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## Plenary 1: Vitamin D; The Great Debate

### How much vitamin D do we need?

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#### Summary

How vitamin D works, what effects it has on health, and how much we need are questions that originated in the early days of nutrition research and have a long history of investigation. Though current understanding is far from complete, we know that vitamin D plays a vital role in maintaining calcium balance and promoting bone health.

In recent years, there has been a resurgence of interest in and excitement about the 'sunshine' vitamin. Several reasons account for this; firstly, evidence shows that vitamin D exerts a range of metabolic effects unrelated to bone health; secondly, there is a growing body of epidemiological evidence – albeit inconclusive – suggesting that lower vitamin D status, at levels not conventionally interpreted as deficient, is associated with increased risk of chronic diseases such as cancer, cardiovascular disease, and diabetes; finally, the results from a number of well-designed, population-based surveys have been interpreted as showing a high prevalence of low vitamin D status. This final point is problematic. All agree that circulating 25-hydroxyvitamin D concentration should be used to assess vitamin D status. Unfortunately, the quality and consistency of the evidence to define cutpoints for serum 25-hydroxyvitamin D has made it difficult to establish with confidence the relevant reference ranges; experts have suggested cutpoints for adequacy and insufficiency between 50 and 80 nmol/L, some argue that optimal serum concentrations should be above 100 nmol/L.

To maintain year round, in every individual, a vitamin D status above the suggested cutpoint for adequacy – even the most conservative one – will require an enormous increase in the population's mean serum 25-hydroxyvitamin D concentration; an increase of 30 to 40 nmol/L may be needed. The next two speakers will debate the ability and relative merits of sun exposure or diet as strategies for improving vitamin D status. The ultimate debate about the benefit of these strategies for population health may have to remain for another day.



## Plenary 1: Vitamin D; The Great Debate

### Vitamin D – the light side of sunshine

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Vitamin D is not strictly a vitamin, since it is produced in skin by the action of ultraviolet radiation (UVR) in the UVB range (290 – 315nm) on 7-dehydrocholesterol, which is present in skin. Absorption of UVB by 7-dehydrocholesterol opens up the B-ring of this molecule, converting it to pre-vitamin D, which at body temperature, thermally rearranges to the more stable vitamin D. The process takes several hours. It is not possible to produce too much vitamin D from sun exposure, since continued irradiation causes breakdown of both pre-vitamin D and vitamin D to “overirradiation products”, which have little effect on mineral metabolism, so short exposures may be more efficient at producing vitamin D. How much sun exposure is considered adequate is difficult to ascertain, as the data is limited. There is some evidence that the standard advice to obtain one-third of a minimal erythemal dose (an MED is the dose of UVR required to just produce faint redness) to 15-18% body surface (face, hands and arms – or equivalent), most days, results in an increase in vitamin D status approximately equivalent to 1000 IU (25 mcg) per day. Vitamin D status is measured as the concentration of the major circulating metabolite, 25-hydroxyvitamin D (25OHD). Even so, the sun exposure required for 1/3 of an MED depends on many factors, including latitude, altitude, season, time of day, clothing and skin type, which makes the message complex and difficult for the public to understand, although attempts at simplification are underway.

Vitamin D is present in some foods, including oily fish, (more if not farmed), wild mushrooms and fortified foods, including milk, though there seem to be undue restrictions on the degree of fortification, particularly for milk products. The amounts available in most diets have been estimated to provide less than 10% of that required to maintain adequate vitamin D status. What is adequate vitamin D status is also a topic of considerable debate, though it is generally agreed that a 25OHD concentration of at least 50-60 nmol/L should be a target for optimal bone and muscle function, for which we have the best data. There is considerable support for targets of 75-80 nmol/L and even higher to achieve optimal health outcomes – especially in relation to non-classical effects on cancer, cardiovascular and metabolic diseases and immune function, for which there is very limited controlled trial data available, on which to make recommendations. Virtually all the trial data for all outcomes have been obtained using supplements.

For the general population, there is an argument to promote short periods of sun exposure to uncovered skin

outdoors, as part of a balanced lifestyle. An immediate concern is that Australia has the highest rates of skin cancer in the world and promotion of sun exposure, however brief, could be seen to run counter to the effective “slip, slop, slap” campaign. Furthermore, there is no getting away from the fact that it is the same energetic UVB rays which produce vitamin D, that also damage DNA. There is some evidence, however, that skin adaptation occurs with short sun exposures, so that DNA repair mechanisms, for example, are upregulated. There is also some evidence that the active hormone, 1,25-dihydroxyvitamin D and other vitamin D compounds provide protection from UV-induced DNA damage. So because vitamin D is synthesized in skin as a result of UVB irradiation and converted locally to the active hormone and other compounds, there may be less DNA damage than would otherwise be the case.

Largely unknown and ignored are other factors which contribute to maintenance of adequate vitamin D status. The storage site for vitamin D itself is likely to be fat, but it is unlikely that fat will release vitamin D unless the fat stores themselves are broken down. Little is known about storage of 25OHD, but exercise is known to be associated with better 25OHD status. This has been assumed to be due to the location of the exercise outdoors, but this cannot explain all the data. Nevertheless, the general message of getting adequate exercise, at least in part outdoors, could help improve vitamin D levels. The half-life of 25OHD varies between 15 and 50 days in people, but the reasons are not clear. Adequate calcium intakes have been shown to reduce vitamin D catabolism and increased calcium intake has been reported to improve vitamin D status in many studies.

