Functional Food Focus Program

Final Report

July 2016

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Functional Food Focus Program

Information current as of July 2016

Disclaimer

Report prepared by Dr Valeria Torok and Dr Stevphen Lapidge.

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Project Title

Functional Food Focus Program

Organisations conducting the research

This project was undertaken by the South Australian Research & Development Institute in consultation with Food SA, Adelaide University and industry partners. Core funding of $1.1 million was allocated as part of the Labour Government's $6 million 2014/15 Primary Industries and Regions South Australia Agribusiness Accelerator Program, which is aimed to drive the State Government's Premium Food and Wine from our Clean Environment strategic priority.

Program Leader

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Other key personnel

SARDI: Dr Valeria Torok, Dr Pamela Zwer, A/Prof David Stone, Dr Robert Hughes, Dr Janelle Hocking Edwards, Dr Svetlana Rodgers, Dr Stephen Pahl
Food SA: Dr Silvia Estrada-Flores, Ms Samara Miller
FOODplus: Dr John Carragher and Prof Bob Gibson

Location/s of Research:

The research has been conducted at various locations including:

1. Plant Research Centre, Waite Campus
2. FOODplus, Adelaide University, Waite Campus
3. Pig and Poultry Production Institute, Roseworthy Campus
4. Struan Research Centre
5. South Australian Aquatic Sciences Centre, West Beach

Program background and description

Functional foods are those that provide a demonstrated physiological benefit or reduce the risks of chronic disease above and beyond basic nutrition. They are a rapidly advancing area of the food industry due to consumers becoming increasingly concerned about overall health as well as avoiding debilitating illnesses. An aging population and increased disposal income are assisting this growth. To secure the strength of its food industry South Australia (SA) must be at the forefront of developing functional foods. The Functional Food Focus Program or FFFP aims to determine what functional foods can best be developed and produced in SA so as to gain a competitive advantage both nationally and internationally. Year one of the project has initially investigate four avenues of research for new SA developed and produced functional foods, and has undertaken a foresighting study with Food SA into other functional food opportunities with SA food companies. Year two of the project will focus research on the 2-3 most promising developments from Year 1, as well as simple functional food developments with SA food companies, with the aim of taking then to the point of commercialisation by the end of the program in June 2016.

Commented [VT1]: This statement needs to be modified to reflect actual yr 2

Program objectives

The objectives of the FFFP are to:

1. Investigate the most promising areas of functional food development in SA where we natural possess a competitive advantage.
2. Undertake a foresighting study with Food SA to detail future areas of functional food development with SA food SME's.

3. Take the 2-3 most promising functional foods through to commercialisation with an industry partner.

Commented [VT2]: Same as above

Program deliverables

Year one of the project has focused on the below promising areas of functional food development in SA:

1. Improved fatty acid profiles in lamb and/or mutton
2. Improving the nutrient quality of eggs
3. Producing a line of gluten free oats
4. Developing abalone aquafeeds that utilise food waste that enhance product integrity

Additional cross-research projects have included:

5. Path to market: verifying food safety and health benefits and assisting in industry uptake
6. Foresighting with Food SA to benchmark SA functional food production and possibilities
7. Demonstration project

Results of each project will be published in scientific and industry publications except where intellectual property developed can be commercialised, such as with feed formulations. The development of new products based on the outcomes of projects 1 to 4, combined with the deliverables of projects 5 and 7, will occur with an identified SA food industry partner in year 2 and beyond.

Revised program budget

The overall budget for the $1.1 million PIRSA allocation for the project is detailed below, including the $33,000 in approved carry-over from 2014/15.

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~~Project~~		~~2014-15 Actuals~~	~~2015-16 Budgets~~
~~Healthy fat lamb (V. Torok)~~	~~Salaries~~	~~$ 36,000~~	~~$ 71,600~~
	~~Operating~~	~~$ 49,000~~	~~$ 76,000~~
~~Supercharged eggs (V. Torok)~~	~~Salaries~~	~~$ 32,000~~	~~$ 65,200~~
	~~Operating~~	~~$ 43,500~~	~~$ 102,000~~
~~Gluten-free oats (P. Zwer)~~	~~Salaries~~	~~$ 5,000~~	~~$ 4,000~~
	~~Operating~~	~~$ 16,400~~	~~$ 18,100~~
~~Thriving abalone (D. Stone)~~	~~Salaries~~	~~$ 41,000~~	~~$ 45,000~~
	~~Operating~~	~~$ 76,000~~	~~$ 107,500~~
~~Path to market (S. Lapidge)~~	~~Salaries~~	~~$ 30,000~~	~~$ 42,100~~
	~~Operating~~	~~$ 3,000~~	~~$ 22,000~~
~~Foresighting with Food SA + Program Management (S. Lapidge)~~	~~Salaries~~	~~$ 40,000~~	~~$ 40,500~~
	~~Operating~~	~~$ 18,000~~	~~$ 16,100~~
~~PIRSA Corporate overhead~~		~~$ 45,000~~	~~$ 55,000~~
~~**Total**~~		~~**$ 434,900**~~	~~**$ 665,100**~~[‡]

~~‡Note- at the time of writing the $65,000 carryover to 2015/16 had not been formally approved.~~

Project		2014-15 Actuals	2015-16 Actuals
Healthy fat lamb (V. Torok)	Salaries	$ 35,619	$ 70,691
	Operating	$ 49,289	$ 33,360
Supercharged eggs (V. Torok)	Salaries	$ 32,063	$ 70,873
	Operating	$ 43,520	$ 103,202
Gluten-free oats (P. Zwer)	Salaries	$ 5,012	$ 0
	Operating	$ 16,400	$ 18,186
Thriving abalone (D. Stone)	Salaries	$ 41,161	$ 58,140

	Operating	$ 75,914	$ 89,841
Path to market (S. Lapidge) and Demonstration project (S. Pahl)	Salaries	$ 29,998	$ 47,900
	Operating	$ 2,441	$ 26,740
Foresighting with Food SA + Program Management (S. Lapidge)	Salaries	$ 40,679	$ 41,551
	Operating	$ 18,000	$ 4,129
PIRSA Corporate overhead		$ 45,000	$ 55,000
Total		**$ 435,100**	**$ 619,618**

$45,000 carryover

In addition the project has so far attracted $95,000 in external industry cash support along with $387,500 in-kind support, principally to the Thriving Abalone project. Additional co-investment is expected as the project progresses.

Further information is available in the previous FFFP reports (FFFP Proposal, March 2014; FFFP Operation Plan, January 2015) and only pertinent information to the final report is repeated herein.

Individual projects

Healthy-fat lamb

Principal Investigator: Dr Valeria Torok, Plant Research Centre, SARDI (8303 9688; valeria.torok@sa.gov.au)

Project aims

- To investigate the potential for grape marc to reduce saturated fatty acid in sheep meat and increase unsaturated fatty acid ('healthy fat') profiles.
- To determine the impact of diet on product quality and shelf life.
- To determine consumer acceptability of meat.

Project design

- Undertake a literature review on levels of tannin previously reported to alter meat composition and submit animal ethics application.
- Pilot animal feeding trial investigating levels of dietary treatment (grape marc) required to alter meat fatty acid profiles.
- Undertake compositional analysis of fatty acid profiles of meat.
- Evaluation of nutritional strategy on animal production traits.
- Undertake meat quality analysis.
- Undertake sensory evaluation trials of mutton meat.

Project Outcomes

Australians are among the largest consumers of lamb in the world. The national off-farm meat value of the Australian sheep meat industry is $3.9 billion with major prime lamb production in eastern South Australia. Unsaturated fatty acids are abundant in grass and other forage, yet they are present in low concentrations within the meat and milk of ruminants. Microbes within the rumen contribute significantly to ruminant product (meat and milk) fatty acid composition. Rumen microbes transform dietary lipids from the feed (unsaturated fatty acids) to saturated fatty acids by lipolysis and biohydrogenation, thereby restricting the availability of health-promoting polyunsaturated fatty acids in meat and milk. Feeding dietary supplements such as algal meal rich in docosahexaenoic acid (omega-3) or grape marc (skins, seeds, stalk and stems remaining from wine production) have been shown to change the fatty acid profiles in dairy cow milk away from the saturated (linked to heart disease) to unsaturated healthier fatty acids. The aim of this trial was to investigate the potential to feed grape marc to reduce saturated fatty acid in sheep meat and increase unsaturated fatty acid profiles. There are local producers of grape marc and Tarac Technologies (SA) has expressed interest in finding alternative uses for their waste product.

Two sheep (lamb and mutton) feeding trials were conducted during the course of the Functional Foods Focus Program to determine impact of grape marc supplementation on meat fatty acid profiles, animal production, meat quality and sensory quality (mutton only) traits. In year one, lambs (crossbred) were fed a commercial ruminant pellet or grape marc supplemented pellet diet for a total of eight weeks prior to slaughter. Two levels of grape marc supplementation in the pellet (18 or 31%) were evaluated which translated into 15% and 25% supplementation of the animals' overall diet. In year two a mutton (Border Leicester x Merino ewes aged 7-9 years) feeding trial was undertaken. Mutton were exposed to a period of feeding on either dry or green pasture immediately prior to the commencement of the feeding trial. Mutton from each of these pre-treatment groups received either a commercial ruminant pellet diet or a grape marc (30%) supplemented pellet diet (representing a 25% supplementation to the overall diet) for a total of nine weeks.

No significant differences in animal production or meat quality traits were observed in associated with dietary treatment in the lamb feeding trial. Furthermore, no significant differences in retail colour of lamb loins associated with dietary treatment were observed. However, loins from lambs fed grape marc tended to have a higher oxy/met at 48 hrs and 72 hrs of display, indicating a reduced tendency for oxidation and greater level of consumer acceptability at 48 hrs of display. All these measures indicated that feeding grape marc up to 25% of the final diet had no detrimental impact on lamb production or meat quality traits.

No significant differences in mutton production or meat quality traits were observed in associated with dietary treatment in the mutton feeding trial, with the exception of dressing percentage (DP) or percent of the live animal that ends up as

carcass. There were no significant differences in DP of mutton receiving the control or grape marc supplemented pellet diet. However, mutton which had received the dry pasture pre-treatment were significantly higher in DP than those which had received the green pasture pre-treatment. No significant differences were observed in mutton retail display colour with the exception of meat lightness. Meat from mutton receiving the green pasture pre-treatment or supplemented with grape marc were lighter than meat from mutton receiving the dry pasture pre-treatment and control pellet diet.

The intramuscular fat content (IMF %) of lamb and mutton from the feeding trials were approximately 3% and 4%, respectively and did not vary significantly with dietary treatment. The total fatty acid profiles of sheep loin meat did vary significantly with grape marc supplementation. For lamb, significant differences in meat fatty acid profiles were observed in some mono- and poly-unsaturated fatty acids, as well as total omega-3 polyunsaturated fatty acids, 22:1n-9 (erucic acid), 20:5n-3 (EPA) and 22:6n-3 (DHA). For mutton loins, there were significant pre-treatment associated differences noted in the total omega-3 polyunsaturated fatty acids, as well as, in the EPA and DHA levels, with mutton on the green pasture pre-treatment containing significantly higher levels of these fatty acids in the meat. Significant differences in meat fatty acid profiles associated with diet (control versus grape marc supplemented pellets) were only determined for c10,c12 conjugated linoleic acid (CLA), with mutton receiving the grape marc supplemented diet having significantly higher levels in their meat.

The nutritional and health claims for lamb and mutton were evaluated using the relevant Standard within the Australia New Zealand Food Standards Code. The lamb and mutton meat samples all contained a combined saturated and trans fatty acids of greater than the cut-off of less than 28% of the total fatty acid content allowable by the standard. Hence, fatty acid claims linked to polyunsaturated fat, monounsaturated fat, omega-3, omega-6 and omega-9 fatty acids were ineligible and sheep meat did not qualify to carry any nutrient content, comparative or general level health claims associated with these nutrients. Furthermore, both lamb and mutton samples were all slightly above the cut-off point of up to 3 gm total fat per 100 gm meat to qualify for a 'low fat' claim. Results also indicated that none of the lamb and mutton samples met the required minimum of omega-3 (alpha linolenic acid) (200 mg per serve) or EPA + DHA (30 mg per serve) to make a claim about the presence of these omega-3s.

Low value mutton is largely exported, included in processed meat and perceived as undesirable. Consumer preference sensory analysis was undertaken on loin samples from mutton feeding trial. Consumers were selected based on their liking of sheep meat. Consumers were not told the meat being evaluated was mutton. Consumer preference sensory indicated that there were no significant differences among mutton fed the four dietary treatments for attributes in appearance, aroma, flavour, tenderness, juiciness or overall liking. However, the mutton fed the dry pasture pre-treatment followed by grape marc supplemented pellets was the most preferred across all sensory and quality attributes except appearance. Mutton fed the green pasture pre-treatment followed by control pellets was the least preferred across all sensory and quality attributes tested, except appearance. All treatments were well received by the consumers, indicating that none of the treatments' sensory and quality attributes were disliked. There was a significant difference in flavour associated with pre-treatment, with mutton receiving the dry pasture pre-treatment being preferred to mutton receiving the green pasture pre-treatment, regardless of the pellet type received (control versus grape marc supplemented). Furthermore, there was a significant difference in flavour associated with pellet type regardless of pre-treatment, with mutton receiving the grape marc supplemented diet being preferred.

A trained sensory panel was used to identify particular attributes of mutton fed grape marc supplemented diets. Feeding regimes significantly impacted the physical (tenderness and juiciness), flavour and aftertaste characteristics of mutton meat but did not affect aroma. The grape marc supplemented diet resulted in perceived improvement in both physical and flavour characteristics. Mutton with prior exposure to a green pasture followed by a grape marc supplemented pellet diet were significantly lower in 'barnyard' flavour and higher in both juiciness and tenderness. Mutton with prior exposure to a dry pasture followed by a grape marc supplemented pellet diet were significantly higher in 'buttery' flavour and 'oily mouth coating' aftertaste. 'Barnyard' flavour was correlated with total omega-7, omega-9 and monounsaturated fatty acids. 'Buttery' flavor and 'oily mouth coating' aftertaste were correlated with total omega-6 fatty acids and 'juiciness' correlated with the C17:0 fatty acid.

