Stability of six carotenoids, retinol and α-tocopherol after extraction from plasma

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Carotenoids and other essential antioxidant nutrients are receiving considerable scientific attention. Recent data suggest that optimal intake of carotenoids and other antioxidants can help delay or prevent the onset of cancer, cardiovascular diseases, cataracts and macular degeneration (1). Liquid chromatography procedures have been described for determination of carotenoids, retinol and α-tocopherols simultaneously in human serum or plasma (2).

We investigated the stability at various temperatures of carotenoids, retinol and tocopherol extracted from a pooled plasma samples. The plasma, after being deproteinized with ethanol, was extracted with hexane. After evaporation, the residue was dissolved in 30 µL of chloroform, followed by 70 µL of methanol : acetonitrile (1:1). Table 1 presents data obtained from duplicate extracts stored in the dark at 37°C, room temperature or -20°C. Samples were analysed at various time points after extraction.

	37°C			Room temperature			-20°C		
Time (hr)	2	20	116	2	24	100	2	48	120
retinol	101.0	103.0	94.6	102.0	101.1	103.5	100.3	99.5	102.4
α-tocopherol	99.6	96.8	96.7	99.6	99.1	98.6	99.8	98.7	96.4
ß-carotene	98.3	93.4	78.3	102.0	96.3	92.3	100.3	102.6	99.6
α-carotene	98.8	94.2	64.8	101.5	98.7	95.6	100.1	100.5	99.6
lutein/zeaxanthin	98.4	89.7	96.7	101.6	102.6	99.2	100.5	101.0	99.8
cryptoxanthin	100.0	91.5	85.6	101.9	102.3	99.4	100.1	99.8	97.6
lycopene	92.3	90.4	41.2	97.3	92.1	82.1	97.8	87.5	79.9

Retinol, α-tocopherol and most carotenoids were stable in darkness at 4°C and -20°C for at least 24 hr. Lycopene was reduced by about 8% after 24 hr under room temperature storage condition. Retinol and α-tocopherol were stable for up to 20 hr at 37°C in darkness. Most carotenoids were reduced by about 7% and lycopene was decreased by 10% under these conditions. The results show that lycopene is less stable (and therefore more reactive) than other carotenoids. Carotenoids are less stable than retinol and α-tocopherol after extraction.

These data will assist in the design of studies and the assay of large numbers of plasma samples for antioxidant vitamins analysis. When using automated injection systems, samples should be kept at 4°C and analysed within 24 hr.

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Effect of dietary arachidonic and docosahexaenoic acid on polyunsaturated fatty acid levels of retina, liver and heart in the guinea pig

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Our previous studies have shown that retinal function in the guinea pig is influenced by the proportion of docosahexaenoic acid (DHA) in the retina. In these studies guinea pigs were fed for 3 generations on defined diets containing [1] safflower oil (SO rich in linoleic acid and poor in alpha-linolenic acid), [2] guinea pig chow or [3] a canola oil (CO) diet. Retinal DHA values were measured and compared with electroretinographic (ERG) function. The results showed that the safflower oil diet led to more than a 90% reduction in retinal DHA relative to the CO diet, and that the ERG signal was significantly reduced in guinea pigs on the safflower oil diet.

The aim of the present study was to determine the effect of dietary DHA and arachidonic acid (AA) on the long chain polyunsaturated fatty acid (PUFA) composition of retina, liver and heart in the guinea pig. Female pigmented guinea pigs (3-weeks old) were fed for 12 weeks on one of five semi-synthetic diets (n=14/group), containing 10% (w/w) lipid. All diets were designed to provide approx. 17% of the fatty acids as linoleic acid. In diets S and C the lipids were mainly provided by safflower oil and canola oil, respectively. These diets had linoleic acid/linolenic acid ratios of >200:1 and 2.3:1, respectively. Diet A was based on mixed vegetable oils with a fatty acid composition similar to that of human milk (linoleic acid/linolenic acid ratio of 17:1). In diet A1, some of the oleic acid was replaced by AA and DHA (1% of the fatty acids as AA and 0.7% as DHA simulating the level found in human milk), and diet A3 contained 3% AA and 2.1% DHA. The AA and DHA were obtained from microalgal sources and the tissue phospholipid fatty acids were examined by capillary GLC.