There are three main groups of people known to be at risk for significant vitamin D deficiency. These are older people, particularly in aged care, dark-skinned people, especially if they wear modest dress for religious or cultural reasons and those who are chronically ill. Access to sunny spaces is often limited for older individuals and those who are chronically ill, particularly if they are less mobile. In dark-skinned people, the melanin absorbs UV, as does clothing, meaning that the longer periods of bare skin exposure required for them to make adequate vitamin D are impractical. Sun exposure is also a major problem for people who are immunosuppressed, since their risk of skin cancer is up to 80 times higher than that of the general population. So while the message of “get out, get active, clear your head and get a bit of sunshine” might be, on balance, reasonable for most of the population, as an alternative to mass medication, there remain substantial groups of people for whom this message is unhelpful.

#### Source of funding

National Health and Medical Research Council, NSW Cancer Council, Cancer Institute of NSW.



## Plenary 1: Vitamin D; The Great Debate

### The public health benefits of oral supplementation of vitamin D

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**Abstract not received at time of going to print**



## Concurrent Session 1: Omega-3

### PUFA status and response to n-3 PUFA treatment in children with ADHD: role of phospholipase A2

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#### Background

Phospholipase A2 (PLA2) enables functions of polyunsaturated fatty acids (PUFA) by releasing them from membrane stores. Thus abnormalities in PLA2 activity could account for altered PUFA status in ADHD and variation in the response to omega-3 (n-3) PUFA.

#### Objective

To investigate associations between PLA2 activity and erythrocyte PUFA status and whether PLA2 activity modulates responses to n-3 PUFA supplementation in children with ADHD.

#### Design

Ninety children with ADHD symptoms were recruited for a double-blind, placebo-controlled trial and randomised to take 1000 mg EPA + 120 mg DHA/day, 240 mg EPA + 1000 mg DHA/day or n-6 PUFA (safflower oil) for 4 months. Erythrocyte PUFA status, assessments of attention, cognition and literacy and Conner's Parent Rating Scales were evaluated at baseline and 4 months; activity of PLA2 in plasma was assessed at baseline.

#### Outcomes

Seventy-five children at baseline and 44 children at 4 months provided blood samples for assessment of erythrocyte PUFA and PLA2 activity. Higher PLA2 activity was associated with lower erythrocyte EPA status at baseline ( $P=0.017$   $r=-0.28$ ). However, baseline PLA2 activity did not influence change in PUFA status over the 4 months. Including PLA2 activity in regression analyses strengthened relationships between changes in PUFA status and parent-reported restless/impulsiveness and ADHD symptoms over 4 months: lower PLA2 activity was associated with greater improvement. PLA2 activity was not a significant moderator of any other relationships between change in PUFA status and change in cognitive/behavioural outcomes.

#### Conclusion

PLA2 may modulate responses to PUFA supplementation, whereby lower activity is associated with greater improvements in behaviour and cognition. This is a preliminary finding and the influence of PLA2 activity should be further assessed in ADHD and other mental health problems.

#### Source of funding

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### Long chain omega-3 fatty acids suppress adipose tissue inflammatory pathways

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#### Background

Diets rich in long chain omega-3 fatty acids are potent regulators of inflammatory responses. Adipose tissue is a significant source of pro-inflammatory mediators, yet few studies have investigated the actions of long chain omega-3's (EPA, DPA, DHA) compared with shorter chain omega-3's (ALA) on inflammatory mediators in adipocytes.

#### Objective

Therefore the current study analysed the intracellular stress and cytokines generated in adipose tissue samples in rodents fed diets either composed of safflower oil (devoid of omega 3's), flaxseed oil (with little or no long chain omega 3's), versus diets with added fish oil.

#### Design

40 male Sprague-Dawley rats were fed one of four diets containing the following lipid sources, as 10% of energy [canola oil; safflower: safflower and flaxseed oil: safflower and flaxseed oil and tuna oil (0.1%)] for 8 weeks. Subcutaneous adipose tissue was harvested and analysed for pro-inflammatory adipokine gene expression, including monocyte chemoattractant protein 1 (MCP1), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF $\alpha$ ). Stress and inflammatory related signalling, including the MAPK pathway member (ERK1/2), nuclear factor kappa B members (IkkB), toll like receptor 4 (TLR4) and cytokine signalling (suppressor of cytokine signalling 3, SOCS3) was also measured.

#### Outcomes

The diet deficient in omega 3's (safflower oil) resulted in a marked activation of the gene expression for MCP1, IL-6 and TNF $\alpha$ . The addition of shorter chain omega-3's (flaxseed oil) halved the expression of these genes ( $p>0.01$ ), however the addition of tuna oil further reduced these pro-inflammatory genes by a further half again ( $p>0.01$ ). Consistent with this ERK activation and IkkB concentrations were lowest in the tuna oil supplemented group and highest in the omega-3 deficient diet. Unexpectedly, TLR4 protein levels were increased by the addition of tuna oil to the diet.

