POULTRY CRC LTD

FINAL REPORT

Sub-Project No: 1.5.4

SUB-PROJECT LEADER: Dr Tamsyn Crowley

Sub-Project Title: A new test for the measure of poultry welfare

DATE OF COMPLETION: 30/6/16

ISBN 1 921010 54 1

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Sub-Project No. 1.5.4

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Published in 2016

Executive Summary

Welfare is of prime concern in the Australian Egg Industry and is highlighted with the ever increasing demand for free range eggs. There have been huge changes in the egg industry in recent times to accommodate for a laying hen's welfare, but what is driving this change? Some argue it is supermarket monopolies others suggest it is people's perceptions of hen welfare. Here we present a new scientific biomarker test that can used to determine the welfare and stress level of laying hens. The preliminary research presented in this report has surveyed cage, barn and free range management systems to determine their welfare/stress state. This test has the potential to be used as an auditing tool for future investigations into the welfare/stress of laying hens.

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Introduction

Heavily driven by public perception and large supermarket monopolies, welfare is of major concern for the poultry industry. The past few decades has seen development of poultry practices with a heavy focus on the welfare of poultry. There have been huge changes implemented to accommodate improvements in welfare particularly in the layer industry. There are now a number of production systems including caged and free range which provide the consumer a conscious choice on how their eggs have been produced. These advances are closely regulated in most countries with regular inspections and audits to ensure the industry it committed to improving welfare standards.

While these developments have undoubtedly facilitated an increase in perceived welfare standards there is little research to confirm that there has been an actual increase in welfare standards and reduced stress on the birds. Many of the demands placed on the industry in terms of welfare are essentially based on public perception and interpretation, thus making it difficult to objectively assess the real welfare situation. Currently there are a number of behavioural and other tests (cortisone levels) that have provided an insight into the welfare of poultry, but to date there is no clear scientific test that can be attributed to welfare. This project will develop a clear cut test that will determine the welfare/stress on layers in various production systems. This will enable the industry to provide accurate scientific information on the welfare status of their production systems. This test will also enable auditors to directly test welfare during the course of their routine inspections.

Over the last 30 years a dramatic increase in society's interest in the welfare of farm animals has arisen (David Fraser 2001; Coleman 2008) and consequently there has been increasing scrutiny of the use of farm animals. A current weakness in studying animal welfare is that there are differing definitions of animal welfare (D. Fraser 2003; Sandøe, Forkman, and Christiansen 2004). Together with a limited number of evidence based assessments of welfare there is need to develop further scientific quantitative assessments to allow producers and the industry to make decisions about the improvement of welfare in these systems. A commonly used biomarker of stress in avian species is measurement of corticosterone in blood. Corticosterone is the major adrenal glucocorticoid hormone that increases in birds under conditions of stress. Corticosterone has short-term effects on the physiology and behaviour of laying birds and also on their long-term performance. A wide variety of stressors, including environmental, temperature and humidity, housing space, feed and water restrictions and transport conditions, increase serum corticosterone concentrations in poultry. Unfortunately, there are major practical difficulties with the measurement of blood concentrations of corticosterone as a biomarker of stress responses due to the act of sampling serum from birds can have a profound effect on corticosterone levels as early as 45 seconds after restraint (Beuving and Vonder 1978). Non-invasive techniques have been developed looking at the corticosterone in egg yolk and albumen (Singh et al. 2009; Royo et al. 2008) and faecal droppings (Sophie Rettenbacher and Palme 2009; S. Rettenbacher et al. 2004). These techniques have reduced the sampling stress on the birds but can be time consuming and measure total corticosterone levels and does not distinguish between free (biologically active) and bound (not biologically active) corticosterone (Hemsworth and Coleman 2011).

Recently, a class of small non-coding RNAs, namely microRNAs (miRNA) that regulate gene expression and have a critical role in many biological and pathological process have been discovered. Studies investigating diseases in humans and other animals have shown clear differences in the expression patterns of miRNAs in serum from healthy compared to disease states (Chen, Hu, et al.; R. Liu et al. 2011; H. Zhao, Shen, et al. 2010; Gilad et al. 2008; QiQi Zhou and G Nicholas Verne 2011; Schrauder et al. 2012). These studies suggest that these profile patterns of serum miRNAs are useful as biomarkers in a range of conditions, including welfare status. Serum miRNAs are packaged in exosomes and these encapsulated miRNAs have been found also in human breast milk (Q. Zhou et al. 2012; Kosaka et al. 2010) and bovine milk (Chen et al. 2010; Hata et al. 2010).

Objectives

The aim of this project was to develop an evidence based diagnostic test for welfare. Our primary objective was to investigate chronic stress in layer hens. The diagnostic test will determine a bird's state of welfare by examining its miRNA profile and ultimately allowing this profile to be used as a biomarker for stress. This test will need to be applicable on a per flock basis rather than at an individual bird level to ensure its future value in the industry. This project will validate the diagnostic test by testing samples generated in our laboratory, other Poultry CRC project samples and industry samples.