In conclusion, it was found that feeding grape marc up to 25% of the sheep's overall diet for 2 months prior to slaughter did not impact negatively on animal production traits, meat quality attributes or retail acceptability. Although feeding of grape marc did alter meat fatty acid profiles, these differences did not translate into levels which would allow the making of nutritional or health claims. Feeding grape marc to sheep did impact on the sensory properties of the meat resulting in

increased consumer acceptability. A trained sensory panel identified significant meat attributes associated with feeding regime. Mutton with prior exposure to a green pasture followed by a grape marc supplemented pellet diet were significantly lower in 'barnyard' flavour and were higher in both juiciness and tenderness. Mutton with prior exposure to a dry pasture followed by a grape marc supplemented pellet diet were significantly higher in 'buttery' flavour and 'oily mouth coating' aftertaste. The results of this work show the feasibility of using an abundant local low value viticultural waste product (grape marc) in sheep finishing diets to improve sensory attributes and value add to mutton. Based on the assumption that Tarac Technologies produces approximately 130,000 ton of grape marc a year, this would be sufficient to feed over 9 million sheep per year on our diet formulation.

Publications to date

No scientific publication have been published to date.

The following internal reports have been prepared:

- ***Healthy Fat Lamb – Path to Market*** prepared by Dr Lenka Malek.
- ***SARDI Functional Foods Focus Program Health and Nutrient Content Claims Report – Eggs and Lamb*** prepared by Sharon Natoli.
- ***Consumer Acceptance Testing of Mutton: Summary of Results*** prepared by Tamira Thompson and Jessica Tan.
- ***SARDI Functional Foods Focus Program – Trained Sensory Panel Assessment of Mutton*** prepared by Dr Sandra Olarte Mantilla.
- ***Functional Foods Focus Program Altered Fatty Acid Profiles in Sheep Meat – Final Report*** prepared by Dr Valeria Torok

Oral Presentation

V Torok. Improving the fatty acid profile of sheep meat. National Lamb Supply Chain Meeting, Glenelg, March 4 2015.

V. Torok. Dietary intervention to improve sheep meat qualities. SA Livestock Consultants Group, PRC Adelaide, November 10 2015

V. Torok. Functional foods focus program: playing to SA's food strengths. Institute of Hospitality in Health Care Breakfast, Lirra Lirra Café Adelaide, May 19 2016.

Data Location

G: SARDI Food Safety/Functional Foods/Healthy sheep meat.

Supercharged eggs

Principal Investigator: Dr Valeria Torok, Plant Research Centre, SARDI (8303 9688; valeria.torok@sa.gov.au)

Project aims

- To investigate the beneficial additives to human health which could be used to enrich eggs.
- To enrich eggs with identified additives.
- To determine consumer acceptability and impact on product quality and shelf life.

Project design

- Conduct literature review on nutritional deficiencies within the Australian community and the potential to use eggs to address these.
- Conduct layer feeding trials designed to enrich eggs with identified additives.
- Conduct compositional analysis of eggs to determine degree of enrichment.
- Undertake egg quality analysis.
- Undertake shelf life analysis (egg quality and compositional analysis).
- Conduct sensory evaluation of product.

Project Outcomes

Eggs have excellent nutritional value and are a rich source of protein, vitamins and minerals. In 2011, the Australian egg industry produced 392 million dozen eggs or 12.9 million eggs each day with the annual national consumption estimated at 213 eggs per person. New science shows that egg consumption can help people with diabetes and assist in reduction of obesity within the community. The production of enriched eggs as a functional food to improve human health is not new - eggs enriched with omega-3 fatty acids are already available in the market place.

Eggs are an easy and convenient vehicle to address nutritional deficiencies within the community. A review entitled ***Supercharged Eggs: Path to Market*** was undertaken regarding the nutritional status of the Australian population and provides the rationale for functional egg development. The most common nutritional deficiencies in Australia include calcium, iodine, iron, zinc, vitamin D and omega-3 fatty acids. In addition to considering the above nutrients, carotenoids (specifically, lutein and zeaxanthin) were also examined as potential targets for egg enrichment due to their role in eye health. Based on the literature review, the following novel functional eggs were identified: 1) eggs enriched with the carotenoids, lutein and zeaxanthin, which protect eyes from harmful high-energy blue light and oxidative damage and could be targeted at elderly Australians; 2) eggs enriched with iodine for optimising iodine intake of childbearing aged women, in particular pregnant and lactating women with increased iodine requirements; 3) eggs enriched with vitamin D to reduce prevalence of vitamin D deficiency, especially in the winter months, and among institutionalised individuals with limited mobility who have limited sun exposure, and the elderly who have reduced ability to synthesise vitamin D from sunlight; 4) and eggs enriched with all of the above nutrients, to improve intakes of all nutrients and therefore, potentially provide multiple health benefits.

Levels of compounds for addition to layer feed were derived from published hen feeding studies, potential for toxicity to the animal and desired levels to be achieved in the egg based on recommended daily intake (where available). Relationships between in-feed levels and egg concentration were observed across many studies. In some cases these appeared to have a linear relationship (i.e. iodine), while in others it was not linear (i.e. lutein/zeaxanthin) making determination of targeted in-feed levels more difficult. Natural product containing lutein/zeaxanthin extracted from marigold was used for enrichment. This product is already used by some of the layer industry to naturally improve yolk colour for aesthetic reasons. Recommended feeding levels for egg yolk colour enhancement are much lower than the levels targeted for egg enrichment of lutein/zeaxanthin.

In this study, 216 layer hens (Hyline Brown) were fed experimental diets enriched with iodine, a combination of vitamin D [D3 or 25(OH)D3], lutein or a combination of all of these for six weeks during early lay (commencing at 30 weeks of age) or late lay (commencing at 65 weeks of age). None of the experimental diets impacted negatively on egg production, egg quality or bird performance. Hens receiving diets enriched with lutein had significantly higher yolk colour scores as compared to eggs from the other dietary treatments investigated. Feeding the experimental diets to hens for six week was

sufficient to enrich the egg nutrient content for that individual nutrient and in most cases in the combination enrichment group. Measurements of vitamin D as vitamin D3 was variable within treatments for both feeding trials and may indicate a technical issue with sample analysis. Measurement of 25(OH)D3 was more consistent. Both D3 and 25(OH)D3 contribute to the overall vitamin D content of food with suggestion that 25(OH)D3 is more potent and bioavailable. In the combination enriched eggs it also appeared that the inclusion of iodine and/or lutein may impact on the vitamin D content, although this was not conclusive from the results.

Nutrient claims could be made for all the enriched eggs although allowable health claims were restricted to the iodine and vitamin D enriched eggs. Iodine enriched eggs from early and late lay could make the following nutrient content claims: **high in iodine**; **good source of iodine;** and **provide 100% of the RDI for iodine** per serve or per egg, respectively. Iodine enriched eggs could also make the following or similarly worded general health claims: necessary for normal production of thyroid hormones; necessary for normal neurological function; necessary for normal energy metabolism; contributes to normal cognitive function; contributes to the maintenance of normal skin; contributes to normal growth and development in children.

Eggs enriched with vitamin D either as a combination of D3 and 25(OH)D3 or 25(OH)D3 alone could make a nutrient content claim of either **good source of vitamin D** or **source of vitamin D** per serve (feeding trial 1) or per egg (feeding trial 2). Large variations in vitamin D3 measurements in eggs were observed within treatments which may indicate issues around testing. However, vitamin D enrichment of eggs should include both vitamin D3 and 25(OH)D3 supplementation in the layer diet for the most effective outcome. Vitamin D enriched eggs could also make the following, or similarly worded general health claims: necessary for normal absorption and utilisation of calcium and phosphorus; contributes to normal cell division; necessary for normal bone structure; contributes to normal blood calcium levels; contributes to the maintenance of normal muscle function; contributes to the maintenance of normal teeth; contributes to the normal function of the immune system; contributes to normal growth and development in children.

For lutein enriched eggs a claim may be made about the presence of lutein in eggs, such as **source of lutein**, **contains lutein**, **provides lutein** and ✓ (tick) **lutein**, however, no descriptors can be used about the amount of lutein present in the eggs. Comparative claims are considered nutrient content claims and this provides an additional option for claims about lutein. When comparative claims are made on a pack, a statement must also be included that states the identity of the reference food (e.g. 'compared to standard eggs') and the difference between the amount of lutein in the claimed food and the reference food (in this case, the % difference needs to appear or the amount in micrograms). The lutein enriched eggs contained 2.4 to 2.7 mg of lutein per serve of two eggs and was 45 to 70 times higher as compared to the control eggs. The Australia New Zealand Food Standards Code does not allow for any health claims about lutein that link its presence in eggs with a health effect, unless the claim is substantiated by a systematic review. A systematic review was not undertaken as part of this project, although quotes to undertake this were obtained and ranged from $26,500 to $55,000 ex GST and may provide an avenue for producers to make a unique health claim in relation to their product.

Consumer preference sensory was undertaken on enriched eggs. Overall, egg enrichment had no detrimental impact on consumer acceptability, and in fact for the attributes of appearance and aroma egg enrichment increased consumer acceptability. Consumers significantly preferred the appearance of hard boiled eggs enriched for iodine, lutein and a combination of iodine, lutein and vitamin D as compared to standard eggs. Consumers also significantly preferred the aroma of hard boiled eggs enriched with a combination of iodine, lutein and vitamin D as compared to standard eggs. For both hard boiled and scrambled eggs, there were no significant differences in flavour, texture, aftertaste or overall liking of enriched eggs as compared to standard eggs.

The average cost of egg production in Australia is around 95 cents per dozen or $1.45/kg. The main factors influencing egg production are: 1) cost of feed ingredients; 2) cost of rearing pullets from day-old to point of lay, or purchasing pullets at point of lay; 3) level of mechanisation on the farm (larger, more mechanised and automated farms have lower labour costs but higher equipment costs); and 4) mortality. Supplementing layer hen diets with vitamin D, iodine and/or lutein is not expected to impact the mortality of the layer hens or the cost of rearing pullets. The only factors that are likely to be influenced are price of feed ingredients and potentially the level of mechanisation on the farm. Based on the levels targeted in the **Supercharged Eggs** project, additional costs per tonne feed would be as follows: $10.50 for iodine enriched eggs; $6.95 for vitamin D enriched eggs; $125.30 for lutein enriched eggs; and $142.75 for combination enriched eggs. Commercial layer feed used in this trial cost approximately $800/tonne.

The gross value of Australian and South Australian egg production has experienced consistent growth in recent years. While the gross value of egg production in Australia increased by 8.7% from 2012-13 to 2013-14, SA alone experienced a growth of 31.4% during this same period (ABS, 2015). The estimated value of egg production at farm gate prices in 2013-14 was $625.5 million for Australia and $33.4 million for SA; an increase of 10.9% and 48.4% since 2012-13, respectively. Outcomes of the **Supercharged Eggs** project could put a competitive edge on value added SA eggs within the local market and support the investment already made by government into expansion of the layer industry in SA.

Publications to date

No scientific publication have been published to date.

The following internal reports have been prepared:

- ***Supercharged Eggs: Path to Market*** prepared by Dr Lenka Malek.
- ***SARDI Functional Foods Focus Program Health and Nutrient Content Claims Report – Eggs and Lamb*** prepared by Sharon Natoli.
- ***SARDI Functional Foods Focus Program – Consumer Acceptance of Enriched Eggs*** prepared by Valeria Torok, Jessica Tan and Anna Crump.
- **Functional Eggs: Prior Art and Patent Search** prepared by Dr Valeria Torok.
- **Functional Foods Focus Program Supercharged Eggs – Final Report** prepared by Dr Valeria Torok

Correspondence

- Berg Layers - Letter dated 14th June 2016. Regarding potential options to patent the enrichment of eggs from the Supercharged Eggs project. It was recommend that a prior are search be undertaken to determine the extent of disclosure or discussion that had occurred in the public domain in relation to the technology.
- Berg Layers - Memorandum date 26th July 2016. The following recommendation was revived in relation to enrichment of eggs "it would appear that the prior art that exists in relation to the enrichment of eggs with particular additives, and the complexities of the commercial arrangements being considered, does not set up a justifiable base for expenditure of funds to achieve a financial return from a licensing strategy".

Oral Presentation

Informally discussion with JoJo Jackson, R&D Manager, Australian Egg Corporation Ltd, on the 22nd May 2014 during her visit to SARDI Roseworthy Campus. A general presentation on SARDI Food Safety and Innovation capability was given during the meeting entitled "SARDI Food Safety and Innovation – Waite Campus". Following the meeting JoJo Jackson, Carolyn Dekoning, Kelly Drake and Valeria Torok visited Days Eggs Free Range Farm, Two Wells and were given a tour of the operation by owner Anne Andary.

V. Torok. Enriching eggs to improve human health outcomes. Southern Star Poultry Alliance Scientific Meeting, Veterinary School Roseworthy Campus, November 12 2015.

V. Torok. Functional foods focus program: playing to SA's food strengths. Institute of Hospitality in Health Care Breakfast, Lirra Lirra Café Adelaide, May 19 2016.

Data Location

G: SARDI Food Safety/Functional Foods/Eggs.

Gluten free oats

Principal Investigator: Dr Pamela Zwer, Waite Building 4C, SARDI (8303 9485; pamela.zwer@sa.gov.au)

Project aims

- To confirm the genetic variation for avenin content, the glutinous protein in oats similar to the gluten within wheat, in 12 oat varieties and lines.
- To determine the effect of environment on avenin content.
- To provide evidence that specific oat varieties are safe for people suffering from coeliac disease as they contain less than 20 ppm avenin content.

Project design

- Twelve breeding lines/varieties of oats with 3 replications were sown at two sites in 2013, 2014, and 2015.
- Grain was harvested, cleaned, dehulled, and milled.
- Milled samples will be evaluated for their ability to stimulate avenin-specific T cell lines/clones isolated from coeliac disease patients *in vitro*.
- The second *in vivo* study will confirm the immunogenicity of oat varieties using fresh T cells isolated from coeliac disease volunteers challenged orally with oats.