#### Conclusion

Diets containing short-chain omega-3 oils reduce inflammatory signalling and cytokine gene expression relative to diets deficient in omega 3's. However, with the addition of longer-chain omega-3's, inflammatory signaling and cytokine gene expression were further attenuated.

#### Source of funding

Molecular and Medical Research Strategic Research Centre, Deakin University



## Concurrent Session 1: Omega-3

### Investigating the metabolic fate of <sup>14</sup>C-DPA (22:5n-3) in rats

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#### Background

A recently published study has shown that, in rats, DPA supplementation led to deposition of DPA in various tissues. Part of the supplemented DPA was retro-converted to EPA with minimum conversion into DHA. However, it is not known what proportion of ingested DPA is  $\beta$ -oxidised to form CO<sub>2</sub> compared with deposition of DPA in various tissues.

#### Objective

The aim of the current study was to determine the extent to which the <sup>14</sup>C-DPA was catabolised to CO<sub>2</sub> compared with EPA and DHA and to examine the incorporation of label into the various tissue lipids.

#### Design

Twenty 3-wk-old male weanling Wistar rats were administered a single oral dose of 2.5 $\mu$ Ci of 1-<sup>14</sup>C-DPA or 1-<sup>14</sup>C-EPA or 1-<sup>14</sup>C-DHA or 1-<sup>14</sup>C-oleic acid (OA). After dosing, the animals were immediately placed in a metabolism chamber for the next 6 hours. The exhaled <sup>14</sup>CO<sub>2</sub> was bubbled into a trapping solution and counted for radioactivity. 24 hours after dosing the animals were euthanized and tissues were removed. Tissue lipids were extracted and counted for radioactivity. Thin layer chromatography was performed to separate the lipid fractions, which were then counted for radioactivity.

#### Outcomes

It was observed that OA led to significantly greater label ( $P < 0.05$ ,  $n = 5$  per group) in the collected CO<sub>2</sub> after 6 hrs compared with EPA (by 1.4 fold) DPA (by 3.6 fold) & DHA (by 4.1 fold). The amount of <sup>14</sup>CO<sub>2</sub> collected for EPA was significantly higher than that of DPA and DHA by 2.5 and 2.8 fold, respectively. The results from analysis of tissue lipids showed that DPA was highly incorporated in heart (153 picomoles/g tissue) compared with OA (10 picomoles/g tissue). EPA and DHA were highly incorporated in liver (190 and 158 picomoles/g tissue, respectively), compared with OA (5 picomoles/g tissue). All the three n-3 LCP including DPA showed an approximately 3 fold higher incorporation into the phospholipid fraction compared with OA in all tissues.

#### Conclusion

In conclusion, this study showed that DPA is more conserved from  $\beta$ -oxidation compared with OA and EPA. It is mainly deposited in tissues like adipose, heart and liver. Higher incorporation of DPA in heart and in PL fraction, might suggest that DPA has a beneficial role in heart tissue. Further analysis of conversion of DPA into EPA or DHA in these animals is currently underway.

#### Source of funding

Meat and Livestock Australia

### Platelet-derived microparticle activity is inhibited by omega-3 fatty acid supplementation

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#### Background

Platelet-derived microparticles have known procoagulant activity and are produced upon platelet activation or physical stimulation. Platelet aggregation also occurs upon activation and is the crucial step leading to a thrombotic event. Supplementation with omega-3 polyunsaturated fatty acids (eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) has been shown to reduce platelet aggregation; however their effects on microparticle activity have not been previously reported.

#### Objective

To examine the effects of dietary supplementation with a single dose of EPA or DHA rich oils on microparticle activity and platelet aggregation (ex-vivo) in male and female subjects over 24 hours.

#### Design

A placebo controlled trial was conducted in healthy males and females ( $n = 90$ ). Microparticle activity and platelet aggregation was measured at 0 and 24 hours post supplementation with either a placebo or EPA or DHA rich oil.

#### Outcomes

EPA and DHA effectively reduced platelet aggregation at 24 hours post supplementation relative to placebo (-13.3%,  $P = 0.006$  and -11.9%,  $P = 0.016$  respectively). Microparticle activity was reduced following EPA (-22.6%,  $P = 0.002$ ) only. When grouped by gender, males showed a reduction in microparticle activity (-22%,  $P = 0.018$ ) following EPA, but not DHA compared with placebo. In females, DHA significantly reduced platelet aggregation (-13.7%,  $P = 0.04$ ), while EPA was not effective. Microparticle activity was not reduced following DHA supplementation.

#### Conclusion

The results demonstrate for the first time that EPA but not DHA reduce microparticle activity and platelet aggregation in males. In contrast, in females, DHA but not EPA reduce platelet aggregation without any change in microparticle activity. The differential effects of EPA and DHA on platelet aggregation and the observed gender-based differences in microparticle activity merits further investigation.