In the retina, the DHA levels increased in the order S<A<C<A1<A3. Supplementation with DHA and AA (at x1 and x3) compared with the diet A increased the retinal DHA proportion from 9.7% to 17.6% and 25.5%, respectively. The proportion of retinal DHA on the C diet (LA to ALA of 2.3:1) was similar to that obtained on the A1 diet (+ 0.7% DHA). In the heart, the DHA proportions were very low for diets without LCP. The DHA values increased from 0.4% for diet A to 5.9% and 5.6% for diets A1 and A3, respectively. In the liver, the DHA proportions were less than 1% for diets S, C and A. Supplementation with DHA increased the value to 6.1% and 14.6% for diets A1 and A3, respectively. The proportion of retinal AA was between 8.7% to 9.2% for all diet groups. In the liver, supplementation with AA increased the value from 7.6% on the A diet to 15.6% and 19.6%, respectively on the A1 and A3 diets. In the heart, the AA levels were highest for all three tissues (>20%), and AA supplementation had a relatively minor effect on heart AA proportions.

These data show that retinal DHA levels can be substantially increased by dietary manipulation of n-3 PUFA (either as alpha linolenic acid or as DHA). Consumption of both DHA and AA by weanling guinea pigs, as a balanced addition to their diet, was associated with accretion of DHA in retina, liver and heart, without affecting the tissue AA levels in the retina.

Oil usage by Greek migrants in Melbourne: validating dietary survey questions

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Intake of olive oil and other vegetable oils may partly account for the low incidence of coronary heart disease and a number of other chronic diseases in some populations. It is thus important to be able to quantitate oil intake in dietary surveys. Fifty six Greek-born men and women aged 48-65 years old, who had lived in Melbourne for the last 15-30 years, took part in a study to validate results being collected in a large epidemiological study. The subjects answered six questions about the types and uses of fats and oils used in their household, including a multi-choice question estimating the amounts of olive and/or vegetable oil/blend used. This question is being used in the Melbourne Collaborative Cohort Study (MCCS) by the Anti-Cancer Council of Victoria (ACCV) which is investigating the influence of diet and lifestyle on the incidence of a number of chronic diseases in Australian born men and women and Greek and Italian born migrants. The validity of answers to this question regarding monthly household oil usage was evaluated by comparison with the subsequent quantitative measurement of oil(s) used in a month.

Most subjects used a combination of olive oil and vegetable oil/blend in their household: the mean measured household usage was 4.1 L/month, with a range of 0.75 to 8.05 L/month. The mean intake of olive oil was 2.2 L/month. The answers to the question where the subjects were required to tick a marked category range of oil intake were similar to the measured intake. From the measured quantities of olive oil and vegetable oil/blends used and knowledge of the number of individuals per household, the mean individual intake in grams per day was considered to be 22 g/day of olive oil and 13 g/day of vegetable oil/blend. The olive oil was used mainly as a dressing for salads and cooked vegetables, whereas vegetable oil/blend was used for frying.

Comparison with an identical question asked of the same sample of subjects in 1987, revealed that there has been a statistically significant ($P<0.05$) increase in the mean household and individual intake of olive oil, but an apparent decrease in the intake of vegetable oil/blend although the later was not significant.

The question used in the study, and being used in the MCCS study, appears to be a reasonable survey tool for estimating household oil intake in a population study with a large percentage of Greek-born subjects.

Brain gangliosides: variation in sialic acid concentration among eight mammalian species

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Sialic acids play an important role in brain development and in the transmission and storage of information in the central nervous system (1). In this study, we determined the concentration of bound and free sialic acid in ganglioside extracts of brain cortex in eight different mammals at several stages of development. These comparisons are unique and have implications for evolutionary development and learning ability. Sialic acid was determined by the thiobarbituric acid assay described by Lorant (2) and modified by Aminoff (3).

