restrictions	Ranging definitions/rules/comments
Australia	Free-range	FREPA	FREE-RANGE EGG & POULTRY AUSTRALIA LTD. FREPA FREE-RANGE EGG STANDARDS 2015	10 (if up to 1000 birds on site) 9 (if up to 2000 birds on site) 8 (if up to 3000 birds on site) 7 (if up to 4000 birds on site) 6 (if over 4000 birds on site)	Only wholesome foods are permitted, with the addition of vitamins, minerals and amino acids as required for the welfare of the birds.	The land where birds are permitted to range must have shade, shelter and palatable vegetation. The range area must be capable of continued production of vegetation.
European Union	Free-range	European Union	Hens Directive, supra n.81, Art. 4 (1) (1) (e).Id. Art. 4 (1) (4).	9	None applicable	8.1.2. Free-range, open-air exercise areas, or open-air runs must, if necessary, provide sufficient protection against rain, wind, sun and extreme temperatures, depending on the local weather conditions and the breed concerned.
USA	Cage-free	United Egg Producers	United Egg Producers. Animal Husbandry Guidelines for U.S. Egg Laying Flocks 2016 Edition. http://www.unitedegg.org/information/pdf/UEP-Animal-Welfare-Guidelines2016.pdf	11 The density can vary between 7 and 11 depending on the 'cage free' system	1. Access to fresh feed must be provided at all times. Feed must not become stale, mouldy, rodent or insect infested, or contaminated with litter or faeces.	The area should be designed and managed to ensure it is kept in good condition and does not become infested with parasites, rodents or insects.
Australia	Cage	Commonwealth of Australia and each of its States and Territories	Primary Industries Standing Committee. Model Code of Practice for the Welfare of Animals. Domestic Poultry 4th Edition. SCARM Report 83. 2002	18 (if 3 birds per cage under 2.4kg/bird) 16.7 (if 3 birds per cage over 2.4kg/bird) 15 (if 2 birds per cage) 10 (if 1 bird per cage)	N/A	N/A
European Union	Enriched cages	European Union	Hens Directive, supra n.81, Art. 6 Id. Art. 6 (1) (a) & (b) and Art. 2 (2) (d).	16.7	N/A	N/A
USA	Cage	United Egg Producers	United Egg Producers. Animal Husbandry Guidelines for U.S. Egg Laying Flocks 2016 Edition. http://www.unitedegg.org/information/pdf/UEP-Animal-Welfare-Guidelines2016.pdf	23 *The range is 18-23 birds per square metre. Calculated from 1 bird per 67 to 86 square inches.	N/A	N/A
European Union	Organic	European Union	COUNCIL REGULATION (EEC) No 2092/91. of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs (OJ L 198, 22.7.1991, p. 1) 1991R2092— EN— 14.05.2008 — 031.001— 1	6 (each poultry house must not contain more than 3000 laying hens)	Cannot contain GMOs Cannot contain feed produced using synthetic fertilisers Cannot contain organic ingredients above 5% total	Poultry, must have access to an open-air run whenever the weather conditions permit and, whenever possible, must have such access for at least one third of their life. These open-air runs must be mainly covered with vegetation be provided with protective facilities, and permit animals to have easy access to adequate numbers of drinking and feeding troughs.

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