#### Source of funding

Not applicable



## Concurrent Session 1: Omega-3

### Dietary omega-3 PUFA prevent stress induced memory impairment in the rat

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#### Background

Anxiety and depression can result in memory impairment, including reductions in visuospatial memory performance. Dietary omega-3 polyunsaturated fatty acids (PUFA) have been demonstrated to have beneficial effects relating to both mood disorders and memory performance.

#### Objective

This study examined the effect of dietary omega-3 PUFA on a novel model of stress induced memory impairment and learned helplessness in the rat.

#### Design

72 male Sprague Dawley rats were placed on one of two semi-synthetic experimental diets six weeks prior to behavioural testing. Diets were either deficient in (DEF; 7% safflower oil) or supplemented with (SUP; 5.5% safflower oil, 1% flaxseed oil, 0.5% tuna oil) omega-3 PUFA. Groups underwent 15-minute forced-swim-stress (F1) or no swim-stress (C1). The following day animals were either tested in a Y-maze (Y2), to assess visuospatial memory performance, or for a second time (5-minutes) in the forced-swim apparatus (F2), to assess learned helplessness/depressive behaviour. Therefore, in total there were 6 groups SUP-F1/F2, DEF-F1/F2, SUP-F1/Y2, DEF-F1/Y2, SUP-C1/Y2 and DEF-C1/Y2 (n=12/grp).

#### Outcomes

Stress reduced memory performance in the Y maze (DEF-F1/Y2), when compared with the unstressed group (DEF-C1/Y2), for animals on a DEF diet. The SUP diet completely prevented the memory impairments associated with stress. Also, SUP animals (SUP-F1/F2) spent less time immobile in the forced swim test than the DEF group (DEF-F1/F2).

#### Conclusion

Similar to previous reports, our results demonstrate that omega-3 PUFA reduce learned helplessness/depressive behaviours in the rat. In addition, the results show that dietary omega-3 PUFA can prevent stress-induced memory impairment.

**Source of funding** LTU Internal Grants Scheme

### Dietary intake of omega-3 long chain EPA, DPA and DHA of Australian children

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#### Background

Sufficient dietary intake of omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) is necessary for supporting normal physiological functions, brain and cognitive development, and also modifying some disease states.

#### Objective

To examine the intake contribution of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) and its food sources to the dietary n-3 LC-PUFA intake of Australian children.

#### Design

Data files were acquired from the Australian Social Science Data Archive for 4487 children aged 2-16 years in the Kids Eat Kids Play 2007 survey and the median and inter quartile range (IQR) of the dietary EPA, DPA and DHA intake was determined for each age group. The amount of fish and seafood, n-3 enriched foods and meat, egg and their contribution to EPA, DPA and DHA intakes was also determined.

#### Outcomes

The median (IQR) EPA, DPA, and DHA intake (mg/d) for 2-3 y, 4-8 y, 9-13 y, 14-16 y were: EPA 15 (8-27), 18 (9-33), 24 (12-42), 28 (11-52) respectively; DPA 19 (11-32), 23 (13-40), 29 (16-54), 35 (18-65) respectively; and DHA 9 (3-34), 12 (4-39), 16 (6-44), 19 (7-48) respectively. Fish and seafood (mean±SD =12±33 g/d) was the main source of DHA, providing a mean±SD of 35.6±118.6 mg/d, but only 20% of total children consumed fish/seafood. Fish-eaters consume 12 fold higher intake of DHA adjusted energy (24.6±27.3 mg/d) than non-fish consumers (2.1±2.7 mg/d). Intake of EPA, DHA and DPA were increase by age ( $p<0.05$ ). The meat and egg (102±90 g/d) food groups were the largest contributors for EPA (20.3±20.3 mg/d) and DPA (32±32.4 mg/d). Omega-3 enriched foods provided minor contributions to intakes of EPA, DPA and DHA (0.5±2.8, 0.1±0.3 and 1.2±6.0 mg/d respectively).

#### Conclusion

Although fish and seafood was the largest contributor to DHA, only 20% children consumed fish/seafood. Children consumed 8.5 times more meat than fish and seafood, which explained meat being the largest contributor to DPA and EPA intakes.

#### Source of funding

Directorate General Higher Education Indonesia.



## Concurrent Session 2: Domesticated Animal Production

Sponsored by Specialty Feeds

### Transforming salmonid aquaculture from a consumer to a producer of long chain omega-3 fatty acids

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#### Background

Dietary intake of omega-3 fatty acids (n-3 LC-PUFA) should increase for promotion of public health, however this conflicts with the sustainability of wild fish stock, the primary dietary source of n-3 LC-PUFA.

#### Objective

The objective of this study was to evaluate the role of salmonid aquaculture as a supplier of n-3 LC-PUFA as a means of understanding the potential of the sector in conserving wild fisheries.

#### Design

A case-study feeding trial was implemented on rainbow trout up to commercial size, in which fish were fed a fish oil or a linseed oil diet. Harvested fish were analysed for fatty acid composition and consumer acceptance. The n-3 LC-PUFA input / n-3 LC-PUFA output ratio was computed.