Project Outcomes

Milestone 1 - Sow and harvest 12 entry replicated field trial sown plots at two sites at Pinery and Riverton in 2013 and 2014. Twelve oat varieties and breeding lines were selected to represent low, medium, and high avenin (gluten equivalent in oat) content. This was based on a preliminary assessment completed by Biomedal in Spain. The entries had four replicates in each trial.

Milestone 2 – Harvest grain, weigh, clean, and collect data on grain quality traits. The grain was harvested from the trials, weighed to determine grain yield and cleaned. Grain quality traits, oil, protein, and groat percent, were assessed using NIR predictions. Physical quality traits, hectolitre weight, screenings, and 1,000 grain weight were assessed.

Milestone 3 – Dehull and mill four replicates of 12 varieties/lines from Pinery and Riverton in 2013 and 2014. 192 samples were milled in preparation for assessment using the Biomedal GlutenTox ELISA Sandwich.

Milestone 4 – Produce experimental design for ELISA plates and perform the ELISA. Dr Beverly Gogel, Statistics for the Australian Grains Industry (SAGI), prepared the experimental design for the randomization of samples in the ELISA plate.

Mrs. Jan Gooden from the SARDI Plant and Soil Health conducted the GlutenTox ELISA sandwich utilising 12 plates. Two dilutions were used 1:20 which detected the lowest quantification limit of 0.6 to 10 ppm and 1:200 detecting 6.2 to 100 ppm avenin. The results were below the detectable limits of the standard curve for the 1:20 dilution. Although the GlutenTox ELISA sandwich kit was promoted to measure avenin, it was not sensitive enough for avenin detection. Therefore, quantification of avenin was not possible and statistical analysis could not be undertaken.

The failure of the ELISA to measure avenin has resulted in no further dealing with Biomedal, the company that manufactured and promoted the kit for measurement of gliadin, secalin, hordein, and avenin.

The failure of the ELISA prompted Dr Zwer to contact Dr. Melinda Hardy, Researcher in the Immunology Division and Dr. Jason Tye-Din, Head of Coeliac Research at the Walter and Eliza Hall Institute. They co-authored a paper about ingestion of oats and barley with coeliac disease. Mrs Jan Gooden and Dr Zwer along with Mr. John Pitcher, Head of R&D for Cereal Partners Worldwide (Uncle Tobys) met with Melinda and Jason. The questions discussed were: 1) Is there a method to accurately quantify avenin content? and 2) Does it correlate with the physiological effects of avenin in a coeliac patient? As a result of the meeting the milestones for 2015/16 were changed. Another meeting was held at UTC (Cereal Partners Worldwide) in Rutherglen to further discuss the new research objectives. Dr. Tye-Din and Dr. Hardy's team attended the meeting in November 2015 along with Mr. Pitcher, Dr. Pamela Zwer, and Mr. Peter McCormack.

Milestone 5 – Assess the 10 lines/varieties selected for the field experiments and rank the response based on the reactivity to the peptide-specific T cell clones. T cells will be expanded to enable functional T cell assays. This is a marker of oat "toxicity" based on the content of dominant immunogenic T cell epitopes. The peptide-specific T cell clones are the parts of oat avenins that cause an immune response in 8% of people with coeliac disease. Oat varieties and breeding lines will be ranked based on T cell immunogenicity. This research is currently underway.

Milestone 6 – Based on the ranking of the 10 lines/varieties Dr Hardy and Dr. Tye-Din will conduct an *in vivo* short term (3 day) oral challenge for about 30 coeliac patients. This *in vivo* study will confirm the immunogenicity of oat varieties using fresh T cells isolated from coeliac disease volunteers challenged orally with oats. A low, intermediate, and high immunogenic variety of oats will be selected. Three separate groups of patients (10 per group) with coeliac disease will undertake a three day oat challenge. Patients will eat 100 gm per day of oats for three days and blood will be collected on Day 0 before the challenge and on Day 6 after the challenge is started. Blood will be collected on day 6 to isolate gluten-specific T cells and IFN-gamma ELIspot assays will be performed to measure immunogenicity. This research is currently underway.

Publications to date

No publications have been produced at this stage.

Oral Presentation

P. Zwer. National oat breeding program: "Gluten free" oat – fallacy or reality. Waite Seminar Series, Plant Research Centre, August 18 2016.

Data Location

G://Avena/OATS/Michelle Quality/COELIAC

Thriving abalone

Principal Investigator: A/Prof David Stone, Aquatics Science Centre, SARDI (8207 5350; david.stone@sa.gov.au)

Project aims

- To evaluate the use of macro- and micro-ingredients derived from grape seeds, macroalgae, peanuts and other ingredients in feed for high value aquaculture abalone, to improve growth performance, health and product quality.

Project design

- This collaborative project brought together food producers (TARAC Technologies, Peanut Company of Australia; Venus Shell Systems, Agricure Pty Ltd, Lesaffre Feed Additives), all the Australian abalone aquafeed manufactures (Aquafeeds Australia, Eyre Peninsula Aquafeeds, and Skretting Australia), the Australian Abalone Growers Association and a group of Research providers (SARDI, Flinders University, University of Adelaide, University of Sydney, University of Southern Cross) to investigate the incorporation of micro- and macro-ingredients derived from a range of food waste products into aquafeeds for abalone to improve growth performance, health and product quality.

- A series of steps were chosen to evaluate the chosen ingredients in aquafeeds for abalone. The steps included:
  1. Sourcing ingredients
  2. Characterising ingredients
  3. Determining the ingredients application in aquafeeds
  4. Processing or modifying ingredients
  5. Evaluate biological performance and economics of ingredients with greenlip abalone in the laboratory and in on-farm commercial systems

- Steps 1 to 4 were evaluated using a desktop study and some chemical analyses and processing in conjunction with discussions and input from project participants.

- Step 5 involved a series of laboratory and on-farm trials which tested a range of feeds containing ingredients derived from food waste products with live abalone.

Macro ingredients identified and tested included:

- Acti-meal (steam distilled grape marc meal) provided by TARAC Technologies (Nuriootpa, SA)
- Peanut meal (normal and high aflatoxin levels) provided by Peanut Company of Australia (Kingaroy, QLD)
- Live and dried natural and protein enriched *Gracilaria* cliftonii and *Ulva* sp. meals cultured by SARDI and supplied by Venus Shell Systems (Narrawallee, NSW, Australia).
- Dried *Ulva* sp. protein extract meal supplied by Venus Shell Systems (Narrawallee, NSW, Australia).
- Mixed drift cast macroalgae provided by Eyre Peninsula Aquafeeds (Lonsdale, SA))

Micro ingredients identified and tested included:

- Grape seed extract (G-SeedEx) provided by TARAC Technologies (Nuriootpa, SA)
- Green tea extract provided by Eyre Peninsula Aquafeeds (Lonsdale, SA)
- Peanut testa extract provided by Eyre Peninsula Aquafeeds (Lonsdale, SA)
- Orego-Stim® supplied by Meriden Animal Health Limited (Australia)
- Probiotics (*Bacillus nato*, *Bacillus subtilis* and *Bacillus licheniformis*) supplied *by* Agricure Pty Ltd (Braemar, NSW) and International Animal Health Product Pty. Ltd. (Huntingwood, NSW).
- Prebiotics (SafMannan yeast) provided by Lesaffre Feed Additives (France)
- Vitamins (vitamin C and vitamin K_1) provided by Agricure Pty Ltd (Braemar, NSW)

Project Outcomes

Australian abalone feed companies have used information arising from this project to improve commercial diets for abalone production. Australian abalone farmers, in direct collaboration with feed companies and project research providers, have also undertaken a number of on-farm trials to evaluate a range of the products evaluated in this project. For example, TARAC grapeseed extract to improve survival and TARAC Acti-meal as macro-ingredient to improve growth and feed

utilisation were investigated in on-farm trials. Results were promising and several of the micro- and macro-ingredients evaluated had positive impacts on the health, growth and product quality of abalone.

Macro-ingredients that have shown positive results for inclusion into abalone grow-out aquafeeds to improve growth performance and feed utilisation include TARAC steam distilled grape marc meal, now marketed as Acti-meal (improved growth and economically priced) (Currie *et al*., Submitted a), dried *Gracilaria cliftonii* meal (improved growth and abalone lip and shell colour, but is currently too expensive) (Bansemer *et al*., 2015 ; Bansemer *et al*., 2016a; Bansemer *et al*., 2016b; Duong *et al*., In press; Thanh *et al*., 2017; Thanh *et al*., 2017) and *Ulva* sp. protein extract (positive growth and feed efficiency, but is currently too expensive) (Bates *et al*., Submitted). Additionally, positive information pertaining to the kinetics of lip and shell colour change in response to dietary macroalgal manipulation in market sized greenlip abalone was provided. As a result, commercial diets formulated to contain a dried mixed drift cast macroalgae meal, sourced from a local commercial SA collector/supplier, were tested on-farm in an attempt to improve growth performance, shell colour and survival. Dietary inclusion of macroalgal meals is an important area of abalone dietary research that warrants further investigation in terms of growth performance, product quality and health.

Laboratory work with Acti-meal has identified a low value waste product that has significant potential to improve abalone growth and feed utilisation when included into aquafeeds at levels up to 20% (Currie *et al*., Submitted a). This application of the product has the significant potential to value add to TARAC's existing stocks of low value grape marc. Initial results originating from laboratory trials from this project indicate that with minimal processing grape marc, a product that TARAC currently has difficulties selling for <$50 tonne, may be sold into the global aquafeed market for >$400 tonne. However, on-farm testing of laboratory results are required to demonstrate and validate the incorporation of Acti-meal in aquafeeds. This has led to two newly funded industrial research projects to validate Acti-meal inclusion in aquafeeds:

1. "Value adding to TARAC Acti-meal by incorporation into abalone aquafeeds". David Stone (Principal Investigator); Co-investigators, Tarac Technologies, Aquafeeds Australia and Southern Ocean Mariculture. Funded by the South Australian River Murray Sustainability (SARMS/Rural Solutions SA) Program. 19 September 2017 – 28 February 2019.
2. "Scoping study: Value adding to TARAC Acti-meal by incorporation into aquafeeds for juvenile Silver perch and Barramundi" October 2017-July, 2018.

Both projects are important steps towards value adding to TARAC's stocks of lower value raw grape marc, with a view to market the more refined and valuable Acti-meal product globally as an affordable and sustainable ingredient for aquafeeds. Both projects have also generated considerable industry and media attention in SA regional newspapers and also on a global basis on a range of industry relevant and non-government organisation internet websites.

Micro-ingredients that have shown positive results in laboratory trials for potential inclusion into summer abalone grow out aquafeeds to improve survival at high summer water temperatures include TARAC grape seed extract (G-SeedEx; potential to improve survival but economically boarder-line) (Currie *et al*., Submitted b; Duong *et al*., 2016; Sheil *et al*., 2017). Further research into the active constituent of G-SeedEx in relation to abalone immune function and survival is recommended as a priority. Green tea extract also displayed potential to improve survival and was economically viable but results were variable, and inclusion of this product requires more research (Duong *et al*., 2016; Currie *et al*., Submitted c). Pre- and probiotic products tested in this project exhibited potential to reduce *Vibrio* sp. levels in the digestive tract of greenlip abalone, but did not improve survival of abalone when challenged with a temperature stress. Further research investigating nutrient utilisation and health of abalone fed with pre- and probiotics is also recommended, particularly in the area of digestive tract microbiomics.

Rapid extension of project results to participants has been a strong component of this project. Information arising from this project was extended directly to project participants as it came to hand. Information has also been presented at a range of domestic industry workshops and domestic and international scientific conferences. Nineteen manuscripts have been published, accepted or submitted for publication in international peer reviewed scientific journals. Eight more are currently in preparation for submission for publication and many more will follow. Three PhD, one Masters and three Honours theses have also been completed.

Student training has also been a strong component of this project. The project has trained a large contingent of industry ready undergraduate and post graduate students which includes five PhD students, one Masters student, four Honours

students, two undergraduate student projects and numerous undergraduate students undertook extra mural work placements.

Milestones for 2014-2015

Accomplishments against each milestone are detailed below:

Milestones for 2014-2015 were completed

Q1- Complete Experiment 1: Temperature challenge test for grape seed extract and other antioxidant ingredients.

Laboratory-based trials carried out at the SARDI South Australian Aquatic Sciences Centre (SAASC) Nutrition laboratory in a flow-through system with water temperatures of 22 °C and 25 °C assessed the effects of graded levels of grape seed extract (G-SeedEx) (0.05, 0.125, 0.25, 0.5, 1, 2 and 5%; Currie *et al*., Submitted b) and oregano extract [Orego-Stim®] (0.5, 1, 2 and 4%; Buss, Submitted) inclusion on the survival and immune function of 3-year-old greenlip abalone at summer water temperatures (25°C). The grape seed extract (G-SeedEx) was produced and provided by TARAC Technologies (Nuriootpa, SA), while the Orego-Stim® was provided by Meriden Animal Health Limited Australia. Results confirmed the potential for the inclusion of 5% grape seed extract as a feed additive in a commercial formulated diet to reduce high water temperature associated mortality on land-based farms in southern Australia (Currie *et al*., Submitted b; Duong *et al*., 2016). As a result of these trials the 5% grape seed extract diet was taken and used in two SA on-farm trials at Kangaroo Island and Port Lincoln over the summer of 2014/15 (see Q3 2014-15).

The inclusion of Orego-Stim® led to a significant improvement ($P<0.05$) in feed intake. However, mortality rate significantly increased as inclusion level increased ($P<0.05$; Buss *et al*., Submitted). Based on this research, one of the Australian abalone feed manufacturers stopped using Orego-Stim®] in their commercial diets. This resulted in a feed cost saving.