Methodology

This project can be divided into three distinct phases with associated methodologies as set out below;

1. **Chronic stress (social/hierarchical competition) animal trials and sample collection**

Initially, a chronic stress animal trial was completed which included the following steps;

- Hens were housed in cages in pairs, food and water was freely available.
- Hens were randomly allocated to either a control group (n=20) or a stress group (n=20).
- Hens in the stress group were taken out of their cage and put in another cage on alternate days for 10 days. Half the animals were moved to a different cage in the first day and the other half were moved to another cage in the following day. These cycles were repeated over 10 days so that all the hens are moved to a different cage a total of 5 times and without sharing the cage with the same hen more than once.
- Hens in the control group were kept undisturbed in their cages during this period.
- A 1 ml blood sample was taken on day 1 and day 5 for corticosterone and miRNA determination in plasma in all hens.
- On the last day of treatment (Day 10), the hens were evaluated with a tonic immobility test. A hen will be placed in a supine position in a cradle with its head suspended from the side of the cradle. The right hand of the experimenter is placed on the breast of the bird, while the left hand gently forces the bird's head down. Birds will be restrained in this position for 10 s. If after releasing, the hen remains in this position for at least 10 s, TI duration will be recorded until the hen returns to an upright position. If the hen return to an upright position within 10 s after release, tonic immobility will be induced again, with a maximum of five attempts at inducing tonic immobility. The number of induction attempts needed and the duration of tonic immobility (i.e. latency to self-righting) will be recorded. The maximum duration of tonic immobility allowed will be 5 min, at which point the bird will be gently removed from the cradle and returned to its cage. A blood sample was taken at the beginning of the test and 15 min after the start of the test for corticosterone determination in plasma.
- Eggs were collected and identified on the same day that we collected blood samples.

Collected samples (serum and eggs) from birds in the controlled welfare model were assessed for their corticosteroid level (serum only) and also miRNAs were extracted ready for sequencing.

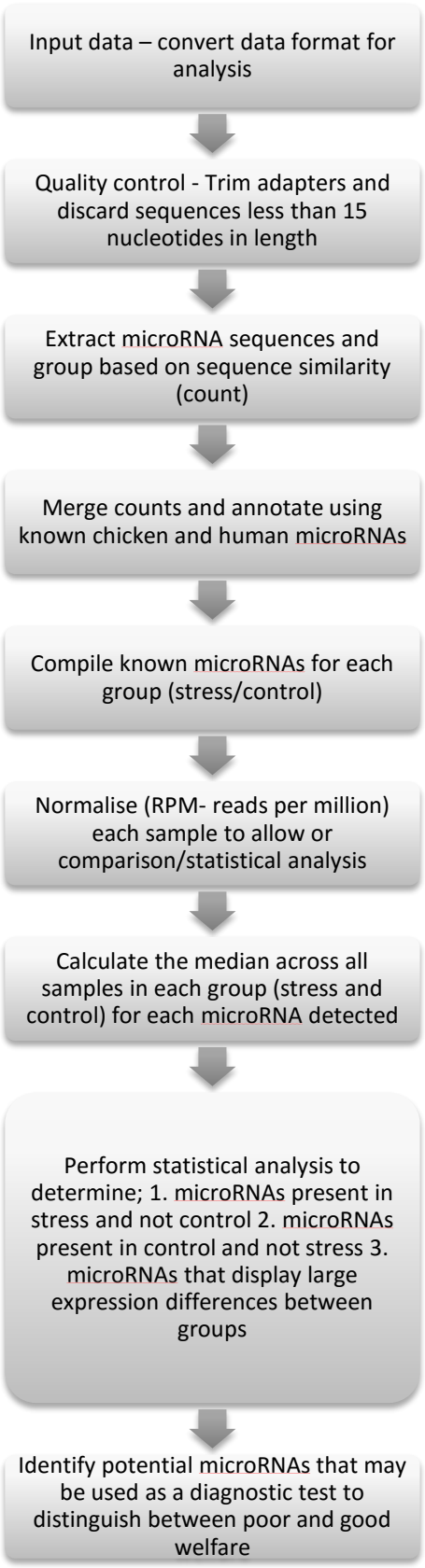

Figure 1 – Summary of the bioinformatics pipeline used to identify known and unknown miRNAs.

2. Development of a diagnostic miRNA biomarker test for stress

miRNA extraction and High throughput sequencing

miRNAs were extracted from both serum and egg samples and sent to Macrogen for high throughput sequencing (HiSEQ). This was performed twice to allow error reduction. The resulting sequence information for each sample was then put through a rigorous in-house bioinformatics pipeline that included quality control and analysis as outlined in Figure 1.
In short this pipeline ensures all sequence output files pass quality control which includes looking at the sequence output and determining the quality of the reads using FastQC. Prior to being run through FastQC each sample was groomed and adapters trimmed. A report was generated for each sample including a graphical output of the sequence quality. The sequence data is then extracted and counted (this puts all the reads with the same sequence together) and then annotated with a known miRNA database (MIRBASE). For each analysis there were three outputs;

1. Annotated miRNAs – this group included known miRNAs from chicken and human (MIRBASE) and allowed up to 2 mismatches, and/or 2 missing or extra nucleotides.
2. Mature miRNAs – this group included only known mature (exact match) miRNAs from chicken and human (MIRBASE).
3. Unknown miRNAs – this group included all sequences that fit the profile of a miRNA but were not annotated using the latest MIRBASE (21) release.