#### Outcomes

The fatty acids of the fillets were significantly modified by the diets. On the input side, for the production of 100 grams of fish fillet, it was necessary to use 8640 mg of n-3 LC-PUFA to produce an output of 1857 mg of n-3 LC-PUFA in the fish oil- fed fish; in contrast it was only necessary to use 270 mg of n-3 LC-PUFA to produce 560 mg of these fatty acids in the linseed oil- fed fish. Consumers showed no preference, but were able to distinguish between samples.

#### Conclusion

It was shown that the substitution of fish oil with linseed oil in aquafeed is an easily implemented tool to transform salmonid farming from a consumer into a net producer of health promoting n-3 LC-PUFA and accomplish its role in conserving wild fisheries into the future.

#### Source of funding

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### Use of algae in sheep feeding systems

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#### Background

The major issues concerning consumers and food manufacturers are food safety, environmental impact of production and distribution, animal welfare and human health. The use of plant algae as a livestock feed supplement is one option to maintain or improve productivity and quality of meat while potentially reducing methane emissions.

#### Objective

To investigate the effect of algae as a feed supplement for lambs on yield and quality of meat.

#### Design

One hundred and twenty lambs were randomly assigned to twelve groups, based on liveweight and allocated to four dietary treatments. Lambs were fed a basal diet (annual ryegrass hay and clover hay = Trt1); basal diet with flaxseed (Trt2); basal diet with algae (Trt3); basal diet with flaxseed + algae (Trt4). The experiment was conducted in two runs with the six heaviest groups allocated to run 1 and the rest allocated to run 2, to account for the difference in weight gain over time. The design was a full factorial with runs used as a blocking factor. Lambs were maintained in group pens and fed *ad libitum* for eight weeks. The crude protein content of the rations offered was 10.6-11.6%.

#### Outcomes

Lambs fed the basal and algae diets had similar carcass yield and fatness. Carcass yield and fatness were greater in lambs fed flaxseed alone or with algae compared with other groups. Carcass yield and fatness values for Trt1, Trt2, Trt3 & Trt4 were 19.7, 22.5, 20.2 & 21.1 kg ( $P < 0.005$ ; SED 0.31) and 10.4, 15.3, 11.7 & 13.1 mm ( $P < 0.03$ ; SED 0.84), respectively. Ultimate pH (5.62-5.67) and redness ( $a^*$ -value; 15.0-15.2) of fresh meat were within the range for quality meat. Preliminary intake of feed on an air-dry basis show that lambs fed algae supplement consumed less than the other groups (1.28 vs 1.48 kg, raw data).

#### Conclusion

Increase in hot carcass weight with flaxseed may be due to an increase in fatness. With lower intake, the performance of lambs fed the algae diet compared with the basal diet indicate that algae fed lambs may have better feed efficiency, which needs further investigation.

#### Source of funding

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## Concurrent Session 2: Domesticated Animal Production

Sponsored by Specialty Feeds

### Effects of dietary $\alpha$ -linolenic acid (18:3n-3) / linoleic acid (18:2n-6) ratio on growth performance, fillet fatty acid profile and finishing efficiency in Murray cod

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#### Background

Due to environmental and economical concerns, the substitution of dietary fish oil with readily available and sustainable terrestrial alternative oils is a key issue for the aquaculture feed industry. Thus, knowledge of fatty acid metabolism of farmed fish is paramount.

#### Objective

The objective of the present study was to evaluate the effect of different dietary  $\alpha$ -linolenic acid (ALA, 18:3n-3), to linoleic acid (LA, 18:2n-6) ratios, while employing 100% fish oil deprived diets on growth performance and flesh fatty acid composition of Murray cod and subsequent finishing (wash-out) efficiency.

#### Design

An experimental feeding trial was implemented on Murray cod in which the ALA/LA ratios of the five experimental diets were gradually increased from 0.3 to 2.9, with a constant total content of ALA+LA of 60% for the grow-out period. Fish oil was used for the sixth (control) diet, which was also used during the subsequent finishing period.

#### Outcomes

It was shown that the dietary ALA/LA ratio significantly impacts on final fatty acid make-up and nutritional quality of fish fillet. In particular, fillets of fish fed with a higher ALA/LA ratio recorded a significantly higher content of EPA and DHA (eicosapentaenoic acid, 20:5n-3, and docosahexaenoic acid, 22:6n-3, respectively). The deposition of EPA and DHA during the finishing period was affected by previous feeding history, with fish previously fed high amounts of LA depositing significantly lower amounts of these fatty acids in comparison to fish previously fed a diet rich in ALA.

#### Conclusion

The results of the present study suggest that high dietary LA contents may have significant negative impacts on the efficiency of a finishing strategy, and in general, that alternative oils rich in LA should be used cautiously when replacing fish oil.

#### Source of funding

This research was supported by the Australian Research Council's Discovery Projects funding scheme (project DP0772271). The views expressed herein are those of the authors and are not necessarily those of the Australian Research Council.

### Reducing inflammation in sheep by feeding silage high in omega-3

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#### Background

Omega-3 fatty acids are associated with reduced inflammation in several disease models. Decreased inflammation *in utero* during conception may improve embryo survival and, therefore, the reproductive performance of sheep.

#### Objective

To determine whether improving the omega-3 status of sheep can reduce markers of inflammation.