As a result of the studies in this Milestone, evaluation of micro-ingredients similar to grape seed extract, but more cost effective, such as peanut meal extract and green tea extract, were evaluated for incorporation into diets to improve survival of abalone at high water temperatures (Duong *et al*, 2016). In the subsequent studies, Duong et al. (2016) confirmed that 5% TARAC grapeseed extract was effective at reducing heart related mortality in greenlip abalone in the laboratory, whereas, peanut meal extract was not. Duong *et al*. (2016) also suggested that green tea extract may have potential to reduce heat related mortality in greenlip abalone and recommended further research with this product (section Q2. 2015-2016; Currie et al., Submitted c).

Q2- Complete Experiment 2: Laboratory based growth trial with steam distilled grape marc meal (TARAC Acti-meal) and protein enriched macroalgae.

Laboratory based trial - Protein enriched macroalgae growth trial (Bansemer et al., 2016a)

One-year-old juvenile greenlip abalone were fed seven experimental formulated diets, a basal diet (0% diet), and three inclusion levels of SARDI grown and enriched *Ulva* sp. meal (5, 10 and 20% inclusions) and *Gracilaria* sp. meal (5, 10 and 20% inclusions) and growth performance was measured (Bansemer *et al*., 2016a). The study was carried out at the SARDI SAASC Nutrition laboratory at a water temperature of 22 °C. Abalone fed 5% *Gracilaria* sp. meal or *Ulva* sp. meal exhibited superior growth to abalone fed 0%. Increasing dietary *Gracilaria* sp. meal inclusions (>10%) led to further growth improvements (Figure 1). In contrast, abalone fed >10% *Ulva* sp. meal inclusions exhibited similar growth to those fed 0 and 5% *Ulva* sp.

Dietary inclusion of 10% *Gracilaria* sp. meal or 5% *Ulva* sp. meal are recommended to improve abalone growth (Bansemer *et al*., 2016a). Although *Ulva* sp. and *Gracilaria* sp. meals are currently not commercial viable, this study has provided useful information to the project participant, Venus Shell Systems, and clearly demonstrates the potential to develop abalone feeds with inclusions of dried macroalgae meal. This study also demonstrated that increasing the inclusion of *Gracilaria* sp. inclusions made the shell and lip of abalone redder and greener, respectively (Thanh *et al.,* 2016; Thanh *et al.,* 2017). This finding has now been re-examined, using larger 3-year-old abalone with SARDI grown *Gracilaria* sp., to assess whether this colour change can be translated to larger abalone (see section Q1. 2015-2016). In addition, the time taken for the colour change to occur is being been evaluated. Both research questions returned positive outcomes (see section Q1. 2015-2016).

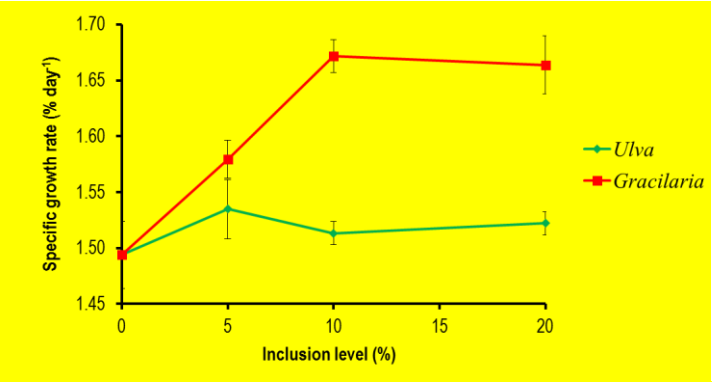

Figure 1: Specific growth rate of juvenile greenlip abalone fed diets containing either dried *Ulva* sp. meal or *Gracilaria* sp. meal at inclusion levels 0, 5, 10 and 20%. Data is presented as mean ± standard error (SE), n=4 (Bansemer *et al.*, 2016a).

Laboratory based trial - Steam distilled grape marc meal (Now commercially registered as Acti-meal) as an energy source for growth of greenlip abalone (Currie et al., Submitted a)

In this experiment, juvenile 1-year-old greenlip abalone were fed five experimental formulated diets, a basal diet (0% diet), and four inclusion levels of TARAC Acti-meal (5, 10, 15 and 20% inclusions) and the test ingredient and diet nutrient composition growth performance, feed and nutrient utilisation and survival were measured (Currie *et al.*, Submitted a). The Acti-meal was produced and provided by TARAC Technologies (Nuriootpa, SA). The study was carried out at the SARDI SAASC Nutrition laboratory in a flow through system supplied with seawater at a water temperature of 22 °C. Abalone fed the diets containing Acti-meal, up to and including 20%, exhibited superior specific growth rates compared to the basal diet (Figure 2).

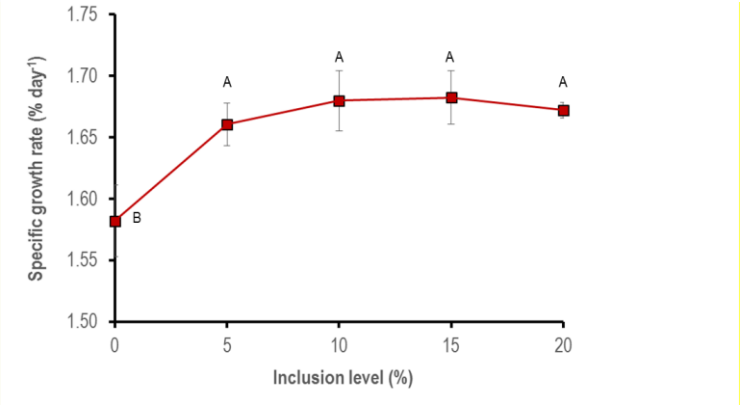

Figure 2: Specific growth rate of juvenile greenlip abalone fed diets containing TARAC Acti-meal (0, 5, 10, 15 and 20%) (Currie *et al.*, Submitted a). Data is presented as mean ± SE, n=4. Different letters represent significant differences between diet means (P<0.05; one-factor ANOVA; SNK test).

Feed utilisation, in terms of feed conversion ratio (FCR), was also significantly improved with the inclusion of Acti-meal (P<0.05). The survival of all abalone fed diets containing Acti-meal was 100%. Protein and energy deposition were significantly improved (P<0.05) in abalone fed diets containing >10% Acti-meal (Currie *et al.*, Submitted a).

Based on the results from our study, 5-20% dietary inclusions levels of Acti-meal in commercial abalone diets are recommended as an energy source to promote optimal growth for (Currie *et al.*, Submitted a). In contrast to dried

macroalgae, the Acti-meal produced by TARAC Technologies is commercially viable, sustainable and available. The cost of the meal is significantly cheaper than current ingredients used as energy sources in commercial diets for greenlip abalone, making this a highly promising alternative ingredient. However, the growth performance of abalone fed commercial diet formulations containing Acti-meal needs to be validated in a pilot scale on-farm trial before results are taken up commercially by industry. Additionally, we recommend that TARAC Technologies ensures the nutrient composition and physical characteristics of their Acti-meal product are consistent between batches and meet the specifications required by feed companies, especially in relation to particle size (<0.3 mm), to ensure the product is consistent and accepted for use by feed companies.

A pilot scale on-farm trial has now been organised and funded and details are provided below::

- "Value adding to TARAC Acti-meal by incorporation into abalone aquafeeds". David Stone (Principal Investigator); Co-investigators, Tarac Technologies, Aquafeeds Australia and Southern Ocean Mariculture. Funded by the South Australian River Murray Sustainability (SARMS/Rural Solutions SA) Program. 19 September 2017 – 28 February 2019.

The new on-farm project has also generated considerable industry and media attention and has been broadcasted widely in SA regional newspapers, and world-wide, on a range of industry relevant and no-government organisation internet websites.

- Associate Professor David Stone was interviewed by Andrew Spence from The Lead South Australia which led to the following article "Abalone thrive on wine waste diet" (8/6/17). (http://www.theleadsouthaustralia.com.au/industries/primary-industries/abalone-thrive-on-wine-waste-diet/)
- Associate Professor David Stone was interviewed by Casey Treloar from the Port Lincoln Times which led to the following articles: Port Lincoln Times and Barossa Herald article. "Abalone to try wine waste" (1/8/17). (http://www.barossaherald.com.au/story/4823126/abalone-to-try-wine-waste/)

Tarac Technologies are also interested in marketing the global application of their Acti-meal product in aquafeeds for fin fish. In collaboration with Associate Professor David Stone, Tarac Technologies have also commercially funded an additional project to investigate the potential of Acti-meal as an ingredient for inclusion into aquafeeds for carnivorous and omnivorous fish entitled:

- "Scoping study: Value adding to TARAC Acti-meal by incorporation into aquafeeds for juvenile Silver perch and Barramundi" October 2017-July, 2018.

Q3- Complete first on-farm trial to evaluate the efficacy of a dietary additive to reduce the mortality of abalone at high summer water temperatures.

Based on previous laboratory results that demonstrated the use of 5% TARAC grape seed extract as an effective dietary additive to reduce the mortality of abalone at high water temperatures (Currie *et al*., Submitted b; Duong *et al*., 2016), two South Australian farms (Kangaroo Island Abalone, and SAM at Port Lincoln, SA) participated in separate 4 month on-farm trials testing a 5% grape seed extract diet compared to a standard commercial diet. The addition of 5% grape seed extract did not reduce feed intake or growth or impact on survival. Unfortunately, water temperatures at both farms that participated in the trials during the summer of 2014/15 were relatively mild (<23°C). As a result, abalone were not exposed to a challenging event and the effectiveness of the diet at reducing mortality could not be adequately assessed.

Milestones for 2015-2016

Milestones for 2015-2016 are completed

Q1- Complete Experiment 3: Growth trial of peanut meal, Ulva sp. protein extract and mixed draft cast macroalgae.

Laboratory based trial - Peanut meal and aflatoxin growth trial (Currie et al., Submitted d)
This study evaluated seven experimental diets, a basal diet (0% diet) and six diets containing graded levels of peanut meal (5, 10 and 20%) at two aflatoxin levels (<20 ppb and >50 ppb). Aflatoxin was produced by *Aspergillus* sp. Each graded level of peanut meal were tested with a different inclusions of the mould, *Aspergillus* sp., in order to evaluate if higher levels of aflatoxins present in peanut meal altered the growth, health and survival of greenlip abalone. The study was carried out in the SARDI SAASC Nutrition laboratory at a water temperature of 22 °C. The peanut meals were

produced and provided by the Peanut Company of Australia (Kingaroy, QLD). The growth performance, feed utilisation and survival of the juvenile 1-year-old greenlip abalone were measured and no significant differences were observed for inclusion level (Currie *et al.*, Submitted d). However, an increase in peanut meal inclusion level led to a numeric decline in growth parameters. There was also no difference observed between abalone fed the low or higher aflatoxin diets, indicating that in the short term, the tested aflatoxin levels do not pose a problem to the growth of greenlip abalone. Inclusions for >5% peanut meal in abalone diets are not recommended (Currie *et al.*, Submitted d). However, alternative forms of peanut meal may be applicable for further research for inclusions into abalone diets.

Laboratory based trial - Protein enriched Ulva sp. extract growth trial (Bates et al., Submitted)
This study evaluated four experiments diets, a basal diet (0% diet) and three diets containing graded levels of *Ulva* sp. protein extract (5, 10 and 20%). The *Ulva* sp. protein extract was produced and provided by the Venus Shell Systems (Narrawallee, NSW). The study was carried out in the SARDI SAASC Nutrition laboratory at a water temperature of 22 °C. Growth performance and feed utilisation of juvenile 1-year-old greenlip abalone was measured (Bates *et al.*, Submitted). Compared to the control basal diet, there was no significant effect of *Ulva* sp. protein extract inclusion level on the growth performance (Figure 3), feed utilisation or survival of greenlip abalone (Bates *et al.*, Submitted). Although *Ulva* sp. meals or extracts are currently not commercial viable, this study has provided useful information to the project participants, Venus Shell Systems, the feed companies and abalone producers, and confirms the potential to develop abalone feeds with inclusions of dried macroalgae meals from previous research (section Q2. 2014-2015). An Australian aquafeed company now uses low dietary inclusion levels of this product in their commercial abalone diets.

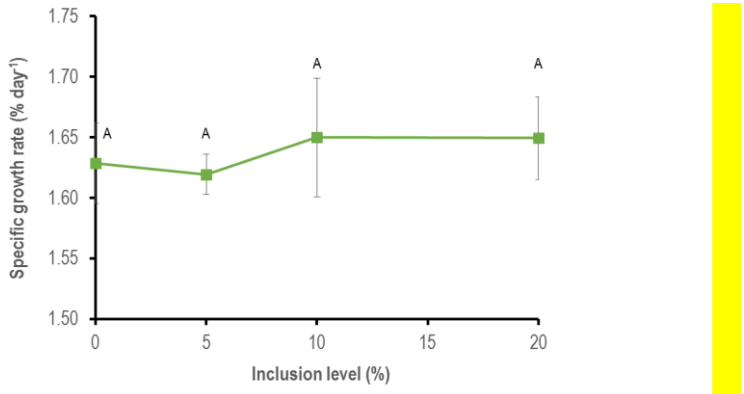

Figure 3: Specific growth rate of juvenile greenlip abalone fed diets containing Venus Shell Systems *Ulva* sp. protein extract (0, 5, 10, 15 and 20%) (Bates *et al.*, Submitted). Data is presented as mean ± SE, n=4. Different letters represent significant differences between diet means (P<0.05; one-factor ANOVA).

