Year 1 Final Report

Fish Silage Project 2012

Funding Recipients: United Fisheries and Lincoln University

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(The Schedule Tasks have been included below, and the trial work undertaken in each is noted in bold italics underneath)

Project Objectives

There are two principal fields of investigation in this project:

A) the development of the optimal manufacturing process for the desired product

B) The assessment of the animal production benefit of the product in typical NZ cattle and sheep operations.

Section A – Fish Silage Processing Decisions

The specific trials designed to provide the necessary information for the product development are, correspondingly:

- An analytical profile of the lipid, protein, carbohydrate and mineral content of the silage produced by each of four potential combinations of fish carcasses and associated by-products, scored by Jim Gibbs against the established recommendations for ruminant intake and the typical diet mixes considered for the product.
    - ***This work is complete, as the only change in product between seasons is the bone structure, and the manufacturing process removes all material greater than 1cm in size. The assessment of the product with extra bone out and bone in revealed there was no practical differences, except mineral content, which is of no significance to the end use.***
- An assessment of the flow through typical delivery mechanisms used in the livestock industries of fish silage of a range of particle sizes produced by alternative grinding protocols.
    - ***The particle size for the final screened product is 750 microns, which is far smaller than ever required in industry use. The standard fish silage bone particles are removed above 1500 microns, which has been deemed as acceptable for the feeding systems in which it is to be used.***
- An assessment of the odour (manual and via livestock preference assessment) and the peptide profile by a combination of high pressure liquid chromatography and Western Blot techniques (Jim Gibbs), compared between three different protocols of enzyme concentration and time.
    - ***The final peptide size distribution has proved more difficult than scheduled for and is continuing, but the selected process for silage manufacturing is now final, and it***

involves a single enzyme concentration and time of hydrolysis, which has obviated the need for serial assessments.

- For the temperature, organic acid selection and the physical processing assessment, the final pH and storage capacity of the product will be compared between a range of each in house, and evaluated against the input costs of production of each.
    - ***The physical processing and storage time of the selected process have been deemed acceptable, and the organic acid initially used (acetic) has been retained for practical reasons of effectiveness and cost. The only aspect of the physical process that is still open to question is the temperature, as this may alter the fat profile of the final product by some oxidation above 45 degrees Celcius. However, as there are important practical reasons for a temperature of 65 degrees at certain points, this in unlikely to change, and the results of the changes in milk fat when using fish silage do not indicate any problems with this potential oxidation.***

Section B – Fish Silage Use in Stock

Year 1: ***Part A***: Rumen methanogenesis research at LU:

1) Experimental assessment of fish silage effects on rumen methane production in high production cows and sheep on high quality pastures. There are two separate experiments – dairy cows and breeding ewes.
    - Cows: 6 non lactating dairy cows (fistulated) will be used in a 2 X 3 crossover design. The cows will be grazed for 21d on typical ryegrass pastures, with 3 receiving fish supplement and 3 without supplement as controls. The cows will be rumen sampled across the diurnal cycle every 4h on day 3 and day 21. The groups will then be spelled for 21d, then swapped, and the process repeated. The samples will be analysed for 16S RNA and mRNA expression of mcrA gene to simultaneously plot the methanogen community profile and the methane profile across the diurnal cycle between the treatment groups.
        - ***This work is complete***
    - Sheep: Eight mature sheep (fistulated) will be grazed on typical high quality ryegrass for 17d, then fed fresh cut ryegrass indoors for 4 days with intakes measured. Four will be supplemented daily with fish silage over the entire period, and four will be controls. The sheep will be rumen sampled across the diurnal cycle every 4h on day 3 and day 21. The samples will be analysed for 16S RNA and mRNA expression of mcrA gene to simultaneously plot the methanogen community profile and the methane profile across the diurnal cycle between the treatment groups. On each day 22 -25 a sheep from either treatment or control groups that has maintained normal intake will be placed into a methane measurement chamber for 24h, and total methane emissions calculated. The groups will then be spelled, then swapped, and the process repeated.
        - ***This work is completed, with methane yields established directly via methane chamber.***
2) Milk composition in cows, conducted twice, in spring and autumn: Four ruminally fistulated lactating cows grazing at the Lincoln University Dairy Farm were used. The cows were milk sampled am and pm at day 14, and milk yields and fat and protein composition were obtained.