All samples are then normalised to ensure that each sample may be statistically tested to determine the differentially expressed miRNAs. Stringent t-testing (EdgeR) including multiple testing correction (p=0.05) was used to determine miRNAs that were significantly different between groups. Each of these outputs were then assessed to determine miRNAs that have the potential to be used in the diagnostic test.

Following identification of the miRNA biomarker candidates from the high throughput sequence analysis each candidate was validated using RT-PCR on the original welfare animal trials. Once validated a multiplex miRNA RT-PCR was then optimised for further validation in both serum and eggs.

3. Validation of the miRNA biomarker stress test

The resulting stress test was tested using a range of different industry samples as detailed below. We also tested some ‘blind’ samples to ensure the validity of our test.

Results and Discussion

Samples collected from the chronic stress trial were tested for corticosterone levels and tonic immobility. As shown in Table 1 there was a significant difference (P=0.03) in the corticosterone levels between the stress and control at 15 min, this is shown further in Figure 2. Samples (serum) from these trials were collected and sent for sequencing according to Table 2. Egg samples were also collected from this trial.

Table 1. Chronic stress. Hens were housed in pair cages. Stress hens were subjected to 12 days of daily social mixing (unstable social groups) whereas control hens were kept in stable pairs for the duration of the test. Data presented are LSMean ± SEM or back-transformed LSmeans ± SEM

			Corticosterone ng/mL1,2		Tonic immobility (TI) 2	
	n	Hens with all plasma samples (n)	Basal	15 min	# attempts to induce TI	Latency to stand (s)
Control	20	13	1.2 ± 0.2 (n=16)	1.3 ± 0.2 (n=17)	1.4 ± 0.3 (n=20)	131.8 ± 21.7 (n=20)
Stress	20	12	1.2 ± 0.2 (n=14)	2.1 ± 0.3 (n=17)	2.1 ± 0.3 (n=20)	138.1 ± 22.8 (n=18)
			p = 0.85	p = 0.03	p = 0.1	p = 0.8

1 Data was log transformed

2 Model allows for "cage pairs" effect

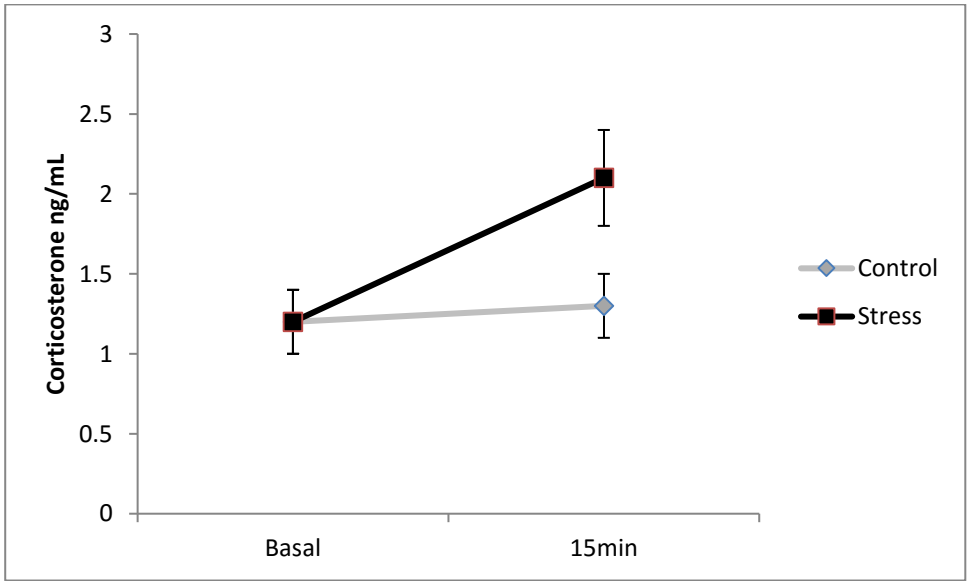

Figure 1. Plasma corticosterone response to tonic immobility test back-transformed LSMeans ± SEM (allowing for cage-pair number)

Table 2 – Calculations for the corticosteroid serum levels resulting from the chronic stress test. Details of shaded cells is as follows; red = stressed; green = controls; yellow = selected samples for preliminary sequence analysis.

RANK	sample	result (15min-base)		RANK	sample	FOLD diff
1	218	5.98		1	245	11.7619
2	230	3.06		2	218	5.496241
3	239	2.97		3	239	5.496241
4	212	2.20		4	5	4.380952
5	245	2.26		5	8	3.794118
6	6	2.18		6	223	3.952381
7	8	1.90		7	219	3.809524
8	15	1.84		8	212	2.981982
9	10	1.43		9	15	2.586207
10	225	1.24		10	225	2.589744
11	7	0.91		11	10	2.201681
12	5	0.71		12	230	2.037288
13	249	0.64		13	249	2.122807
14	223	0.62		14	6	1.95614
15	14	0.60		15	238	1.904762
16	219	0.59		16	7	1.784483
17	238	0.57		17	14	1.722892
18	214	0.42		18	214	1.461538
19	228	0.19		19	228	1.193878
20	235	-0.29		20	246	0.832432
21	229	-0.30		21	235	0.766129
22	246	-0.31		22	229	0.724771
23	4	-0.61		23	247	0.71916
24	3	-0.86		24	4	0.625767
25	250	-1.01		25	250	0.602362
26	247	-1.07		26	3	0.494118
27	232	-1.42		27	248	0.264059
28	248	-3.01		28	232	0.211111