Laboratory based trial - The effect of dried macroalgae dietary inclusion on shell and foot colour and growth performance
The study was carried out at the SARDI SAASC at a water temperature of 22 °C. The shell, lip and foot colour of 3-year-old greenlip abalone were evaluated using three diets: Diet 1) control diet; commercially available formulated abalone diet; 2) 15% dried mixed drift cast macroalgae meal diet; produced by Eyre Peninsula Aquafeeds (Abgrow Premium diet with 15% dried mixed drift cast macroalgae meal), and Diet 3) a formulated experimental diet containing 15% dried enriched *Gracilaria* sp. The *Gracilaria* sp. was grown and enriched at SARDI SAASC (Bansemer *et al.*, 2016a). Abalone were photographed monthly over a three-month period to evaluate the effects of diet on colour change, as well as growth performance. There was no difference in the colour (shell, lip or foot) of abalone fed either the control diet or the 15% dried mixed drift cast macroalgae diet. The addition of 15% dried enriched *Gracilaria* sp., confirmed the results of previous research in this project using juvenile abalone, that the addition of the *Gracilaria* sp. meal made the lip of greenlip abalone greener, compared to the control diet (Thanh *et al.*, 2017). Additionally, the addition of 15% *Gracilaria* sp. also improved the growth performance of abalone compared to the control diet. These results demonstrated that the improvement of growth performance of juvenile greenlip abalone fed a dried *Gracilaria* sp. diet also translated to larger greenlip abalone (Bansemer *et al.*, 2016a). This is a significant finding, as improvements in the growth performance of the slower growing

larger 3-year-old greenlip abalone are rare. Unfortunately, as previously mentioned, this meal is currently not commercially available. Further research is warranted in this area in relation to lip and foot colour and growth.

Q2- Complete Experiment 4: Laboratory based trials - Temperature challenge test with peanut meal extract and other micro-ingredients (Duong et al., 2016)

In addition to previous temperature challenge tests (see Q1. 2014-2015), several other diet micro-ingredients have been evaluated at graded levels at 22ºC (positive water temperature control) and 25ºC (negative water temperature control) at the SARDI SAASC Nutrition laboratory to assess the effects of dietary additives on the survival of 3-y-old greenlip abalone. Experimental diets were compared back to a basal diet (0%) and a 5% grape seed extract diet was used a positive control diet (Duong *et al*, 2016). A summary of results is proved below.

Laboratory based trial - Peanut testa extract
Peanut testa extract was evaluated at inclusion levels of 0.5, 1, 2.5 and 5%. There was no significant improvement in the survival of greenlip abalone at 25ºC (Duong *et al*., 2016).

Laboratory based trial - Green tea extract
Green tea extract was evaluated at inclusion levels of 0.5, 1, 2.5 and 5%. There was a significant improvement in the survival of abalone fed the 0.5 and 2.5% diets. However, inclusions levels of 1 and 5% negatively affected the survival of abalone (Duong *et al*., 2016). Additionally, abalone fed the 5% green tea extract diet had depressed cellular immune function compared to the basal diet 25ºC (Duong *et al*., 2016; Currie *et al*., Submitted c).

Due to promising results from the above experiment, the green tea extract inclusions levels were refined (0.125, 0.25, 0.5 and 1%) and another study was conducted (Currie *et al*., Submitted c). Results from the follow up study demonstrated that green tea extract is not a suitable additive to improve the survival of greenlip abalone at high water temperatures (Currie *et al*., Submitted c).

Laboratory based trials - Vitamin C and K_1 fortification (Duong *et al*., 2016; Thomson *et al*., Submitted).
The effect of vitamin C and Vitamin K_1 fortification were assessed in two separate experiments:

- Experiment 1. This experiment used 6 experimental diets. Diets 1 and 2: The control commercial diet with or without 1% vitamin C over fortification; Diets 3, 4, 5 and 6: two other diets containing 1% green tea extract or 1% peanut testa extract with or without over fortification with 1% vitamin C (Duong *et al*., 2016).
- Experiment 2.Vitamin K_1 (0.0, 0.5, 1.0, 5.0 mg/ kg feed) was evaluated (Thomson *et al*., Submitted).

Results indicated that no diet, in either Experiment 1 (Duong *et al*., 2016) or Experiment 2 (Thomson *et al*., Submitted), led to a significant improvement in the survival of greenlip abalone at high water temperatures.

Laboratory based trial - Pre and probiotics
Several pre and probiotic and combination diets were tested, including probiotic *Bacillus nato* (0.1, 0.2 and 0.4%); probiotic (Pro) *Bacillus subtilis* and *Bacillus licheniformis* (0.1 and 0.5%); prebiotic (Pre) SafMannan yeast (0.1 and 0.2%); and a combination of the previous two (0.1% Pro 1 + 0.1% Pre; 0.5% Pro1 + 0.2% Pre). There was no significant influence of these dietary micro-ingredients on the survival at a high water temperature compared to the control.

Q3- Complete second farm trial to evaluate the efficacy of a dietary micro-ingredient to reduce the mortality of abalone at high summer water temperatures.

Two on-farm trials were conducted during the summer of 2015/16:
Trial 1: Assessed the effects of dietary micro-ingredients (5% GSE) to reduce mortality during summer at Kangaroo Island Abalone (KIA), SA.

Unfortunately, results from the on-farm trial at Kangaroo Island Abalone were inconclusive. Against the advice from researchers, the diet used in the 2015/16 on-farm summer trial at Kangaroo Island Abalone was conducted using a 0.05% GSE diet compared to the current commercial diet, rather than the recommended level of 5% GSE (Currie *et al*., Submitted b; Duong *et al*., 2016). Based on laboratory results the 0.05% GSE diet was not effective at reducing mortality at a high

water temperature. However, in laboratory based study the inclusion of 0.05% GSE reduced oxygen consumption rates in 3-year-old greenlip abalone, as did all other GSE inclusion diets (0.05-5%) (Currie *et al*., Submitted b).

Trial 2: Assessed the effects of the inclusion of 15% dried mixed drift cast macroalgae in a commercial diet on the shell, lip and foot colour development in greenlip abalone (Coastal Seafarms, Portland, VIC).

One-year-old greenlip abalone were fed two separate diets: Diet 1) control diet; commercially available formulated abalone diet (n=4 raceways), and Diet 2) 15% macroalgae meal diet; Eyre Peninsula Aquafeeds (EPA) Abgrow Premium with 15% dried mixed drift cast macroalgae meal (n=4 raceways). The abalone were fed the trial diets over the summer – autumn period (February 2016 – May 2016). Abalone were held in concrete production raceways and cultured using normal commercial practices. Samples were collected from each replicate raceway at the beginning of the trial in late February 2016, and at five week intervals, thereafter, until the conclusion of the trial in mid May 2016. On-farms results indicated that the use of the commercial diet containing 15% dried mixed drift cast macroalgae did not alter the shell, lip or foot colour of greenlip abalone. These results were also confirmed with laboratory results at SARDI SAASC with 3-year-old greenlip abalone fed the same diet at 22 °C (see section Q1. 2015-16).

Visiting Scientist, post graduate and undergraduate student programs and progress

Training was extensive in this project. A visiting scientist, five PhD students, one Masters student, four Honours students, two undergraduate student projects and numerous other extra mural work placements students were trained in this project.

Visiting Scientist

Dr Debasis De (Visiting Scientist on a 6 month Endeavour Fellowship; April 2015-October 2015) is a visiting Scientist from the Nutrition, Genetics and Biotechnology Division of the Kakdwip Research Centre, Central Institute of Brackishwater Aquaculture, South 24 Parganas, West Bengal, India. Investigated the effects of pre- and probiotics on the survival of greenlip abalone at high summer water temperatures. Work is completed, and manuscripts are being prepared for publication.

PhD students

The two PhD students who were awarded operating scholarships from this project are nearing completion (March 2018). Both PhD scholarship projects addressed specific relevant industry issues, are progressing well and are on track for completion within the prescribed PhD project timelines. An outline of their PhD project progress is below:

1. Nathan Danckert, PhD student. Project entitled "Characterisation of the digestive tract microbiome of cultured abalone in response to temperature and diet". University of Sydney, Faculty of Agriculture and Environment, Department of Plant and Food Sciences, Eveleigh, NSW, Australia. Supervised by Dr Brian Jones, Dr Kim-Yen Phan-Thien, Dr Neil Wilson and Assoc. Prof. David Stone. Commenced February 2015 - current.

Nathan's PhD project is progressing well. The scope of Nathan's project is to investigate the microbiology of abalone, specifically the digestive tract microbiome in both controlled laboratory and commercial aquaculture systems. The project aims to:

- Characterise the core digestive tract microbiome of Australian abalone (*Haliotis laevigata* and *Haliotis laevigata* × *rubra*) in commercial aquaculture systems;
- Identify changes in the digestive tract microbiome in response to dietary intervention, both in controlled laboratory and commercial aquaculture experiments; and
- Determine the gene expression and digestive tract microbiome of abalone when exposed to chronic temperature stress compared to optimal growth temperatures.

Work is in progress to determine the effects of dried macroalgae (*Gracilaria cliftonii* and *Ulva* sp.) inclusion in the feed on gene expression and digestive tract microbiome in greenlip abalone (*H. laevigata*). This component complements the work done in the Q2 section of Milestones for 2014-2015 (Bansemer *et al.,* 2016a).

- Analysis of selected gene targets to identify differences between larger and smaller abalone, distinguish important growth-related genes, and determine any correlations between animal size and stress sensitivity.

- Characterisation of digestive tract microbiome in response to dietary treatments; in particular, the microbiome of abalone fed on dried macroalgae (*Gracilaria cliftonii* and *Ulva* sp.) and commercially formulated feeds will be compared.
- Statistical exploration of correlations between digestive tract microbiome, dietary treatments, animal size, health indicators, and other variables of interest.

Subsequent research will investigate:

- The influence of chronic temperature stress and dietary intervention on the digestive tract microbiome and gene expression in greenlip abalone (*H. laevigata*) in laboratory experiments that simulate summer mortality. Literature suggests bacterial disease to be the main contributor to summer mortality throughout Europe. This research will determine the role of bacteria in summer mortality in Australian aquaculture systems.
- The ecological succession of the digestive tract microbiome in greenlip (*H. laevigata*) and hybrid (*H. laevigata* × *rubra*) abalone over a 12-month period. Abalone will be sampled from two commercial aquaculture sites (SAM, Port Lincoln, SA and Southern Ocean Mariculture, Port Fairy, Vic) to examine changes in digestive tract microbiome of juvenile abalone through their development to the early grow-out stage.

2. Krishna-Lee Currie, PhD student. Project entitled "Optimisation of formulated diets to maximise growth and survival of cultured greenlip abalone, *Haliotis laevigata*, at optimal and suboptimal temperatures". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Dr James Harris, Assoc. Prof. David Stone and Prof. Gordon Howarth (University of Adelaide, School of Animal and Veterinary Sciences). Commenced 17th March 2014 - current.

Krishna-Lee's PhD project is on track and progressing well. Krishna-Lee has completed all of her experimental work for the studies investigating antioxidants and survival of greenlip abalone at high summer water temperatures (Components of Sections Q1 and Q2 for Milestones 2014-2015 and 2015-2016, respectively). She has also completed her series of projects investigating the addition of grape marc meal and peanut meal as macro-ingredients in abalone production diets (Components of Sections Q2 and Q1 for Milestones 2014-2015 and 2015-2016, respectively). Krishna is now in the analysis and write up phase of her PhD project.

Three PhD students have graduated (Matthew Bansemer; Duong Noc Duong, Thanh Hai Hoang).

3. Matthew Bansemer (PhD student) Project entitled "Digestive physiology and utilisation of macroalgae as feed for Australian abalone". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Prof. Jian Qin, Assoc. Prof. James Harris, Assoc. Prof. David Stone and Prof. Gordon Howarth (University of Adelaide, School of Animal and Veterinary Sciences). Graduated.
4. Duong Ngoc Duong (PhD student) Project entitled "Bioenergetics of greenlip abalone, *Haliotis laevigata*". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris, Assoc. Prof. David Stone and Prof. Jian G. Qin. Graduated.
5. Thanh Hoang Hai (PhD student) Project entitled "Colour of greenlip abalone fed diet of macroalgae and pigment supplementation". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Prof. Jian Qin, Assoc. Prof James Harris, Assoc. Prof. David Stone. Graduated.

Masters Student

1. Skordas, P. (Masters student). Project entitled "Ventral videographic assessment of the feeding behaviour of juvenile greenlip abalone, *Haliotis laevigata,* in response to diet and an acute water temperature challenge". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris, Assoc. Prof. David Stone and Dr Matthew Bansemer. Graduated.

Honours students

1. Amy Bates (Honours student) Project entitled "Does *Ulva* sp. protein extract enhance greenlip abalone (*Haliotis laevigata*)?" School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia, Supervised by Prof. Gordon Howarth and Assoc. Prof. David Stone. Graduated, 1st Class Honours.
2. Jessica Buss (Honours Student). Project entitled "The use of dietary additives to improve the survival of cultured greenlip abalone, Haliotis laevigata". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris and Assoc. Prof. David Stone. Awarded the Playford Scholarship. Graduated, 1st Class Honours. Awarded the Flinders University Medal.

3. Mark Purvis (Honours Student) Project entitled "Kinetics of colour change in greenlip abalone, *Haliotis laevigata*, fed dried red macroalgae meals. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris, Assoc. Prof. David Stone and Prof. Jian G. Qin. Current.
4. Nicole Thomson (Honours Student). "The potential of vitamin K1 to improve greenlip abalone survival at summer water temperatures". School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia. Supervised by Prof. Gordon Howarth and Assoc. Prof. David Stone. Graduated, 1st Class Honours.

Summer Scholarship and other student

Amy Bates (SARDI summer scholarship student) Project entitled "Improving the binding of diets to reduce leaching loss for diets with novel feed ingredients" School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia, Supervised by Prof. Gordon Howarth and Assoc. Prof. David Stone. November 2015 - February 2016

Caleb McFadden (3rd Year Student project) Project entitled "Ventral videographic assessment of the feeding behavior of juvenile greenlip abalone *Haliotis laevigata* in response to diet and an acute water temperature challenge. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris, Assoc. Prof. David Stone and Dr Matthew Bansemer. August 2016 - December 2016.