The concentration of bound sialic acid in gangliosides and free sialic acid in the brain cortex of human, chimpanzee *(Pan troglodytes)*, rat *(Rattus norvegicus)*, mouse *(Mus musculus)*, rabbit *(Oryctolagus cuniculus)*, sheep *(Ovis aries)*, cow *(Bos indicus)*, and pig *(Sus scrofa)* is shown in the table. Humans showed the highest content and there was no significant difference between human males (916 ± 136 μg/g) and females (865 ± 77 μg/g). Apart from the cow vs the sheep, the differences between species were statistically significant ($P < 0.05$).

Species	Total SA (μg/g wet weight tissue)			Free SA (μg/g wet weight tissue)		
	Mean	SD	n	Mean	SD	n
Human	890[a]	103	6	41[a]	3	4
Rat	493[b]	23	12	32[b]	3	6
Mice	445[c]	29	16	25[b]	5	10
Rabbit	380[d]	18	6	0		6
Ovine	323[e]	43	6	0		6
bovine	304[e]	14	6	0		6
pig	252[f]	14	6	0		6

*Data in the same column with different letters are statistically different (P<0.05-0.001)

In the mouse, cow and sheep, total sialic acid concentration increased during maturation by 18-32% ($P < 0.05$). In 2-year-old chimpanzee, the sialic acid concentration in the left lobe of the brain cortex was 25% higher than that of right lobe at 6 weeks of age ($P< 0.05$). Free sialic acid was higher in the human brain cortex (41 ± 3 μg/g) than that of the rat and mouse (32 ± 3 and 25 ± 5 μg/g respectively), and absent from other species.

In the cortex of adult brain, the SA level decreased in the rank order of human, rat, mouse, rabbit, sheep, cow and pig. The human brain had 2-4 times more sialic acid than that of the other mammals, including the young chimpanzee. To our knowledge these comparisons have not been reported before. There are significant regional differences in the sialic acid concentration because the different brain regions perform different neurological functions. We found that brain sialic acid concentration increased with age but there were no sex differences in human brain tissue. We postulate that brain sialic acid concentration is an indicator of intelligence-learning ability and that environment and diet may influence sialic acid accumulation.

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Preliminary studies of phytoestrogens in Australian foods

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Plant foods contain a large number of compounds such as phytoestrogens, which appear to have bioactive roles. Phytoestrogens due to similarities in structure to the natural hormone, estrogen, may have a role in protection against certain hormone-dependent cancers.

There is only very limited published information on phytoestrogens in Australian foods (1). The present study determined the isoflavones (daidzein, genistein, formononetin and biochanin A) and a coumestan (coumestrol) in Australian plant foods using isocratic HPLC techniques (2) developed by the authors.

Dried legumes and canned legume products were collected from retail outlets in Sydney and extracted with ethanol and hydrochloric acid. Assay portions were neutralised before injection into a Waters liquid chromatograph connected to photodiode array detector. The optimised mobile phase was 30% acetonitrile and 70% water at flow rate of 0.8 ml/min. Analytes were identified by comparison with pure standards and quantified by computer integration.

The separation of all five analytes was achieved in under 24 minutes. The isocratic methods also exhibited good repeatability and high linearity (0.999) under safer conditions less detrimental to column life than other methods described in the literature. This method was also suitable for automation and treatment of large numbers of legume samples.

Preliminary results for phytoestrogens in legumes available in Sydney are as follows: Daidzein was found in dried soybean seed and dried berlotti beans, within the range 6 to 20 mg per 100 g wet weight. Genistein was found in dried soybeans, canned and freeze-dried peas, canned and dried red kidney beans, dried berlotti beans, dried haricot beans and canned butter bean, within the range 1 to 30 mg/100 g wet weight. Formononetin was detected in black eye beans and butter beans within the range 1 to 9 mg/100 g wet weight. Biochanin A was detected in canned and freeze-dried peas and dried chick peas within the range 3 to 13 mg. Coumestrol was also detected in some foods at low levels. Confirmation of identity of all analytes is being carried by mass spectroscopy studies. Further studies are being carried out to compare results using different HPLC methods in order to ascertain comparability of results with studies carried out elsewhere. The studies will be extended to include other legume products as well as other plant foods available in Australia, including the forms as prepared for consumption.