Since the overall aim of this project is to develop a welfare assessment using non-invasive samples (ie. Yolk or albumin) it was imperative to determine if we could detect miRNAs in these type of samples. It was for this reason we processed and sent a small subset of samples (serum and albumin) for high-throughput sequencing (MiSEQ). From this initial test we are able to detect miRNAs in both sample types, however the samples were very variable in terms of the number of miRNAs detected; this is due to low yield of miRNA extracted initially. Also MiSEQ sequencing does not allow for a large read depth when multiple samples are run together as was the case here. This is the first time that miRNAs have been detected in albumin. This also confirmed that our sampling, transport (from Armidale NSW), storage and extraction protocols were sufficient allowing us to plan a larger scale sequencing experiment.

All serum samples sent for NGS passed QC and were successfully sequenced twice (following 3 attempts). We opted to send the serum samples in preference to egg samples to see if we could identify systemic differences in the hens. We have begun checking the profile of miRNAs in the eggs however, we have yet to optimise this in eggs and hence this requires further development. We hypothesise that we will be able to determine a miRNA profile that distinguishes the welfare state of the hen directly from the egg. This is not an unrealistic hypothesis as it has been previously shown in corticosterone levels where a high systemic level of the hormone does transfer into the egg via the blood (Groothuis, & Schwabl, 2008). All samples were put through our bioinformatic pipeline and 20 miRNAs and 1 control were selected for further analysis using RT-PCR.

Given our aim of generating an industry suitable assay for measuring stress we decided to concentrate upon a subset of miRNAs from those previously identified through our sequencing efforts. We looked at 21 miRNAs in total (Table 3), 20 which have been previously described and one novel miRNA discovered in our sequencing. The suitability of these miRNAs were confirmed using RT-PCR, with probes specific to each miRNA. We used serum samples from our experimentally induced stress trials against which each probe was tested. Extensive preliminary testing of these probes in RT-PCR showed that there was variation between individual birds within a treatment group and through trial and error, we found that the pooling of extractions from at least 10 individuals and performing the analysis on these pools produced the most reliable, reproducible and representative results. Whilst we were interested in miRNAs that showed differential expression between stressed and unstressed conditions, with an industry applicable assay in mind, we were also concerned about both reproducibility and sufficient expression of a particular miRNA in the sample when selecting candidate miRNAs.

We selected six miRNAs which could reliably and reproducibly generate a distinct profile able to differentiate stress and unstressed pools derived from our experimentally treated birds. These probes (Table 3) include five previously identified miRNAs and one novel miRNA and these represent miRNAs both up and down regulated during stress. We found through extensive testing across our library of samples that one of these miRNA, the novel miRNA we term Mir-control, whilst not necessarily being responsive to stress, proved a useful endogenous control which could be used for confirming sample quality. Figure 3 shows the miRNA profiles derived using this set of six probes against the two treatment conditions. We found that instead of using an absolute quantification measure when analysing the RT-PCR data it was more informative to employ a relative measure in which the number of cycles between the appearances of a probe compared to the one that preceded it is recorded. The key metrics we use when analysing such a profile are these differences, typically beginning with Mir-2188 as well as the difference between Mir-215 and Mir-2188, identified as a potential measure in previous sequencing analysis. The endogenous control, Mir-control, is not included in these metrics as it is only used as quality control. Of particular importance was the difference between Mir-2188 and Mir-215, which increased during stress.

Table 3. MicroRNA tested for suitability with RT-PCR

MicroRNA	Detected?	Expression during stress?	Highly expressed enough?	Reproducible?	Suitable?
Mir-10a	Y	↓	Y	Y	Y
Mir-10b	Y	-	Y	Y	N
Mir-22	Y	↑	N	N	N
Mir-26a	Y	-	Y	Y	N
Mir-30c	Y	↑	Y	Y	Y
Mir-30c-1	N	N/A	N/A	N/A	N
Mir-30c-1-3p	Y	-	Y	Y	N
Mir-92a	Y	↓	Y	N	N
Mir-99a	Y	-	Y	N	N
Mir-100	Y	-	Y	Y	N
Mir-142-3p	Y	↑	Y	Y	Y
Mir-146c	Y	-	Y	Y	N
Mir-183	Y	↑	N	Y	N
Mir-204	Y	↑	Y	N	N
Mir-215	Y	↓	Y	Y	Y
Mir-486-3p	N	N/A	N/A	N/A	N
Mir-1388-5p	Y	↑	N	N	N
Mir-2188	Y	N/A	Y	Y	Y
Mir-2970-5p	N	N/A	N/A	N/A	N
Let-7f	Y	↓	Y	N	N
Mir-Control	Y	-	Y	Y	Y

Y; Yes, N; No, N/A; not applicable, ↑; expression increased during stress, ↓; expression decreased during stress, those in bold were selected as suitable for use in the assay