Publications

Manuscripts published, accepted or submitted

1. Matthew S. Bansemer, Jian G. Qin, Krishna-Lee Currie and David A.J. Stone (2015). Temperature-dependent feed consumption patterns of greenlip (*Haliotis laevigata*) and hybrid (*H. laevigata* × *Haliotis rubra*) abalone fed fresh macroalgae or a formulated diet. Journal of Shellfish Research, 34, 3, 885-892.
2. Matthew S. Bansemer, Jian Qin, James O. Harris, Duong N. Duong, Krishna-Lee Currie, Gordon S. Howarth and David A.J. Stone (2016a). Dietary inclusions of dried macroalgae meal in formulated diets improve the growth of greenlip abalone (*Haliotis laevigata*). Journal of Applied Phycology. 28, 3645-3658.
3. Matthew S. Bansemer, Jian G. Qin, James O. Harris, Duong N. Duong, Thanh Hai Hoang, Gordon S. Howarth and David A.J. Stone (2016b) Growth and feed utilisation of greenlip abalone (*Haliotis laevigata*) fed nutrient enriched macroalgae. Aquaculture, 452, 62-68.
4. Matthew S. Bansemer, Jian G. Qin, James. O Harris, Gordon, S. Howarth., David A.J. Stone (2016c). Nutritional requirements and use of macroalgae as ingredients in abalone feed. Reviews in Aquaculture, 8, 121-135.
5. Amy L. Bates, Gordon S. Howarth, Krishna-Lee Currie, Mark Purvis, Matthew S. Bansemer, David A.J. Stone (Submitted). Growth and nutrient utilisation of greenlip abalone (*Haliotis laevigata*) fed Ulva sp. protein extract. Aquaculture.
6. Jessica J. Buss, Dylan A. Jones, Alyssa Lumsden, James O. Harris, Matthew S. Bansemer, David A.J. Stone (2015). Restricting feed ration has more effect than diet type on the feeding behaviour of greenlip abalone *Haliotis laevigata*. Marine and Freshwater Behaviour and Physiology 48, 51-70.
7. Jessica J. Buss, James O. Harris, Krishna-Lee Currie, David A.J. Stone. (Submitted). Orego-Stim® is a successful feed attractant, but does not improve survival of greenlip abalone (*Haliotis laevigata*) at peak summer water temperatures. Aquaculture.
8. Krishna-Lee Currie, Mark Purvis, James O. Harris, David A.J. Stone (Submitted a). Dietary inclusions of Acti-Meal improves feed utilisation and growth of greenlip abalone (*Haliotis laevigata*). Aquaculture.
9. Krishna-Lee Currie, Jessica J. Buss, James O., Harris, David A.J., Stone (Submitted b). Grape seed extract improves the survival of greenlip abalone, *Haliotis laevigata*, at high water temperature. Aquaculture.
10. Krishna-Lee Currie, Nicole Thompson, Duong N. Duong, James O. Harris, David A.J. Stone (Submitted c). Effect of green tea extract on the immune function and survival of greenlip abalone, *Haliotis laevigata*, exposed to a chronic water temperature challenge. Journal of Shellfish Research.
11. Krishna-Lee Currie, Mark Purvis, James O. Harris, David A.J. Stone (Submitted d). Peanut meal substitution in formulated diets for greenlip abalone (*Haliotis laevigata*). Aquaculture.
12. Krishna-Lee Curries, Brett Lange, Elizabeth W. Herbert, James O. Harris, David A.J. Stone (2015). Gastrointestinal evacuation time, but not nutrient digestibility, of greenlip abalone, *Haliotis laevigata* Donovan, is affect by temperature and age. Aquaculture 448, 219-228.
13. Krishna-Lee Currie, Hannah Davidson, James. O. Harris, Matthew, S. Bansemer, David A.J. Stone (2016). The effect of diet type and water temperature on the feeding behaviour of greenlip (*Haliotis laevigata*) and hybrid abalone (*H. laevigata x Haliotis rubra*). Journal of Shellfish Research. Journal of Shellfish Research 35, 641-651.

14. Duong N. Duong, Jian Qin, James O. Harris, Thanh H. Hoang, Matthew S. Bansemer, Ashley Dowell, Krishna-Lee Currie, Kim-Yen Phan-Thien, David A.J. Stone (2016). Effects of dietary green tea extract, grape seed extract and peanut extract supplementation on metabolism and survival of greenlip abalone (*Haliotis laevigata* Donovan) at high temperature. Aquaculture 464, 364-373.
15. Duong N. Duong, James O. Harris, Matthew S. Bansemer, Thanh H. Hoang, Jian G. Qin, David A.J. Stone (In press). Bioenergetics of greenlip abalone (*Haliotis laevigata* Donovan) fed nutrient-enriched live macroalgae or formulated diets. Aquaculture.
16. Thanh H. Hoang, Jian G. Qin, David A.J. Stone, James O. Harris, Duong N. Duong, Matthew S. Bansemer (2016). Colour changes of greenlip abalone (*Haliotis laevigata* Donovan) fed fresh macroalgae and dried algal supplement. Aquaculture, 456, 16-23.
17. Thanh H. Hoang, David A.J. Stone, Duong N. Duong, Matthew S. Bansemer, James O. Harris, Jian G. Qin (2017). Colour change of greenlip abalone (*Haliotis laevigata* Donovan) fed formulated diets containing graded levels of dried macroalgae meal. Aquaculture, 468, 278-285.
18. Brett P. Shiel, Nathan E. Hall, Ira R. Cooke, Nicholas A. Robinson, David AJ. Stone, Jan M. Strugnell. (2017). The effect of commercial, natural and grape seed extract supplemented diets on gene expression signatures and survival of greenlip abalone (*Haliotis laevigata*) during heat stress. Aquaculture, 479, 798-807.
19. Nicole L. Thomson, Gordon Howarth, Krishna-Lee Currie, Duong N. Duong, David A.J. Stone (Submitted). Impact of vitamin K1 on immunity and survival of greenlip abalone, *Haliotis laevigata*, at summer water temperatures. Aquaculture.

Manuscripts in preparation for submission.

1. Krishna-Lee Currie, James O. Harris, Matthew S. Bansemer, Duong N. Duong, David A.J. Stone (In preparation). Effects of macroalgal diet supplementations on the immunity of greenlip abalone, *Haliotis laevigata*. Target journal: Aquaculture.
2. Krishna-Lee Currie, James O., Harris, Debasis De, Duong N. Duong and David A.J. Stone (In preparation). Effect of dietary supplementation of the probiotic (*Bacillus subtilis natto*) on growth and survival of green abalone (*Haliotis laevigata*) at high water temperature. Target journal: Aquaculture.
3. Debasis De, Krishna-Lee Currie, Duong N. Duong and David A.J. Stone (In preparation). Effect of dietary supplementation of probiotics and prebiotics on growth and survival of green abalone (*Haliotis laevigata*) at high water temperature. Target journal: Aquaculture.
4. Duong N. Duong, James O. Harris, Jian G. Qin, Matthew S. Bansemer, Thanh H. Hoang and David A.J. Stone (In preparation). Bioenergetics of greenlip abalone (*Haliotis laevigata*) fed dried macroalgae inclusion in formulated diet. Target journal: Aquaculture.
5. Duong N. Duong, David A.J. Stone, Matthew S. Bansemer, Thanh H. Hoang, Jian G. Qin and James O. Harris (In preparation). Seasonal bioenergetics of greenlip abalone (*Haliotis laevigata* Donovan) fed formulated diet with increasing dietary protein levels. Target journal: Aquaculture.
6. Duong N. Duong, David A.J. Stone, Matthew S. Bansemer, Krishna-Lee Currie, Thanh H. Hoang, Jian G. Qin and James O. Harris (In preparation). Bioenergetics of greenlip abalone (*Haliotis laevigata* Donovan) fed probiotic and prebiotic supplementation in formulated diet at different temperatures. Target journal: Aquaculture.
7. Duong N. Duong, David A.J. Stone, Matthew S. Bansemer, Thanh H. Hoang, Jian G. Qin and James O. Harris (In preparation). Effects of dietary feed additives on bioenergetics of greenlip abalone (*Haliotis laevigata* Donovan) prolonged exposure elevated water temperature. Target journal: Aquaculture.
8. Thanh H. Hoang, David A.J. Stone, Duong N. Duong, James O. Harris, Jian G. Qin (In preparation). Effects of diet and water temperature on the colour of greenlip abalone (*Haliotis laevigata* Donovan). Target journal: Aquaculture.

Several additional journal manuscripts that have not been included here will also be prepared for submission in the near future.

Student Theses

PhD Theses

1. Bansemer, M., (2016). Digestive physiology and utilisation of macroalgae as feed for Australian abalone. PhD Thesis, Flinders University (School of Biological Sciences). Bedford Park, South Australia, Australia, pp. 186.

2. Duong, D.N., (2016). Bioenergetics of abalone fed different diets with macroalgae supplements. PhD Thesis. Flinders University (School of Biological Sciences). Bedford Park, South Australia, Australia. pp 174.
3. Hai, T.H., (2016). The effects of diets and water temperature on the colour of Australiam greenlip abalone (*Haliotis laevigata* Donovan). PhD Thesis. Flinders University (School of Biological Sciences). Bedford Park, South Australia, Australia. pp 168.

Honours Theses

1. Bates, A., (2016). Does *Ulva* sp. protein extract enhance greenlip abalone (*Haliotis laevigata*) growth? Honours Thesis (1st Class). University of Adelaide (School of Animal and Veterinary Sciences). Roseworthy, South Australia, Australia, pp. 60.
2. Buss, J., (2015). Orego-Stim® is a successful feed attractant but does not improve survival of greenlip abalone (*Haliotis laevigata*) at peak summer water temperatures. Honours Thesis (1st Class), Flinders University (School of Biological Sciences). Bedford Park, South Australia, Australia, pp. 83.
3. Thomson, N., (2016). The potential of vitamin K1 to improve greenlip abalone survival at summer water temperatures. Honours Thesis (1st Class). University of Adelaide (School of Animal and Veterinary Sciences). Roseworthy, South Australia, Australia. pp. 77.

Conference Abstracts, Presentations and Posters

Abstract and oral presentation at the 2nd International Symposium on Marine and Fisheries Research, Yogyakarta, Indonesia, July 24-25, 2017:

1. Nur, Kurniati Umrah*, Louise Adams, David Stone, Mark Adams, Nicholas Savva. Comparison of three inert markers in measuring apparent nutrient digestibility of juvenile abalone under different culture condition and temperature regimes (Oral).

Abstracts, posters and oral presentations at the World Aquaculture conference, Cape Town, South Africa - June 26-30, 2017:

1. Matthew Bansemer*, Jian Qin, James Harris, Duong Duong, Krishna-Lee Currie, Gordon Howarth and David Stone. Dietary inclusions of dried macroalgae meal in formulated diets improve the growth of greenlip abalone *Haliotis laevigata* (Oral).
2. Krishna-Lee Currie*, Mark Purvis, Amy Bates, James O. Harris and David A.J. Stone. Dietary inclusions of Acti-meal improves the growth and feed utilisation of greenlip abalone *Haliotis laevigata (Oral)*.
3. Paul Skordas*, Caleb McFadden, Krishna-Lee Currie, Matthew Bansemer, James O. Harris and David A.J. Stone. Ventral videographic assessment of the feeding behavior of juvenile greenlip abalone *Haliotis laevigata* in response to diet and an acute water temperature challenge (Poster).
4. David A.J. Stone, Krishna-Lee Currie, Matthew Bansemer, Hannah Davidson, David Connell , James O. Harris. Acti-meal diet substitution in formulated diets for greenlip abalone *Haliotis laevigata*: an on farm trial (Poster, accepted but not presented).

Poster presented at the "On the Pulse, Research Symposium", Tuesday 12 July 2016, Faculty of Agriculture and Environment, University of Sydney the 3rd FRDC Australasian Scientific Conference on Aquatic Animal Health, 6-10th July, Cairns, Australia:

1. Nathan Danckert, Kim-Yen Phan-Thien, Neil Wilson, Brian Jones and David Stone. Evaluation of the digestive tract microbiome of abalone in response to dietary intervention.

Abstract and oral presentation at the 3rd FRDC Australasian Scientific Conference on Aquatic Animal Health, 6-10th July, Cairns, Australia:

1. James O. Harris and David A.J. Stone 2015. Understanding and reducing summer mortality in cultured Australian abalone.

Oral presentations at the SARDI Aquatic Sciences Student Symposium 2015, SARDI Aquatic West Beach, Adelaide, Australia, 25 August 2015:

1. Thanh Hai Hoang. Colour Change of greenlip abalone (*Haliotis laevigata*) fed dried macroalgae Inclusions in formulated diets.

2. Duong Ngoc Duong. Bioenergetics of greenlip abalone (*Haliotis laevigata*) fed dietary macroalgae in nutrient enrichment or commercial diets.
3. Krishna-Lee Currie. Optimisation of formulated diets for the growth, health and survival of greenlip abalone.