#### Design

A parallel-group controlled trial was conducted with 30 Merino x Border Leicester ewes. Ewes were fed a diet containing an oat/pea silage (n=16) or oats/cottonseed meal (n=14) for 52 days. Concentrations of fatty acids were determined in plasma and red blood cells (RBC) prior to and, 14, 28, 43 and 52 days after, the introduction of experimental rations. Effects of experimental rations on the prostaglandin response to an oxytocin challenge were determined by the analysis of the prostaglandin metabolite 13,14-dihydro-15-keto PGF<sub>2 $\alpha$</sub>  (PGFM) in plasma. The time to oestrus following oestrous synchronization was also determined during natural mating with rams.

#### Outcomes

Concentrations of omega-3 fatty acids were significantly (P<0.05) higher in plasma and RBC of ewes fed silage compared with oat grain. The prostaglandin response to oxytocin challenge was lower in ewes fed silage compared with grain. The lower prostaglandin response was associated with a longer time to oestrus (P=0.07) in ewes fed silage.

#### Conclusion

Increased plasma concentrations of omega-3 fatty acids were associated with a reduced prostaglandin response to oxytocin and a longer time to oestrus. Further work will examine the relationship between inflammatory responses and other reproductive traits including embryo survival and duration of gestation.

#### Source of funding

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## Concurrent Session 2: Domesticated Animal Production

Sponsored by Specialty Feeds

### Energy utilisation of broiler chicks on starter diets containing cassava pulp and microbial enzymes

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#### Background

Cassava pulp (CSP) is a low-protein, high-fibre by-product of the starch industry. There is a potential for this ingredient in poultry diets if the nutritive value can be improved.

#### Objective

To assess the energy utilisation of broiler chicks on diets containing CSP, when supplemented with microbial enzymes.

#### Design

Eighty-four day-old chicks were assigned to six treatments, each replicated six times, 12 chicks to a replicate. The birds were fed on diets in which CSP replaced maize by 0, 10 or 15 %, with or without microbial enzymes (Avizyme 1502/Phyzyme 10000, Danisco Animal Nutrition, UK). The diets were fed over the starter phase (0-10 days), followed by sample collection. The AME content of the diets was determined and the comparative slaughter technique was used to assess the energy value and utilisation of diets containing CSP. These data were used to derive values of net energy of production (NEp), heat production (HP), and efficiencies of utilisation of protein and fat for energy production. Data were subjected to multiple regression analysis, using Minitab.

#### Outcomes

The dietary AME content varied from 11.1 to 11.8 MJ; ME intake declined ( $P<0.01$ ) with an increase in CSP content but was improved ( $P<0.05$ ) by enzyme supplementation. Fat intake was reduced ( $P<0.01$ ) while protein intake was increased ( $P<0.01$ ) with rising levels of CSP but both were increased ( $P<0.01$ ) on the enzyme-supplemented diets. The NEp declined with CSP level but this was significant ( $P<0.01$ ) only at the highest level of inclusion. Heat production on the CSP-supplemented diets was lower than on the maize control diet but this was not significantly different. The efficiency of utilisation of ME for energy and protein retention was increased by CSP but only up to the lowest level of inclusion; enzyme supplementation also marginally increased this efficiency.

#### Conclusion

Cassava pulp generally reduced the dietary AME, NEp and HP but did not adversely affect the efficiency of energy utilisation. Microbial enzyme supplementation improved these variables, some of them significantly.

#### Source of funding

This research was supported by funding from Danisco Animal Nutrition and the University of New England.

### Effect of substituting dietary fish oil with linseed oil and sunflower oil in trout growth and hormones (leptin, LepR, ghrelin and GHSR) expression after six month feeding period

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#### Background

After protein, lipid is a major energy source for fish. Fish oil is rich in omega-3 and is the best lipid source for fish nutrition. However, the critical status of fish oil supply and high market demand has caused a trend to substituting fish oil with an alternative oil source. Plant oil has been favoured, but, the fatty acid profile and its effect on fish growth has been an issue. Thus, it is important to understand physiological systems controlling energy homeostasis in fish and how they respond to different diets. Hormones such as leptin, ghrelin are critical regulators of food intake and energy balance, and their gene expression is affected by the lipid profile.

#### Objective

The objective of this study was to examine the expression of leptin, ghrelin and their receptors in rainbow trout that were maintained with different dietary lipid sources.

#### Design

A six month feeding trial was conducted. Fish were maintained with isolipidic, isocaloric and isonitrogenic diets. Three dietary lipid sources were used: fish oil, sunflower oil and linseed oil. The fish were analysed for the expression of the gene of interest using Real Time PCR.

#### Outcomes

In general, the ghrelin and LepR were highly expressed compared to GHSR and leptin. Leptin expression was not significantly affected by the type of dietary lipid. However, fish fed linseed oil had significantly higher expression of leptin receptor (LepR), ghrelin and growth hormone secretagogue receptor (GHSR); fish oil and sunflower oil had no effect on the expression of these genes.