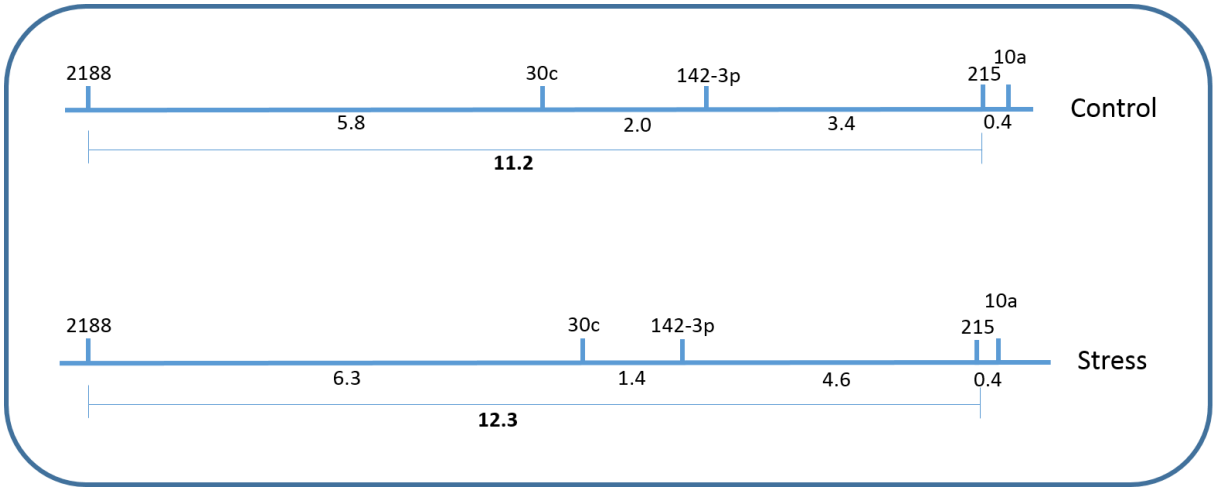

Figure 3. MicroRNA profiles of control and stressed pools. The figure is read left to right, with miRNA on the left being most highly expressed. The numbers between each miRNA represents the cycle difference seen in the RT-PCR and the long bracket with the figure in bold is the difference between Mir-2188 and Mir-215. It is important to note that Mir-control was included in this assay but is not shown here as it is for quality control only. Typically Mir-control is found to be more highly expressed than Mir-2188 in these samples, any significant deviation from that level of expression would lead to the results being rejected.

During this period we went to great efforts to establish industry contacts so as to attain samples from both healthy and stressed animals directly from an industry setting. Initially we were fortunate enough to get a large number of both blood and egg samples from unstressed animals reared in cage, barn and free-range management systems. Using these samples we were able to establish the expression profiles of our six miRNAs of interest in these three different management setting, summarised in Figure 4. We found that each management system displayed subtle differences in their profiles, despite all birds that were sampled having been from the same company, of the same line and same age. We also saw, perhaps not unsurprisingly, that as you moved from caged birds to barn reared birds to free-range animals the levels of variation in the expression of the miRNAs increased. Whether this variation was due to the presence of a subset of stressed animals in these management settings or simply reflective of more diverse behaviours of animals in barn and free-range settings compared to caged animals is unclear. One thing these findings did make apparent was the importance of comparing samples against healthy controls from the same management system as the differences between each system may otherwise interfere with the interpretation of such data.

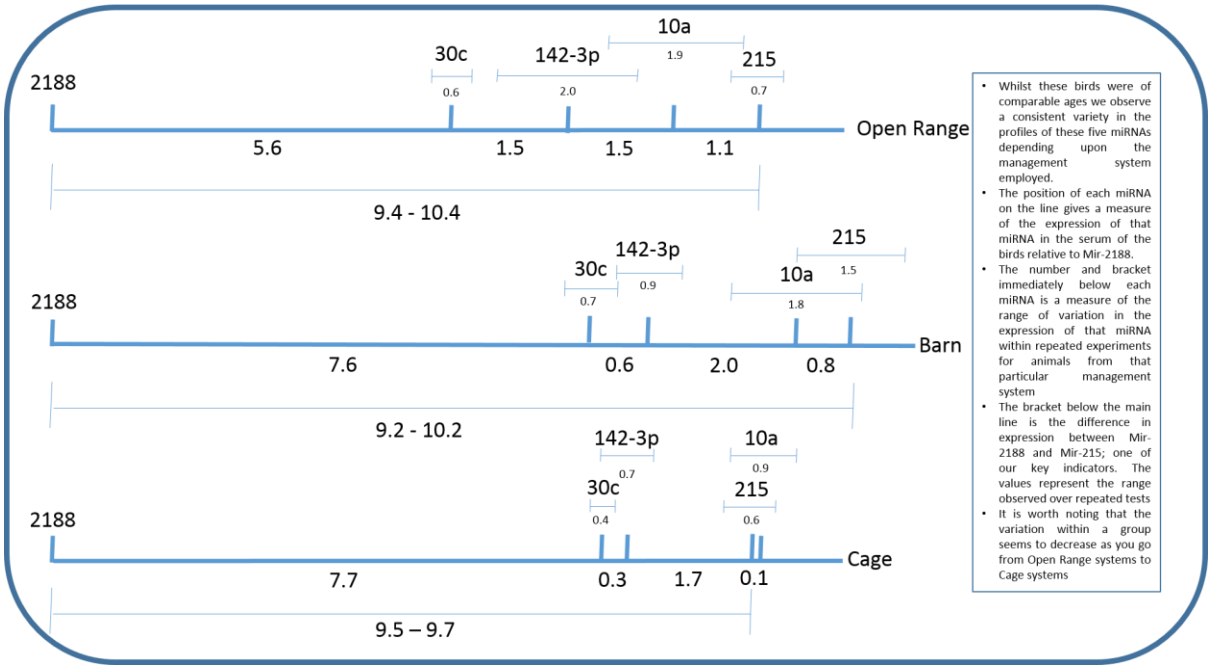

Figure 4. Different miRNA expression profiles in three different management systems. The figure in brackets above each probe demonstrates the level of variation observed between RT-PCR runs for that miRNA.