The cows were then supplemented daily with fish supplement for 14d and the process repeated.

- ***This work is completed, both the two phases, a spring trial of extreme dosages to assess maximal milk fat changes, and a summer/ autumn 2012 trial of standard (minimal) milk fat changes. The milk fat was shown to dramatically increase in the targeted omega group of fatty acids, with a ten fold increase in one isomer (DHA), a 16% increase in another (EPA), and an 87% increase in the important conjugated linoleic acid.***

3) Feeding: Non lactating cows and sheep will be used in preference testing of three compound feeds containing fish silage: palm kernel expeller, barley grain and barley pellets. The desired target inclusion levels will initially be established from the results of 1).

- ***This work is complete – the product was successfully included into barley pellets and fed in several short and long term trials in cattle and sheep. It has also been used in direct mixes with palm kernel expeller (PKE). However, the use of cereal grains as pellets is difficult because it presents an upper limit to the use of the feed due to rumen upsets by the grain content. So the decision has been made to pursue the use of PKE, but also as a pellet, which has not been done to date in NZ.***
- ***The first phase of the year 2 work is to trial a PKE pellet at 40% inclusion rate.***

Part B: Assessment of efficacy of fish silage in reducing nematode burdens in dairy calves and lambs, and the practicality of feeding these livestock classes fish silage.

1) On two separate farms, two groups of 25 calves will be co-grazed in a typical calf ryegrass pasture, split by a hot wire. One group will be supplemented with industry standard pelleted barley mix, and the other with pelleted barley fish silage mix, both via industry standard feeders, between 20-52 weeks. The calves will be serially weighed and faecal sampled across the period, and grazing and supplement feeding behaviours will be recorded at point samples during the period.

    - ***This work was split into two – a large and smaller trial – and the smaller trial conducted in spring. The work used barley pellets with and without silage inclusion, and showed a significant reduction in FEC in the silage treated calves. However, the simultaneous lamb work prompted a postponement of the larger trial (see 2) below).***

2) One hundred twin bearing ewes co-grazing ryegrass pasture were allocated to four equal groups, two supplemented with fish silage barley pellets, two with barley pellets, from lambing to weaning , and then the lambs were retained in their groups and sampled from weaning to slaughter. The lambs were serially weighed and faecal sampled across the period, and grazing and supplement feeding behaviours will be recorded at point samples during the period.

    - ***This work is complete, and used both lactating ewes and lambs in a 6 month trial. The work did not show a satisfactorily large reduction in FEC between control and treatment groups. A significant production issue discovered in this work was the maximum possible inclusion rate of the product in the pelleting process, which at 20% was simply too low for this application. As a consequence, the larger of the two calf feeding projects was postponed pending resolution (see 1) above).***

3) These groups will also have fish silage mix intakes measured and matched with behavioural records of eating behaviour to assess how readily these livestock classes will consume fish silage.

- ***All stock preferred the fish silage enhanced products to conventional feeds – this issue did not materialise as predicted.***

Part C: Setting up farm systems for on farm practical use of silage – involves testing ration formulation, means of delivery.

1) Contemporary dairy and sheep production enterprises are highly industrialised, and to be effectively used in these systems fish silage must be able to be prepared and presented for consumption with some mechanisation. The options (pre-mix, point of feeding mix, and bolus dose) for meal feeding in the dairy bails and in the paddock (pre-mix, point of feeding mix and trough) will be assessed for practicality in typical large dairies and extensive sheep systems.