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Variation in isoflavonoid phytoestrogen content in soybeans grown in Australia

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There is increased evidence that the consumption of soy and its phytoestrogens, the isoflavones, have beneficial effects on blood lipids, hormone-dependent cancers and menopausal symptoms. As a result of these potential beneficial effects, there have been a number of new products containing high levels of soy released onto the Australian consumer market. It has previously been demonstrated that isoflavonoid phytoestrogen concentration varies in different soybean strains, and with the increased use of Australian grown soy, phytoestrogen analysis of soybean strains is warranted.

Fifteen different strains of soybeans were selected. The isoflavones daidzin, genistin and their aglucones daidzein and genistein were assessed using High Performance Liquid Chromatography (HPLC).

Table 1. Isoflavone content of selected soybean varieties.

Soybean Variety	Daidzin mg/g	Genistin mg/g	Daidzein mg/g	Genistein mg/g	Total mg/g
Soy 1	0.684	0.847	0.009	0.011	1.551
Soy 2	0.349	0.458	0.008	0.008	0.823
Soy 3	0.533	0.555	0.009	0.009	1.106
Soy 4	0.503	0.747	0.003	0.008	1.261
Soy 5	0.430	0.534	0.010	0.009	0.983

The level of variation in phytoestrogen content observed in these analyses should be taken into account in the representation of products high in soy, as end product isoflavone content will also vary.

Food and nutrient intakes in naturopathy students

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A study was undertaken to determine food and nutrient intakes of third year students enrolled in a four year Bachelor of Naturopathy degree. Twenty-two students (17 female, five males, age 21-53 years, BMI 18-32, 15 omnivores, six lacto-ovo vegetarians, one vegan vegetarian) provided dietary assessment data from a three day food intake study completed as part of a unit assessment. Dietary intakes were quantitatively analysed by the Serve Nutrition Management System (1) and by the NH&MRC Core Food Group system (2).

Comparison to nutrient goals & RDI's. Nutrients expressed as a % of total energy & RDI (Mean ± SD) (3,4)

Nutrient Goals	Protein 10 -15 %	Fat ≤ 30 %	CHO ≥ 50 %	ETOH < 5 %	Calcium % RDI	Iron % RDI	Zinc % RDI	K:Na 1:1
n=22	16.2 ± 3	32.4 ± 8	48.7 ± 10	2.7 ± 3	96.9 ± 51		82.0 ± 33	1.1 ± 0.6
Female	16.2 ± 3	31.7 ± 8	49.8 ± 10	2.3 ± 3	85.4 ± 28	80.1 ± 21	69.1 ± 20	1.3 ± 0.6
Male	16.4 ± 4	34.9 ± 7	44.7 ± 9	3.9 ± 3	135.8 ± 88	317.4 ± 91	125.9 ± 36	0.7 ± 0.2
Omnivore	17.0 ± 3	31.8 ± 9	48.8 ± 10	2.4 ± 3	96.6 ± 57	93.9 ± 38*	83.1 ± 37	1.0 ± 0.5
Lo vege	14.3 ± 4	32.5 ± 6	49.5 ± 10	3.6 ± 4	93.4 ± 40	88.3 ± 34*	75.3 ± 27	1.3 ± 0.8
Vegan	16.0 ± 0	41.6 ± 0	40.9 ± 0	1.47 ± 0	77.6 ± 0	118.1 ± 0*	105 ± 0	2.1 ± 0

* RDI = 16 mg iron

A key finding of the study revealed that the nutrient intakes of the student population are similar to that of the current Australian diet (5). A criticism of the research suggests that a longer investigation period is indicated as recommended by Basiotis et al. (6). As an adjunct to this investigation, an analysis of food and nutrient intake through a comparison with the NH&MRC Core Food Group guide highlighted discrimination against many common foods consumed by the study population. Foods such as hommous, tofu, seaweed, seeds, miso, and tempeh are excluded from Australian quantifiable food guidance systems. This limits their use as assessment and education tools for diets other than those usually consumed in traditional Western cultures. It was necessary to adapt Australian food selection systems to provide a more extensive list of foods appropriate to the study population.