At the same time that we were receiving these unstressed industry samples we were also lucky enough to receive a number of samples from our industry partners from chickens undergoing stress in the field from both cage and barn management systems. We applied our assay to these new samples, once again pooling them in groups of ten extractions, and found that we got a miRNA profile for the stressed pools distinct to unstressed industry samples from their respective management systems (Figure 5). However we found that, whilst the Mir-2188 to Mir-215 difference did indeed change in the stress groups compared to the unstressed groups, the difference decreased during stress. This is the opposite of what we had seen in our experimentally stressed animals where the difference between these two miRNAs had increased. After multiple experiments it became clear that whilst our chosen miRNAs were responsive to stress the nature of their regulation (up or down regulated during stress) was less predictable. This may be the result of a number of variables such as animal age, line or the nature of the stressor.

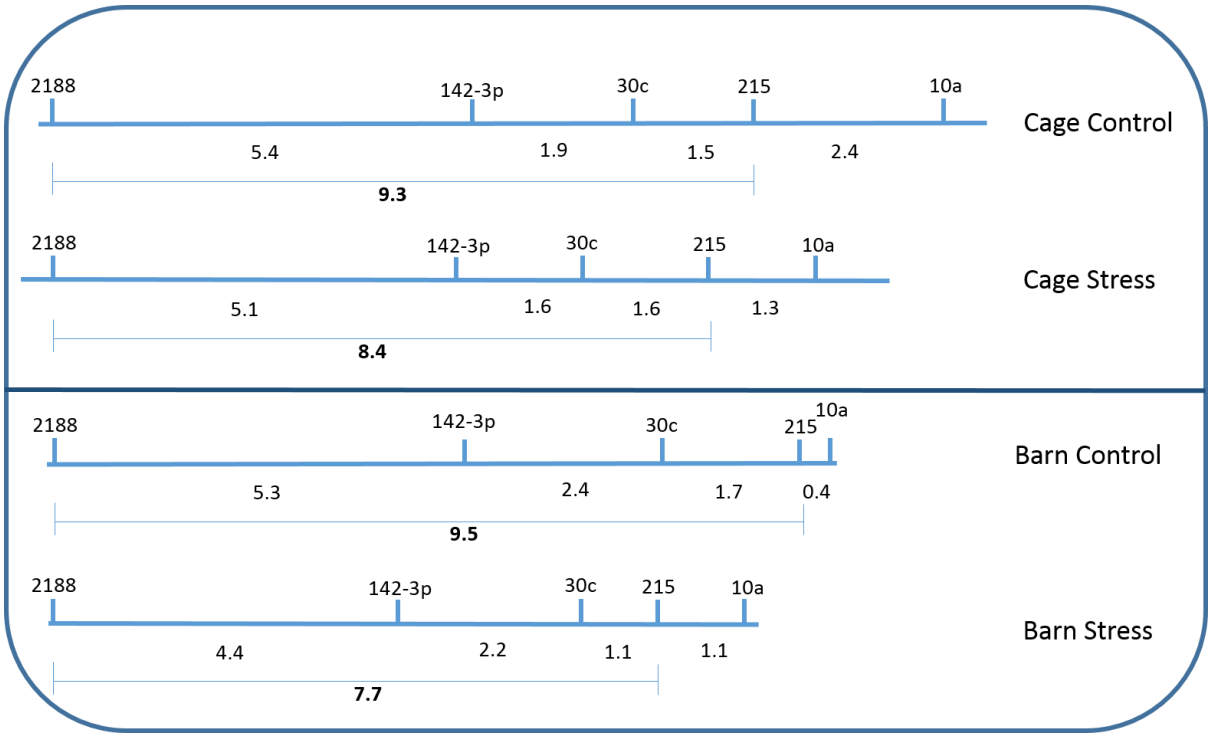

Figure 5.The miRNA profiles of industry samples from stressed animals compared to unstressed industry samples.