#### Conclusion

The data show that the appetite control system of trout is responsive to linseed oil compared to fish and sunflower oil. However, the change in expression appears not be linked to the growth performance of the fish as body weight was similar for each of the dietary regimes and the end of the feeding trial.

#### Source of funding

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## Concurrent Session 3: Glycaemic Index/Load

### Validity of dietary glycaemic load as a predictor of postprandial glycaemia and insulinaemia in lean, young healthy adults

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#### Background

The concept of dietary glycaemic load (GL, defined as the mathematical product of the glycaemic index (GI) and carbohydrate content of a serving of food) is increasingly used in nutritional epidemiology. Its ability to predict postprandial glycaemia and insulinaemia for a wide range of foods or mixed meals is unclear.

#### Objective

To determine the degree of association between calculated GL and glucose and insulin responses in healthy subjects consuming iso-energetic portions of single foods and meals.

#### Design

In study one, groups of healthy subjects consumed 1000 kJ portions of 121 single foods in 10 food categories. In study two, healthy volunteers consumed 2000 kJ of 13 mixed meals. Foods and meals varied widely in macronutrient content, fibre and GL. Glycaemia and insulinaemia were quantified as incremental area under the curve relative to a reference food (=100).

#### Outcomes

GL was the strongest predictor of the observed glucose and insulin responses induced by single foods ( $r = 0.92$  and  $0.77$  respectively, both  $P < 0.001$ ), accounting for 84% and 59% of the variation in glucose and insulin responses respectively. For mixed meals, responses varied over a five-fold range and were strongly correlated with GL ( $r = 0.76$ ,  $P = 0.002$  for glucose and  $r = 0.68$ ,  $P = 0.01$  for insulin). In contrast, macronutrients and fibre were not significant predictors of either response.

#### Conclusion

The findings provide robust support for the physiological validity of GL in predicting both postprandial glycaemia and insulin demand to a wide variety of foods and meals.

#### Source of funding

Research relating to this abstract was funded by internal revenue of the University of Sydney and Harvard School of Public Health.

### Glycaemic and insulinaemic responses to commercially available infant formulas

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#### Background

Commercially available infant formulas include various sources of carbohydrate. High glycaemic index (GI) carbohydrates such as maltodextrins may influence metabolic programming and genetic imprinting in the first year of life, increasing the lifetime risk of type 2 diabetes mellitus and obesity.

#### Objective

The aim of this study was to determine glycaemic and insulinaemic responses to 11 popular infant formulas.

#### Design

Ten healthy subjects consumed 25 g carbohydrate portions of each formula and the reference food (25 g glucose on three occasions) in random order. Fingerprick blood samples were taken at regular intervals over 2 h, and the GI determined according to the Australian Standard. Insulin responses were also assessed.

#### Outcomes

The GI (mean  $\pm$  SEM) ranged from as low as  $17 \pm 3$  to as high as  $71 \pm 7$ . Four of the 11 formulas had a GI greater than 57. Insulin responses also varied over a large range.

#### Conclusion

These findings show that despite apparent similarities in macronutrient composition, infant formulas vary markedly in GI and insulin demand. Differences in carbohydrate source during early life should be considered an additional risk factor for child obesity.

#### Source of funding

Sydney University Glycaemic Index Research Service



## Concurrent Session 3: Glycaemic Index/Load

### Dietary glycaemic index and nutrient adequacy amongst children and adolescents – is there a link?

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#### Background

There has been no investigation into the association between carbohydrate (CHO) intake from foods with different glycaemic indices (GI) and nutrient adequacy amongst children and adolescents.

#### Objective

To investigate the association between low / high GI CHO intake and the risk of not meeting the Australian Nutrient Reference Values (NRVs).

#### Design

Children aged 2 to 16 years who provided two 24-hour recalls in the 2007 Australian National Children Nutrition and Physical Activity Survey were included. Under- and over-reporters were excluded based on the Goldberg cut-off method. A final dataset of 4,184 participants were analysed. CHO from foods with a GI < 52 (median GI of the study population) were classified as low GI. Age, sex, BMI z-score and energy adjusted odds ratios (OR) of not meeting the Australian NRVs were calculated by binary logistic regression.

#### Outcomes

Subjects with higher intakes of high GI CHO were found to be at risk of not meet the NRVs for a wide range of nutrients, eg calcium, iodine and vitamin A (all  $P_{\text{trend}} < 0.001$ ). Compared to subjects in the lowest quartile of high GI CHO intake, those in the highest quartile had more than triple (adjusted OR = 3.36;  $P < 0.001$ ) the risk of not meeting the EAR of calcium, and that for iodine and vitamin A were increased by more than five times (adjusted OR = 5.97 and 5.23 respectively; both  $P < 0.001$ ). On the other hand, subjects with higher intakes of low GI CHO were less likely to meet the adequate intake (AI) of linoleic acid (LA),  $\alpha$ -linolenic acid (ALA) and long chain polyunsaturated fatty acids (LCPUFA) (all  $P_{\text{trend}} < 0.001$ ), despite having lower risks of not meeting the NRVs of most other nutrients.