    - ***A long process of discussion with selected individual farmers and with Synlait farm operations was undertaken in this work. The three possible options, all currently available for use, are pellets, 'liquid aliquot' on dry supplement, and mixing with other supplements (eg. molasses). The preferred method for practicality and ease is liquid aliquot, and this method is marked for commercial trial this coming year.***

Results

The schedule for funding contained four clear "Stop:Go" deliverables that needed to be delivered at the end of year 1 of the project. These were:

1) **A demonstrated methanogenesis reduction of more than 33%**
2) **A demonstrated reduction of faecal egg counts in sheep or calves consuming the silage for >21 days of more than 50%.**
3) **More than 80% of stock consuming the feed (compound mixes of supplement + fish silage) selected as optimal for 1 and 2 within 2 weeks of introduction.**
4) **The demonstrated ability to feed the selected optimal compound mixes of supplement + fish silage to dairy cows, dairy calves and sheep using at least one form of mechanisation already in each of those industries, respectively.**

Methanogenesis

This work has two experimental components, the effect of the constituent fish oils on methane production measured by mRNA, and the effect measured by methane emissions directly via chamber assessment.

The use of pasture fed, ruminally fistulated dairy cows with and without fish oil treatment demonstrated these fish oils had an immediate and lasting effect to reduce the mRNA associated with rumen methanogenesis. This work established the diurnal cycle of methane production in the control (pasture only) fed cows, and demonstrated (Figure 1) a greater than four fold reduction in mRNA production associated with fish treatment at peak methanogenesis period (8h post prandial). This work did display a > 33% reduction in methanogenesis.

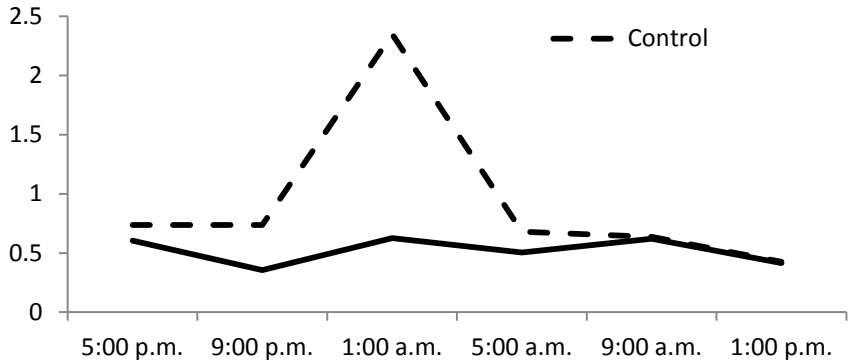

Figure 1 Mean mRNA quantification of key methane production enzyme from 5pm feeding for subsequent 16h period, with control and fish supplement treatments.

The second experimental work used six mature sheep fed conserved pasture, with and without fish supplement at the minimal level possible, and measured methane production directly using the emissions from a sealed methane assessment chamber. The mean methane emission was 17.9g/ 24h without fish supplement, and 13.8g/ 24h when the silage was used, representing a 23% reduction in methane emission. Removing as an outlier a single animal trial that was observed to have no difference between methane emissions with and without fish silage, the methane reduction was 32%.

Faecal Egg Count Reduction

There were two separate trials conducted to establish the efficacy of reducing FEC in sheep and cattle using fish silage supplement. A third trial with calves was postponed after the results of the sheep trial were available.

The cattle work used calves 6-9 months of age grazing pasture in a split group design where the animals grazed together and each day were fed either barley pellets (control group) or barley pellets with fish silage at 20% inclusion. The FEC was conducted from each animal at the trial conclusion, and demonstrated a reduction from a mean of 230 eggs per gram of faeces (epg) in the control group to 90 epg in the fish supplemented group, a 60% reduction.

The sheep work used twin bearing ewes grazing pasture in four groups, two fed barley pellets daily and two fed barley pellets with fish silage at 20% inclusion. The silage dose per ewe per day was sub-optimal, but could not be increased as there is an upper limit to barley intake in these animals, and the manufacturing process could not increase the inclusion rate using barley (see below). FEC were conducted every two weeks from before lambing to lamb weaning, and the lambs were also FEC tested after that until slaughter weights. There was no difference ($P>0.05$) between the treatment groups in FEC at any period in either lambs or ewes, and no difference ($P>0.05$) in liveweights between groups.