Data from this study will from a basis for further investigation. Future research will continue to focus on the food habits and attitudes of the naturopathic student population and the impact of nutrition education on food and nutrient intake.

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A semiquantitative food frequency questionnaire of measuring nutrient intakes for Chinese adolescents

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The food frequency method has rarely been used in food intake studies in China, however such an instrument is needed for estimation of habitual food and nutrient intake in large-scale surveys and epidemiological studies in the country. This study reports the development of a semiquantitative food frequency questionnaire (SFFQ) designed to obtain data on habitual nutrient intakes of Chinese female adolescents in studies of their calcium and vitamin D status and bone health.

A list of foods was developed by means of a data-based approach (1), using food intake data recorded by a 3-day weighed record for a random sample of 3092 Beijing residents in the 1992 National Nutrition Survey (2). Foods were ranked in order of their contribution to the total calcium intake. The top 102 food items representing 94 % of the population's calcium intake were selected as the basic list. Some foods rich in calcium and observed to be popular among Chinese teenage girls were added to the list (eg. dried fish snacks). Chinese measures (bowls and spoons, standard size) were used to quantify some food items. The food composition data were from the Chinese food tables (3). The average frequency of consumption of each food item over the past year, in specified serving sizes, was indicated by marking one of 10 frequency categories. The SFFQ was pre-tested among 10 girls prior to field administration, and reproducibility and validity studies were conducted in a subsample of 200 girls from the survey.

Code	Item (example only)	Portion size	Servings consumed (n)	Frequency Per day 1 2 3	Per wk 1-2 3-4 5-6	Per mo 1 2 3	R/N[1]	Duration Month	Peak
1045	Rice	1 small bowl							
5016	Chinese cabbage	1 spoon							

[1]R/N = rarely or never consumed

The final food list contained 103 food items representing 86 % of calcium intake of Beijing residents. The pretest of the SFFQ showed a correlation of calcium with the 3-24 h dietary recall method of r = 0.56. It was found in the study of calcium and vitamin D status of 1300 study girls that the SFFQ can be self-administrated by girls in this age group with the assistance of a set of food measure models and, in some cases, with the help of parents. The results for nutrient intakes obtained by use of the SFFQ are being further validated by comparison with a 6-day dietary recall and an independent test with another group of same-aged girls.

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Dietary evaluation of full time students at Griffith University, Gold Coast.

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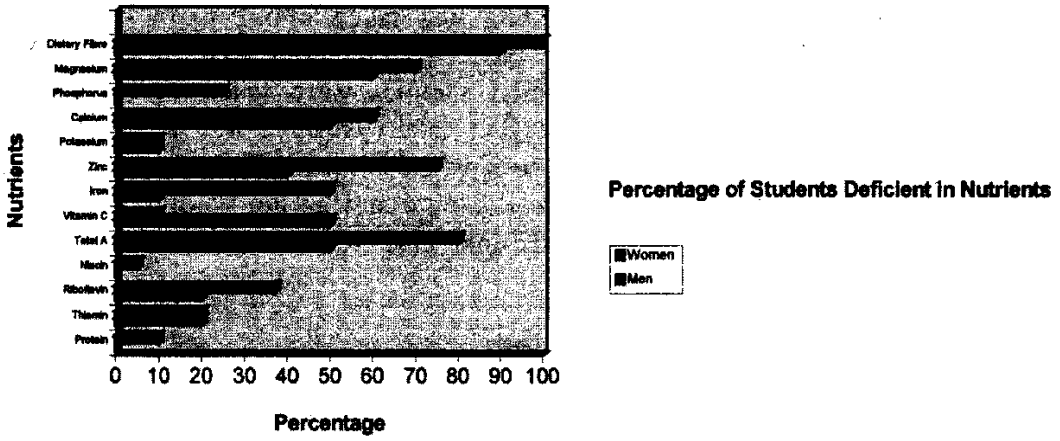
Due to a combination of financial hardship and hectic lifestyle, students often complain that their diet is inadequate. To determine the accuracy of this claim, we have investigated the dietary status of students at Griffith University, Gold Coast.