Abstracts, oral presentations and posters at the 9th International Abalone Symposium, Yeosu, South Korea, 5-10 Oct. 2015:

1. Matthew Bansemer, David A.J. Stone, Jian Qin, James O. Harris, Elise Schaefer, Hanru Wang, Georgia Mercer, and Gordon S. Howarth. Age-dependent response of digestive enzyme activities to dietary protein level and water temperature in greenlip abalone (*Haliotis laevigata*). (Oral presentation, given by Krishna-Lee Currie).
2. Jessica J. Buss*, Dylan Jones, Alyssa Lumsden, Matthew Bansemer, James O. Harris, and David A.J. Stone. Ventral videographic assessment of the feeding behaviour of juvenile greenlip (*Haliotis laevigata*) abalone in response to feed ration and type. (Poster presentation). Awarded prize for Student Poster Presentation.
3. Jessica Buss*, Jones D.A., Lumsden A., Harris J.O., Bansemer M.S., Stone D.A.J. The use of oregano in abalone culture to stimulate feeding and improve survival (Poster presentation).
4. Krishna-Lee Currie*, Jessica J. Buss, James O. Harris, David A.J. Stone. Grape seed extract improves the survival of greenlip abalone, *Haliotis laevigata*, exposed to chronic water temperature elevation (Oral presentation).
5. Krishna-Lee Currie*, Hannah Davidson, James O. Harris, Matthew Bansemer, Debasis De and David A.J. Stone. Ventral videographic assessment of the feeding behaviour of juvenile greenlip (*Haliotis laevigata*) and hybrid (*Haliotis laevigata* × *rubra*) abalone in response to dietary and temperature manipulation. (Poster presentation).
6. Nathan Danckert, Brian Jones, Kim-Yen Phan-Thien, Neil Wilson, David A.J. Stone. Evaluation of the digestive tract microbiome of greenlip and hybrid abalone in response to dietary manipulation and water temperature (Oral presentation).
7. Debasis De, Krishna-Lee Currie*, James O. Harris, David A.J. Stone. Effect of dietary supplementation of probiotics and prebiotics on growth and survival of green abalone (*Haliotis laevigata*) at high water temperature (Oral presentation).
8. James O. Harris*, Krishna-Lee Currie, Matthew Bansemer, Duong, Thanh Hai Hoang, Gordon Howarth and David A.J. Stone. Understanding and reducing summer mortality in cultured Australian abalone. (Oral presentation).
9. Thanh Hai Hoang*, Jian G. Qin, David A.J. Stone and James O. Harris. Colour change of greenlip abalone (*Haliotis laevigata*) fed dried macroalgae inclusions in formulated diets (Oral presentation).
10. Elise N. Schaefer, James O. Harris*, Duong Ngoc Duong, Gordon S. Howarth, David A. J. Stone. Gastrointestinal tract morphology of greenlip abalone (*Haliotis laevigata*) at high water temperatures fed formulated feeds supplemented with antioxidants. (Oral presentation).

Industry Meetings and Workshops

1. Australian Abalone Growers Annual General Meeting and Workshop at Queenscliff, Victoria on the 10 and 11 August 2016:
2. SARDI Aquatic Sciences Student Symposium 2016, SARDI Aquatic West Beach, Adelaide, Australia, 27 July 2016.
3. Australian Abalone Growers Association: Annual Seminar & General Meeting, SARDI Aquatic West Beach, Adelaide, Australia, 9 May 2016. (Organised and hosted by Assoc. Prof. David A.J. Stone).
4. West Java Technical Delegation - Abalone Training Program, SARDI Aquatic Sciences, Adelaide, Australia, 24 Nov. 2015.
5. SARDI Aquatic Sciences Student Symposium 2015, SARDI Aquatic West Beach, Adelaide, Australia, 25 August 2015. (Co-chaired by Assoc. Prof. David A.J. Stone).
6. Australian Abalone Growers Association Annual Workshop, SARDI Aquatic Science Centre, West Beach, South Australia, 13-14th August 2015. (Organised and hosted by Assoc. Prof. David A.J. Stone)
7. Thriving Abalone Project workshop (SA Govt. Functional Food Focus Program), SARDI Aquatic Sciences, West Beach, Adelaide, Australia, 17 June 2015. (Organised and hosted by Assoc. Prof. David A.J. Stone).
8. Thriving Abalone Project Meeting, SARDI Aquatic Sciences Centre, West Beach, Adelaide, Australia, 23 April 2015. (Organised and hosted by Assoc. Prof. David A.J. Stone).
9. Australian Abalone Growers Association "Surviving Summer" Workshop, SARDI Aquatic Science Centre, West Beach, South Australia, 6th September 2014.

Industry Presentations

Oral presentations at the Australian Abalone Growers Annual General Meeting and Workshop at Queenscliff, Victoria on the 10 and 11 August 2016:

1. David A.J. Stone. Thriving Abalone: Quality aquafeeds utilising food wastes

Oral presentations and abstracts at the SARDI Aquatic Sciences Student Symposium 2016, SARDI Aquatic West Beach, Adelaide, Australia, 27 July 2016:

1. David Stone. Progress of Thriving Abalone project
2. Amy Bates. Does *Ulva* sp. protein extract improve growth rates of greenlip abalone (*Haliotis laevigata*)?
3. Mark Purvis. Kinetics of colour change in greenlip abalone, *Haliotis laevigata* fed dried red macroalgae meals.

Oral presentations delivered at the Australian Abalone Growers Association: Annual Seminar & General Meeting, SARDI Aquatic Science Adelaide, Australia, 9 May 2016:

1. David Stone. Progress of Thriving Abalone project.
2. Matthew Bansemer. Macroalgae as feed for greenlip abalone.
3. Krishna-Lee Currie. Using dietary intervention to improve the survival of greenlip abalone at high water temperatures.

West Java Technical Delegation - Abalone Training Program, SARDI Aquatic Sciences, Adelaide, Australia, 24 Nov. 2015.

1. David Stone and Matthew Bansemer. Abalone feeds and nutrition.

SARDI Aquatic Sciences Student Symposium 2015, SARDI Aquatic West Beach, Adelaide, Australia, 25 August 2015. (Co-chaired by Assoc. Prof. David A.J. Stone).
Add presentation.

Oral presentations at the Australian Abalone Growers Annual General Meeting and Workshop at SARDI ASC on the 13 and 14 August 2015:

1. David A.J. Stone. Thriving Abalone: Quality aquafeeds utilising food wastes
2. Nicole Thomson. The potential of vitamin K_1 to improve greenlip abalone survival at summer water temperatures.
3. Duong. Bioenergetics of abalone fed dried macroalgae meal inclusion in formulated diets.
4. Jessica Buss. Oregano extract is a successful feed attract but does not improve survival of greenlip abalone at peak summer water temperatures.
5. Elise Schaefer. The effects of grape seed and green tea extract on the gastrointestinal tract of greenlip abalone.
6. Matthew Bansemer. Dried macroalgal meal inclusions in formulated diets for greenlip abalone (*Haliotis laevigata*).
7. Krishna-Lee Currie. Grape seed extract improves the survival of greenlip abalone exposed to a chronic elevated water temperature challenge.
8. Krishna-Lee Currie. The effect of direct microbial feeding on the immune response and survival of greenlip abalone exposed to a chronic elevated water temperature challenge.
9. Thanh Hai Hoang. Colour change of greenlip abalone (*Haliotis laevigata*) fed dried macroalgae inclusion.

Oral presentations at the Thriving Abalone Project Workshop (SA Govt. Functional Food Focus Program), SARDI Aquatic Sciences, Adelaide, Australia, 17 June 2015:

1. Matthew Bansemer*, Jian Qin, James O. Harris, David A.J. Stone, Gordon S. Howarth. Dried macroalgal meal inclusions in formulated diets for greenlip abalone (*Haliotis laevigata*).
2. Krishna-Lee Currie*, Jessica J. Buss, James O. Harris, David A.J. Stone. Grape seed extract improves the survival of greenlip abalone at high water temperatures.
3. Krishna-Lee Currie*, James O. Harris, David A.J. Stone. The use of graded levels of peanut testa extract on the survival of abalone at summer water temperatures.
4. Krishna-Lee Currie*, James O. Harris, David A.J. Stone. The use of graded levels of grape marc and peanut meal as dietary ingredients for growth of abalone.
5. Nathan Danckert. Evaluation of the digestive tract microbiome of greenlip abalone in response to dietary manipulation and water temperature.
6. Dr Debasis De. The use of pro- and prebiotics to improve growth and survival of abalone at summer water temperatures.
7. Assoc. Prof. James Harris (Jessica Buss Honours Project, Flinders University). Oregano oil improves feed intake but not survival of greenlip abalone at summer water temperatures.

8. Dr Jun Niimi (University of Adelaide). Taste discrimination testing of greenlip abalone fed diets containing 5% grape seed extract from the on-farm trials at SAM and KIA.
9. Camilla Martins (South Seas Abalone). On-farm trials at SAM and KIA with greenlip abalone and grape seed extract.
10. Thanh Hoang Hai. The effects of graded levels of *Ulva* sp. and *Gracilaria cliftonii* on the colour of abalone.
11. Duong Ngoc Duong. Energetics of abalone fed a range of plant ingredients.

Thriving Abalone Project Meeting, SARDI Aquatic Sciences Centre, West Beach, Adelaide, Australia, 23 April 2015. (Organised and hosted by Assoc. Prof. David A.J. Stone).
1. David Stone. Update on progress of the Thriving Abalone project.
2. Dr Debasis Past work with fish and proposed pre-and probiotic project overview.
3. Matthew Bansemer, PhD project progress.
4. Thanh Hoang Hai. PhD project progress.
5. Krishna Lee Currie. PhD project progress.
6. Duong Ngoc Duong. PhD project progress.
7. Nathan Danckert. Preliminary evaluation of the digestive tract microbiome of greenlip abalone in response to dietary manipulation at summer water temperatures at two South Australian farms.
8. Jessica Buss. Honours project progress.
9. Elise Schaefer. PhD project progress.

Australian Abalone Growers Association "Surviving Summer" Workshop, SARDI Aquatic Science Centre, West Beach, South Australia, 6th September 2014.
1. David Stone. Overview of the Thriving Abalone project.
2. Matthew Bansemer, Jian Qin, James Harris, Gordon Howarth, David Stone. Improvement of abalone nutrition with macroalgae addition.
3. Thanh Hoang Hai, Jian Qin, James Harris, David Stone. Effects of dietary macroalgae and commercial diets on shell and flesh colour of greenlip abalone.
4. Krishna Lee Currie, James Harris, David Stone. Lower water temperature and increasing age extend the gastrointestinal evacuation time of greenlip abalone *Haliotis laevigata*.
5. Krishna Lee Currie, James Harris, David Stone. Optimisation of formulated diets for the growth and survival of greenlip abalone (*Haliotis laevigata*).
6. Duong Ngoc Duong, James Harris, Jian Qin, David Stone. The effects of water temperature on the bioenergetics of abalone fed commercial diets and macroalgae supplements.
7. Elise Schaefer, James Harris, Gordon Howarth, David Stone. Summer mortality: A histological examination of crop and stomach epithelial thickness in response to dietary intervention.

Data Location

All of the Key project documents are located on David Stone's computer G drive (G:\Aquaculture\Thriving Abalone) at SARDI ASC and is also backed up on an external hard drive.

Path to market

Principal Investigator: Dr Steven Lapidge, SARDI Plant Research Centre (8303 9616; steven.lapidge@sa.gov.au)

Project aims

- Work with Project Leaders of the lamb, eggs, oats and aquafeed projects to determine:
  - Potential benefits (functional properties) of each new functional food product
  - Potential hazards (pathogens, toxins, allergens)
  - Potential quality issues (interactions, stability of functional properties etc)
  - Production environment requirements
- Market research will be conducted to determine consumer acceptability, target groups, price premiums
- Opportunities to address the needs of particular target groups will be explored e.g. aged food service industry

Project design

- Functional food expert employed to review potential benefit claims
- Commissioning of market research identified as a knowledge gap
- Economic analysis to determine value proposition (cost of production vs price increase)

Progress to date

Dr Lenka Malek, a qualified nutritionist who did her PhD on examining consumer preferences for nutritionally-fortified products, was employed four days a week for six months between February and July 2015. During this time Dr Malek worked closely with the functional food development project leaders to prepare 'Path to Market' reports. The aim of the reports is to provide information regarding the potential functional benefits and possible label claims, quality issues (eating quality, nutrient interactions, stability and shelf-life), hazards (toxins and allergens), specific production requirements and potential market size. The individual reports are included in the appendices as per below:

APPENDIX 1- Path to Market: Healthy-fat Lamb

APPENDIX 2- Path to Market: Supercharged Eggs

APPENDIX 3- Path to Market: Gluten-free Oats

APPENDIX 4- Path to Market: Thriving Abalone

Each report has been written as a stand-alone document. Each report, combined with the corresponding laboratory and field functional food development results will form the basis of a product development package for commercialisation purposes. Information gaps identified in the individual Path to Market reports, as detailed in the Recommendations at the end of each report, will look to be filled in 2015-16.

Milestones for 2015-2016

Q1- Finalise Path to Market reports
Q2- Compile Path to Market report recommendations and establish work program to fill information gaps. Common recommendations for each report include consumer interest, willingness to pay and marketing messages.
Q3- Establish commercial arrangements with SA food industry partners regarding functional healthy-fat sheep meat and supercharged eggs. Solidify commercial arrangements with existing commercial partners- Uncle Toby's for Gluten-free oats, and Aquafeeds Australia, Eyre Peninsula Aquafeeds and Skretting Australia for Thriving abalone.
Q4- Complete final project report.

Commented [VT3]: Steve to update/finalise

Publications to date

Path to Market reports (Appendix 1-4)
Presentation to PIRSA Executive- February 3, 2015

Data Location

G:\TempData\Steve-FFFP

Functional Foods Demonstration Project

Principal Investigator: Dr Stephen Pahl, Plant Research Centre, SARDI (8303 9333; stephen.pahl@sa.gov.au)

Project aims

- Creating one or more functional food or beverage products to demonstrate South Australia's capability to deliver functional foods efficiently and cost effectively.
- Actively engaging and partnering with a number of South Australian food and beverage businesses in the development of functional foods and beverages.
- Demonstrating the effective partnerships developed between PIRSA, SARDI and Food South Australia that could be strengthened under the new SA Food Innovation Centre.
- Providing specific learnings and recommendations that can be used to inform future strategies to build capacity and capability in functional food development and food innovation in South Australia.

Project design

- South Australian food businesses invited to submit an expression of interest to develop functional food products.
- Review and shortlist of concepts based on objectives, resources available and projected commercial feasibility.
- Develop and refine concepts through ingredient selection, product formulation and sensory assessments.
- Undertake compositional analysis to justify any nutritional content and health claims and receive regulatory guidance on claims and labelling.

Project Outcomes

The demonstration project contacted 170 South Australian food businesses and other organisations. The South Australian Food Innovation Centre (SAFIC) was used as the initial point of entry and received eight expressions of interest to develop functional food products from South Australian food businesses. Applications from Riviera Bakery (functional bread), Highway Enterprises (functional purple wheat noodles), SPQR Holdings (functional meal solutions) and Daycone Trust (functional crackers) were shortlisted for product development.