At this point we decided to modify our test so that rather than concentrating on the change between groups in a particular direction (Mir-2188 to Mir-215 increasing for example) the assay would instead generate a more global measure of change in the profile of these miRNAs. We also wanted to make the assay more robust and to allow greater stringency in the face of the variation we had previously seen in field samples from barn and free-range management systems. To this end we generated a measure that we refer to as the difference score (DS). This measure takes each of the five metrics that we record and adds together the difference for each between the control pool and stress (or unknown) pool. If we use the Barn samples from Figure 5 as an example we see the first metric in the control pool is 5.3 and the corresponding metric in the stress pool is 4.4; a difference of 0.9. The next metric is 2.4 vs. 2.2, a difference of 0.2 and so on. The final set of differences is 0.9, 0.2, 0.6, 0.7 and 1.8 which when added together gives a DS of 4.2. Now this figure on its own is not enough to draw any conclusions we must have a more stringent measure against which to compare our DS. To accomplish this and, at the same time, negate the potential effects of the observed variation we included three unstressed control pools per assay instead of only one. Fortunately we had received enough industry samples from unstressed animals so that each of our three pools could be randomised with minimal sample overlap of individual extractions between pools and this randomisation could occur each time the assay was undertaken minimising any unintentional experimental bias and increasing the robustness of the assay. An example of the assay is shown in Figure 6.

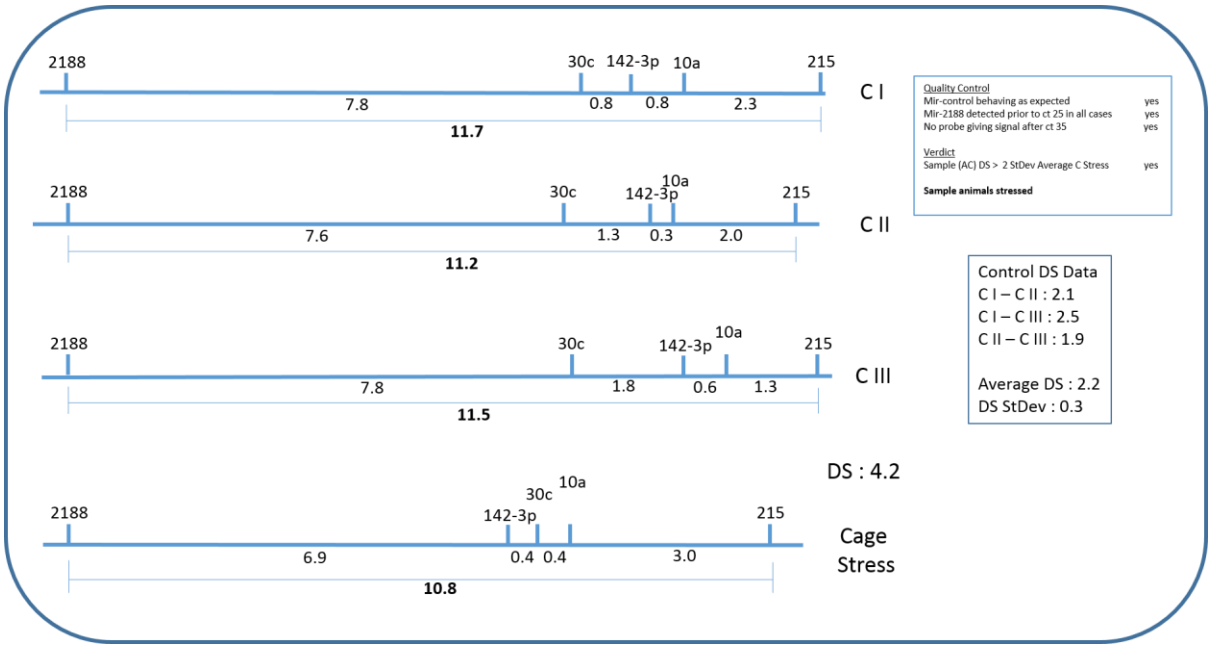

Figure 6. Three unstressed caged animal pool against industry stressed cage pool. C I, CII and C III are three randomised pools of ten samples each from unstressed caged chickens. Note the DS between the caged stress samples and the average metrics for the three unstressed pools is 4.3, greater than two standard deviations from the average DS across the unstressed pools.

Because there are three unstressed control groups they can be compared against one another and each time a control vs control DS is attained. With three control pools we can attain three DSs which taken together give an accurate measure of the level of variation you would expect to see within the unstressed population for these miRNAs. These three DSs can be used to generate an average control DS as well as a standard deviation. The DS for the stress (or unknown) pool can then be generated against the average of each metric produced across the three control pools. For example in the above case the control pools metrics for the Mir-2188 to Mir-215 are 11.7, 11.2 and 11.5; the average for these is (rounded) 11.5, the stress pool has 10.8 for this metric giving a difference of 0.7 and then so on for all metrics. If the resulting DS is two standard deviations greater than the average control DS then the pool is considered sufficiently different to have come from animals undergoing stress. If we look at the example in Figure 6 the average DS across the three unstressed pools is 2.2, with a standard deviation of 0.3. The DS of the stressed pool (compared to the average for each metric across the three unstressed pools) is 4.2, greater than two standard deviations above the unstressed DS and as such is confirmed as stressed. This assay was repeated with the field samples known to come from stressed animals from cage and barn management systems multiple times and was successful in each case in correctly identifying stressed samples.

Having established an assay which could differentiate between stress and unstressed animals when using samples of known providence we once again contacted our industry partners in the hope of obtaining further samples. But this time we specifically asked for blind samples where we would not know whether or not the samples came from stressed or unstressed animals, we would only be told what management system the chickens were raised in. Only after the assay had been undertaken and we communicated our conclusions to our industry partners would they reveal the status of the chickens from which the samples were sourced. These assays were again undertaken using pooled samples. We received a number of blind samples from different management systems and in each case our assay was able to correctly predict the status of the birds.