#### Conclusion

Australian children and adolescents who consumed more low GI CHO and less high GI CHO were more likely to meet the Australian NRVs, ie achieving a diet with better nutritional quality. The results of the present study provide further support for the health benefits of a low GI diet, but further research needs to be conducted into the potential impact on fatty acid intake.

#### Source of funding

Not applicable

### The Australian Paradox: a substantial decline in refined sugars intake over the same timeframe that obesity has increased

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#### Background

The role of sugary foods in the development of obesity remains controversial. Paradoxically, apparent consumption statistics suggest that Australians are eating fewer sugary foods, yet the prevalence of obesity among children and adults has tripled in the same timeframe.

#### Objective

To systematically review and critically evaluate recent literature and statistics on consumption of refined sugars and beverages in Australia.

#### Design

Data on annual apparent consumption of sugar in Australia, the UK and USA were obtained from the Food and Agriculture Organization of the United Nations for the years 1980 to 2003. A systematic literature review was undertaken to document Australian sugar intake as determined by dietary records since 1980. Trends in volume sales of sweetened beverages were sought from the Australian Beverage Industry Council. Government reports on obesity prevalence and population dietary sugar intakes were utilised.

#### Outcomes

In Australia, the UK and USA, per capita consumption of refined sucrose decreased by 23%, 10% and 20% respectively from 1980 to 2003. When all sources of nutritive sweeteners, including high fructose corn syrups, were considered, per capita consumption decreased in Australia (by 16%) and the UK (by 5%), but increased by 23% in the USA. In Australia there was a reduction in sales of nutritively-sweetened beverages by 64 million litres from 2002 to 2006. There was a reduction in percentage of children consuming soft drinks, flavoured waters and electrolyte drinks between 1995 and 2007, by as much as 13% in 2-3 yr olds.

#### Conclusion

The findings confirm an 'Australian Paradox', ie a substantial decline in refined sugars intake over the same timeframe that obesity has steeply increased. The implication is that concerted efforts to reduce sugar intake in other countries (eg by taxation) may reduce consumption but may not reduce obesity prevalence

#### Source of funding

Not applicable



## Concurrent Session 3: Glycaemic Index/Load

### Glycaemic and insulinaemic responses to home-made vs commercial weaning foods

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#### Background

Weaning and complementary foods prepared for the 4-9 month old infant undergo varying degrees of processing (pureeing, grinding, heat treatment and/or sterilisation) in both the home and food factory. Elevated metabolic responses to highly processed foods have implications for metabolic imprinting and risk of type 2 diabetes and obesity later in life.

#### Objective

The aim of this study was to compare the glycaemic index (GI) and insulinaemic index (II) of 7 popular, commercially available complementary foods with the equivalent home-made food.

#### Design

Ten healthy subjects consumed 25 g carbohydrate portions of each test food and the reference food (25 g glucose on 3 occasions) in random order. Fingerprick blood samples were taken at regular intervals over 2 h, and the GI determined according to the Australian Standard. Insulin responses were also assessed.

#### Outcomes

The GI (mean  $\pm$  SEM) of the manufactured infant foods ranged from  $43 \pm 5$  to  $74 \pm 7$  while the home-made versions varied from  $29 \pm 4$  to  $61 \pm 3$ . In three cases, the commercial food had a significantly higher GI value than the home-made equivalent. Two early weaning foods had GI values  $>70$ . Insulin responses also varied over a large range.

#### Conclusion

These findings show that despite similarities in ingredients between commercial and homemade complementary foods, the level of processing increases the rate of digestion and absorption of carbohydrates. Consumption of commercial complementary foods could program the metabolic syndrome later in life.

#### Source of funding

Sydney University Glycaemic Index Research Service

### Do low GI foods prolong satiety and reduce energy intake? A meta-analysis of randomized controlled trials

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#### Background

The glycaemic response to foods may influence satiety and food intake but acute and chronic feeding studies have produced inconsistent results.

#### Objective

We performed a meta-analysis of randomized controlled trials to determine whether high and low glycaemic index (GI) meals improve satiety in acute (duration  $< 1$  day) and in chronic ( $> 1$  day) situations.

#### Design

A web search was undertaken on electronic databases which included CINAHL, EMBASE and MEDLINE. The Cochrane Library was also searched. Randomized controlled trials published between January 1981 and August 2009, limited to English language, were selected. A hand search of the reference lists of review articles was examined to include other eligible studies. Only randomized crossover, within-subject or parallel design studies with matched macronutrient distribution and fibre content were included. Studies involving sugar solutions, alternative methods to lower the glycaemic response and those showing no significant differences in incremental area under the curve (AUC) for glucose and/or insulin responses were excluded. Main outcome measures are satiety, fullness, hunger, appetite and energy intake.

#### Outcomes

In total, 22 acute and 7 chronic studies were identified, which met the inclusion criteria. Acutely, low GI foods or meals increased greater satiety (95% CI: 8.5 - 39.3) compared to foods with a high GI. The pooled estimate shows a statistically significant benefit of low GI on satiety. In chronic studies, differences in satiety and energy intake were not significant.

#### Conclusion

Low GI foods and meals produce greater satiety in the short term, but over the longer term their role in regulating food intake and promoting weight loss remains unclear.

#### Source of funding

Not applicable.