Stock Consumption of Fish Silage

Fish silage was used as a liquid supplement with forages, and as a pellet inclusion with barley, and it was demonstrated to be highly palatable, with effectively no animals refusing the feeds except for the initial 48h periods after introduction.

Commercial Feeding Approaches

The fish silage was demonstrated to be stable at ambient temperatures for the duration of every trial in which it was used, and was stable when used with barley pellets for greater than a month. This interval is well in excess of the period required for commercial use on farm.

The silage is able to be used as a liquid feed, sprayed on dry feed, or used in a pellet form. These are all suitable means to administer the silage to commercial stock.

There is a practical difficulty in raising the silage inclusion rate in barley pellets above 20%. This then reduces the amount of silage that can be dosed using barley pellets per day, as the over consumption of barley can lead to serious rumen problems. This was clearly the issue in the sheep FEC trial, where the silage intake per day was always sub-optimal due to this. As a consequence of this finding, the decision to use PKE rather than barley as a pellet substrate was made at the end of this year's trial work.

"Stop:Go" Summary Conclusions from the results of Year 1 trials

1) A demonstrated methanogenesis reduction of more than 33%
(achieved for cattle, acceptable at +/- 10% for sheep)

2) A demonstrated reduction of faecal egg counts in sheep or calves consuming the silage for >21 days of more than 50%.

(achieved for cattle, not achieved for sheep)

3) More than 80% of stock consuming the feed (compound mixes of supplement + fish silage) selected as optimal for 1 and 2 within 2 weeks of introduction.

(achieved for cattle and sheep)

4) The demonstrated ability to feed the selected optimal compound mixes of supplement + fish silage to dairy cows, dairy calves and sheep using at least one form of mechanisation already in each of those industries, respectively.

(achieved for cattle and sheep)

Research Project Leader Conclusions and Recommendation

The first year of this project has demonstrated that the current United Fisheries fish silage product can readily be used in all livestock classes of cattle and sheep. It was demonstrated to be palatable both 'raw' as a liquid, and in supplement mixes.

The first experiments of the efficacy of this product in reducing methane production in cattle and sheep demonstrate that a significant reduction is obtained, very close to the 33% reduction used as a measure of success in the application for Seafood Innovation Ltd funding. This large a reduction is far in excess of equivalent mitigation strategies at present, and further work using variations in livestock classes, intakes and pasture diets is required to validate this. However, the preliminary results are encouraging, and suggests the work should be continued.

The use of fish silage as a barley pelleted mix to reduce FEC in sheep was unsuccessful, despite the literature in this field amply supporting the use of fish proteins for this purpose both here and internationally. The amount of protein able to be supplied with this barley pellet was too low, and this has identified a significant issue with the use of this product in pellets. In NZ, the current pelletising processes use cereal grains, and as there is an upper limit to the inclusion rate of a low DM product like fish silage in cereal pellets, there is then a difficulty in raising the intake amounts of a cereal pellet to increase the silage intake for fear of rumen dysfunction by cereal overload. This particularly acute in sheep, rather than cattle. This necessitates a shift to PKE pellets, in which neither problem is an issue, but requires some development to do so. This work will be undertaken in the first part of the year 2 project schedule if approved. It should be also noted that there would be significant cost savings with the use of PKE.

Although the use of fish silage was observed to reduce the FEC in calves, the clear impediment of sub-optimal delivery of a daily dose observed in sheep necessitated a postponement of the larger calf trial planned. The reason for this is that a shift to PKE pellet use would need to be validated in calves on a commercial scale, and broader use of the product in calves would also likely demonstrate sub-optimal protein supply when using barley. However, it should be noted that there is no reason in the literature or in this trial work to date to expect that the supply of fish protein via this silage product would not be effective in FEC reduction.

Finally, the palatability and ease of delivery of the fish silage to stock via multiple formulations were found to be, respectively, high and straightforward. There is no known or expected impediment to the commercial and widespread use of this product.