Having now established an assay that could reliably and consistently correctly predict the stress status of animals from the field using serum miRNA profiles we attempted some preliminary experiments using yolk and albumen extractions from the eggs of stressed and unstressed animals. In this case we again used the egg samples collected during our experimentally induced stress study. These studies were made possible by our earlier work which established the first method capable of extracting miRNA from the albumen and yolk of chicken eggs; a method that was recently published. Given that this was a preliminary study and that the number of samples available to us was limited we did not apply our complete assay to this task instead we only looked for a profile capable of differentiating eggs laid by stress and unstressed chickens. After numerous experiments we found that both the abundance and profile of miRNAs in the egg varied to that found in the serum of chickens and so the same six probes used before could not necessarily be utilised. Figure 7 shows an example of a miRNA profile from yolk extractions using RT-PCR, once again these samples are pooled prior to analysis.

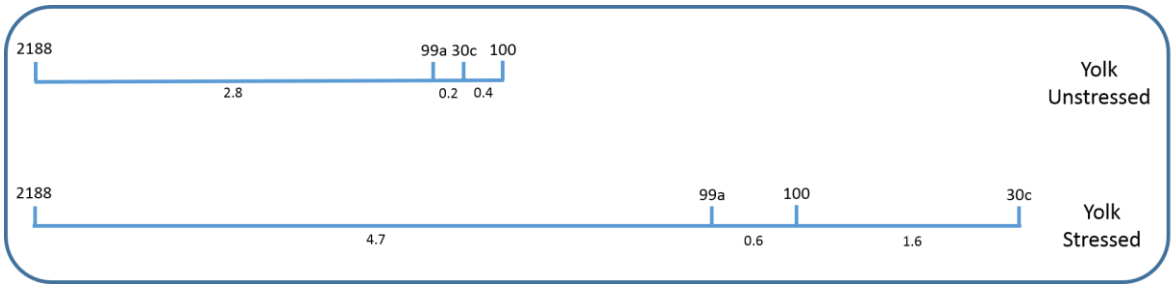

Figure 7. The miRNA profile from pools extracted from stressed and unstressed chickens.

Whilst a clear difference is observed we must stress that these are preliminary results and require further validation. That said the differences observed strongly allude to the possibility that the underpinning mechanisms that form our serum based assay may be applicable to a non-invasive test using eggs; an option that would be more amenable to industry and potentially both quicker and cheaper to perform given that there would be no need to handle live animals.

Implications

Here we present the first miRNA based stress test for laying hens. We believe that this test is ready for testing at the industry level. This test has the capacity to identify if hens are stressed at the flock level by using pooled samples and thus would provide real benefit for assessing stress in a range of farming and management systems. This test has the potential to play a role in the auditing of the stress/welfare of chickens across Australia.

Recommendations

Following the results presented here we recommend that this test be trialled on a much larger scale across the Australian egg industry, ensuring that a range of housing and management systems are tested. We also recommend that the egg assay be further developed as we believe this is a far superior way of testing in terms of welfare of the laying hens.

Acknowledgements

We would like to acknowledge all the groups that provided us with samples to test. We would also like to specifically thank Noel Kratzmann for his continued advice and support throughout this project.

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POULTRY CRC

Plain English Compendium Summary

Sub-Project Title:	**A new test for the measure of poultry welfare**
Poultry CRC Sub-Project No.:	1.5.4
Researcher:	Dr Tamsyn Crowley
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Sub-Project Overview	
Background	Heavily driven by public perception and large supermarket monopolies, welfare is of major concern for the poultry industry. The past few decades has seen development of poultry practices with a heavy focus on the welfare of poultry. There have been huge changes implemented to accommodate improvements in welfare particularly in the layer industry. There are now a number of production systems including caged and free range which provide the consumer a conscious choice on how their eggs have been produced. These advances are closely regulated in most countries with regular inspections and audits to ensure the industry it committed to improving welfare standards. While these developments have undoubtedly facilitated an increase in perceived welfare standards there is little research to confirm that there has been an actual increase in welfare standards and reduced stress on the birds. Many of the demands placed on the industry in terms of welfare are essentially based on public perception and interpretation, thus making it difficult to objectively assess the real welfare situation. Currently there are a number of behavioural and other tests (cortisone levels) that have provided an insight into the welfare of poultry, but to date there is no clear scientific test that can be attributed to welfare. This project will developed a clear cut test that can determine the welfare/stress on layers in various production systems. This will enable the industry to provide accurate scientific information on the welfare status of their production systems. This test will also enable auditors to directly test welfare during the course of their routine inspections.
Research	
Sub-Project Progress	Completed
Implications	Here we present the first miRNA based stress test for laying hens. We believe that this test is ready for testing at the industry level. This test has the capacity to identify if hens are stressed at the flock level by using pooled samples and thus would provide real benefit for assessing stress in a range of farming and management systems. This test has the potential to play a role in the auditing of the stress/welfare of chickens across Australia.
Publications	Ben Wade, Michelle Cummins, Anthony Keyburn and Tamsyn M Crowley. Isolation and detection of microRNA from the egg of chickens. BMC Research notes 2016 (9),