SARDI assisted each business to develop and refine the concepts through ingredient selection, product formulation and sensory assessments, and facilitated the nutritional analysis and input from a nutritional consultant. Functional characteristics where developed through the inclusion of alternative food ingredients, vitamins and/or minerals, or the removal of added sodium. The most common target areas where the businesses were seeking to make general level health or nutrition content claims were in relation to bones and teeth, immunity, brain/attention and cognitive function and sports/energy. A summary of the key outcomes and findings with each of the four businesses are as follows.

Riviera Bakery – Two functional breads (a Swiss Dark Rye and a Multigrain) were developed with a vitamin and mineral premix that did not affect the aroma, appearance, flavour, texture and aftertaste. The research highlighted the need to identify vitamins and minerals already contained within food ingredients and, therefore the lack of advantage in adding these further to the food. For example, wheat flour used for making bread contains mandatory fortification of folic acid and thiamine, and so there was no need to include them in the vitamin and mineral premix. The study also identified the need to source alternative vitamins and minerals. For example, the use of magnesium oxide led to poor loaf structure and unpleasant taste, but the use of magnesium carbonate provided desired health benefits without any negative attributes. Hence, there is a need to source and research other minerals that may help in creating functional food products. Riviera Bakery is looking to commercialise these products and requires assistance in receiving confirmation of their health claims, nutritional information panel and ingredient declaration, shelf-life testing, packaging design and production and product launch.

Daycone Trust – A functional multifibre cracker base (non-seasoned) was developed with a vitamin and mineral premix that did not affect flavour and texture. Two additional premix with added calcium were also trialled. The inclusion of the calcium resulted in a chalkiness texture of the cracker base, however, the mouthfeel of the base was improved by additional changes to the formulation. The study demonstrated the need to test various combinations of vitamins and minerals and that an adequate amount of time is needed to change formulations and run semi-commercial trials. The research identified that one of the challenges to consider in developing functional food is the serving size of the product. When the serving

size is small, it is more difficult to develop a product with sufficient vitamins and minerals to meet the qualifying factors for labelling of health benefits. Daycone Trust is progressing with product development and may require further assistance to finalise testing.

SPQR Holdings - The study was unable to develop a low salt beef lasagne that retained flavour. Even with a salt replacer the reduced sodium claim according to the Food Standards Code (contains at least 25% less sodium than in the same amount of reference food) was not able to be achieved. Hence, the industry needs research and development support to find out what is achievable but also what is not achievable. There is potential that industry may spend time and money exploring new markets for new food products, but they may not be able to improve their current product. This is especially relevant to 'free from' functional food products, where SAFIC could provide evidence for potential food health claims. Industry needs to know what it can't do as well as what it could do.

Highway Enterprises – A functional purple wheat noodle was developed enriched with folate and used a blend of purple wheat and perfections flour. The use of 100% purple wheat flour did not provide acceptable product characteristics. This study highlighted the need for R&D into blending ingredients to maintain the characteristics of the product whilst providing a functional food that can make health claims. Highway Enterprises needs assistance in reviewing the potential health claims in regards to marketing their product, providing samples to existing food service clients, discussing with manufacturers the use of this new flour and the benefits, disseminating information on the new flour and designing new packaging for retail pack launch.

The demonstration project was highly successful; it engaged and partnered with multiple South Australian food businesses and supported the development of four functional food products that are in the process of being commercialised. The partnerships between PIRSA, SARDI and Food South Australia were effective and provided a valuable resource to engage with industry and to ensure the cost effective delivery and review of the project.

A number of specific learning and recommendation where identified and these can be used to inform future strategies to build capacity and capability in functional food development and food innovation in South Australia. The businesses involved in the demonstration project were appreciative of the support and would participate in a similar program in the future. The project identified that there are a limited number of ingredient suppliers that can rapidly supply vitamin and mineral premixes, and that sufficient time and resources should be committed for contracting, product development including nutritional testing and factory trials. The project also identified that health claims must not be made on a food that does not meet the nutrient profiling scoring criterion and a preliminary feasibility assessment should be undertaken prior to commencing research activities. In addition, food manufacturers may require technical support and guidance to identify what nutrition and health claims can be made on food labels and in product advertising and obtaining expert advice in regards to regulatory compliance is beneficial.

Recommendations from the demonstration project included establishing a database of functional ingredient suppliers, and mapping the capability and limitations of food nutritional testing laboratories and test/product development kitchens. The project has also recommended a review of SAFIC members to identify personnel that can provide nutritional advice, and identifying the major roles of each SAFIC member throughout the product development life-cycle from initial assessment, through to product launch and review.

Publications to date

No scientific publication have been published to date.

The following internal and client reports have been prepared:

- S Pahl, S Miller, D Curnow, A Maronich, T Thompson, N Freeman (2016) ***Functional Foods Demonstration Project. Report*** prepared for PIRSA, August 2016
- T Thompson (2016) ***Consumer acceptability of Purple Noodles***. Report prepared for Taing's Noodles, May 2016
- T Thompson (2016) ***Consumer acceptability of Fortified Purple Noodles***. Report prepared for Taing's Noodles, June 2016
- T Thompson (2016). ***Consumer acceptability of Purple Noodles with Sauce***. Report prepared for Taing's Noodles, June 2016

- T Thompson (2016) ***Consumer acceptability of Fortified Bread Products***. Report prepared for Riviera Bakery, June 2016

Data Location

- G:\SARDI Food Safety Projects\Functional Foods\Functional foods demonstration project

Foresighting with Food SA

Principal Investigator: Dr Steven Lapidge, SARDI Plant Research Centre (8303 9616; steven.lapidge@sa.gov.au)

Project aims

1. Undertake a stocktake of functional foods being produced in SA or by SA-owned companies to look for areas of expertise as well as past trends.
2. Understand which existing and new SA food companies could benefit through developing new functional foods based on existing and new technologies.
3. Work with SA food companies to develop new functional foods based on local raw foods, expertise, ingredients or markets.

Project design

- Use Food SA records and the Innova database to ascertain SA produced functional foods, the competitive environment and claims on functionality.
- Ibisworld will be used to understand the potential market size.
- Food SA will directly contact SA food companies to follow up if required.

Progress to date

The Food SA Foresighting report 'Sizing the Opportunity for South Australian Food Manufacturers' was completed in October 2015. The Executive Summary is included below:

Executive Summary

Erosion of product margins is one of the most difficult challenges affecting the Australian food industry in recent times. Yet current annual growth rates for functional beverages and packaged foods in Australia are approximately 12% and 9%, respectively. Understanding the current state of functional food manufacturing in South Australia is critical to ascertaining the potential opportunities for the industry. If margins for functional products are better than their non-functional counterparts, then supporting manufacturers to explore functional products is an important aspect of future food strategies.

Three areas warranting research were investigated; analysing new functional products launched in Australia over the last five years, scanning the current functional products in the South Australian retail market and mapping the opportunities for South Australian food and beverage manufacturers. In addition, a case study on fruit juice manufacturing in South Australia to ascertain the profitability of cost structures and net profit margins for manufacturers in producing functional fruit juice compared to non-functional (traditional) juice was undertaken. Bottled water, sports drinks, energy drinks, bread, breakfast cereals, cheese, milk and spoonable yoghurt were researched as these food and beverage categories either dominate the functional market or are predicted to have the highest growth rate. Functional fresh food was not within the scope of this project. Specifically identifying South Australian companies that could benefit through the development of functional products provides relevance and direction as to how industry could take advantage of the positive trends in the functional food and drinks market.

Opportunities exist for South Australian companies who are manufacturing bottled water to add vitamins and minerals to their existing bottled water products. Companies could also offer more health-related drinks compared to sports-related functional beverages based on the prediction that energy drinks will face scrutiny over their caffeine content in the future. A real opportunity exists for the current eight South Australian juice manufacturers to produce functional fruit juice. Ready-to-drink tea is expected to have the fastest annual growth of all functional beverages over the next four years. Local companies are well placed to exploit this demand in functional ready-to-drink products as they already have functional tea in their product range.

Traditional, low price range apple juice is profitable enough for manufacturers, but not for retailers. South Australian fruit growers are unlikely to benefit from the development of functional juices under the current business model of selling second grade fruit for juice. However, they would benefit from trialling new breeding techniques to increase the contents of phytochemicals to sell these directly to the functional juice manufacturer. Due to ongoing cost pressures set by large retailers on manufacturers, increasing margins for functional juices will be difficult, unless highly innovative products are developed that are difficult to copy by other domestic and overseas manufacturers.

South Australia is a large grain producer and the best cases for functional food are in the bread and breakfast cereal categories. With eight South Australian companies already producing non-functional bread, this category is an attractive option to explore in regards to the additional of functional ingredients. Bread manufacturers who have already capitalised in the gluten-free area are looking to expand the health angle for their products. Limited functional breakfast cereal is produced in this State and therefore there is a case for a functional range.

For dairy products, spoonable yoghurt will have the fastest growth rate over the next four years and only one manufacturer in South Australia is producing functional yoghurt. Out of nine South Australian manufacturers producing cheese, none are producing functional versions. South Australia is not a large producer of milk which may be a disadvantage in choosing dairy products as a platform for functional food products.

For chocolate, benefits in sales may occur from highlighting the positive effects of antioxidants contained in chocolate. For non-chocolate confectionery, no South Australian confectionery manufacturers are producing medicated confectionery and there appears to be growth in demand for this type of product, albeit limited, which is a functional food category to watch in the future.

This research has shown that a multi-tiered approach is needed for the South Australian food and beverage industry. The capabilities for larger companies who may have their own research and development expertise is likely to focus on expansion of their product range, unless researchers have unique intellectual property available that they can share with specific businesses. In contrast, smaller companies are unlikely to have the required resources and capabilities for functional developments, and therefore will need assistance to ensure that they can compete with new functional products. A smaller number of beverage manufacturers exist in South Australia compared to food manufacturers. However, the presence of multi-national companies manufacturing beverages in South Australia may present opportunities to increase the offer of functional foods relatively quickly and efficiently from expanding their existing product range.

The most efficient way to increase the number of functional food and beverage products in South Australia is to investigate whether companies who are already manufacturing products in high growth functional markets are willing to enter this market, through the addition of functional ingredients. Starting a new product or market and developing entirely new product ranges is a high risk and costly proposition. Other value chain analyses need to be conducted for other food and beverage categories to demonstrate the potential profit that could be realised from modifying existing products to become functional.

The complete report forms APPENDIX 5- Foresighting with Food SA: Sizing the Opportunity for South Australian Food Manufacturers.

Milestones for 2015-2016

Commented [VT4]: Steve to update/finalise

Q1- Complete Foresighting with Food SA report and annual progress report
Q2- Gauge interest in developing new functional foods with SA food companies based on the insights provided by the Foresighting report.
Q3- Work directly with SA food companies to modifying existing food products to become functional or develop new functional foods based on the Food Standards Australian & New Zealand Standard 1.2.7- Nutrition, Health and Related Claims.
Q4- Complete final project report.

Publications to date

Foresighting with Food SA: Sizing the Opportunity for South Australian Food Manufacturers

Data Location

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Discussion

Commented [VT5]: Steve to update

The two-year Agribusiness Accelerator Functional Food Focus Program has been researching the development of four new 'naturally functional' foods:

1. Sheep meat with reduced levels of Saturated Fatty Acids to improve its heart health claims so as to add profit to lower value sheep meat such as mutton.
2. Supercharged eggs that address health deficiencies within the Australian community, such as vitamin D deficiency and osteoporosis; iodine deficiency, childbearing and mental health; and carotenoid deficiency and macular degeneration.
3. Gluten-free oats that takes a superfood (oats) and turns it into a functional food for gluten-intolerant coeliacs.
4. Thriving abalone grown on aquafeed formulations that utilise food waste that contain functional ingredients to improve the growth, survival, quality and health benefits of abalone.

Following Proof of Concept studies in year 1 the original aim was to take the 2-3 most promising functional foods through to commercialisation with an industry partner. Commercialisation activities are now occurring within project 3 – gluten-free oats – with Uncle Toby's and project 4 – abalone aquafeeds – with Aquafeeds Australia, Eyre Peninsula Aquafeeds and Skretting Australia. At the time of both project 1 – low SFA sheep meat – and project 2 – supercharged eggs – were delivering promising results and neither is at a stage where it can legitimately be forced to failure. If, however, the final results of the Proof of Concept trials are less promising, or either product fails to attract a commercial partner in 2016, then it will be discontinued and remaining funding will be redirected to other more promising FFFP projects.

The Path to Market reports prepared by Dr Malek and included in Appendices 1-4 detail the likely improved functional properties of each of the four developed functional foods, any potential hazards, possible product quality issues, consumer sensory acceptance indication and/or requirements and indicative price premiums. The reports will form the basis of commercialisation efforts and will be used to determine key remaining questions that need to be addressed in year 2 in determining product viability.

In addition, the foresighting exercise conducted by Food SA benchmarks where functional food production is currently at in South Australia and highlights the opportunity that awaits SA food producers, with the growth of the Australian functional beverages market being 12% p.a. and functional foods 9% p.a. The report details the higher profit margins that can be achieved through functional food and beverages. Functional juices, Ready to Drink tea, bread, breakfast cereals, dairy and medicated confectionary were recommended as the most promising areas of functional food and beverage developments for the state, with specific SA food and beverage companies listed that are best positioned to capitalise on the opportunity. Each of these will be approached in late 2015 and 2016, as well as other SA food producers that have directly expressed interest in working with SARDI to develop new functional foods

It is hoped that this initial two-year project will lead to significant further research, development and commercialisation of functional foods in South Australia through the impending establishment of the SA Food Innovation Centre. The results of this program, combined with the findings of the Functional and Luxury Food Project being conducted by PIRSA Ag, Food & Wine, will be used to guide future developments in this area.

APPENDIX 1- Path to Market: Healthy-fat Lamb

APPENDIX 2- Path to Market: Supercharged Eggs

APPENDIX 3- Path to Market: Gluten-free Oats

APPENDIX 4- Path to Market: Thriving Abalone

APPENDIX 5- Foresighting with Food SA: Sizing the Opportunity for South Australian Food Manufacturers