Thirty students (10 male, 20 female, age 17-35, BMI 17-40) were obtained randomly from the 1997 enrolment of students. These students (1% of campus enrolments) completed a seven day dietary diary and questionnaires about their knowledge and attitude towards health and nutrition. The information from the seven day dietary diary was entered into the computer program *Diet 1* which tabulated the level of nutrients in each diet.



A large proportion of the student population was found to be below the RDI in nutrients (Fig). A greater proportion of women were deficient in nutrients. The students' diets were compared with the CSIRO nutritional survey 1993[1]. The student averages were consistently lower than the Australian averages for the majority of the nutrients. Most female students were deficient in vitamin A, zinc, calcium and dietary fibre, whereas most men were deficient in vitamin A, vitamin C, calcium and magnesium. During the seven days recorded, 65% of women and 50% of men ate no seafood. Only 5% of females and 20% of males ate at least one serving of fruit or vegetables everyday. Women were below the Australian average in 88% of nutrients. Men were below the Australian average in 31% of nutrients. During the interviews students performed poorly at identifying their nutritional deficiencies. For example 43% of students incorrectly identified whether they had sufficient or too little calcium in their diet.This study suggests that the majority of students at Griffith University, Gold Coast have inadequate diets particularly with respect to vitamin A, vitamin C, calcium, zinc, magnesium and dietary fibre.

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Methane emissions of cattle fed on tropical forage or high grain diets

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Methane is a greenhouse gas produced by cattle during the processes of feed digestion, and is estimated to account for 58 % (1) of methane emissions of all Australian livestock, and 73 % (2) of global livestock methane emissions. In the Australian inventory, predictions of methane emissions of cattle are based on measurements (3) made on British breeds of cattle and sheep fed on temperate forage based diets. The chemical structure of temperate forage species differ greatly to those forage species fed to cattle in the tropics. The objective of this experiment was to determine daily methane production of Brahman cattle fed on three different diets that represent those used under commercial conditions in tropical Australia.

Six Brahman heifers, aged 3.5 years, were used in a latin square experiment conducted over six months. During each period two heifers were fed ad libitum with Angleton grass hay (*Dicanthium aristatum*, 0.4 % N), Rhodes grass hay (*Chloris gayana*, 1.5 % N) or a high grain (feedlot, 2.8 % N) diet. Heifers were fed each diet for four weeks prior to measurements being made. Methane production was measured continuously over 24 hours using a confinement type respiration chamber. The table presents measurements made on the day of the respiration chamber experiment, except for liveweight change which was measured over four weeks.

Diet:	Angleton grass (n=6)	Rhodes grass (n=6)	High grain (Feedlot) (n=6)	Sig.
Liveweight (kg)	359±10	361±10	369±10	ns
Liveweight change (kg/day)	-0.84±0.19a	0.52±0.08b	1.34±0.15c	P<0.01
DMI (kg/day)	3.6±0.4a	6.7±0.3b	7.9±0.6b	P<0.01
Methane production				
(g/day)	107±9a	240±13b	168±17c	P<0.01
(g/kg DOMI)	75±4a	65±2b	33±3c	P<0.05

DMI = dry matter intake; DOMI = digestible organic matter intake; ns = not significant

These are the first published measurements of methane production for cattle fed tropical forages. Methane production per DOMI was highest for cattle fed Angleton grass and lowest for the feedlot diet. We predicted methane emissions of our cattle using NGGI methodology (1), and found that they were similar to our measurements for Angleton grass and the feedlot diet, but underestimated (~40 %) that for Rhodes grass. We conclude that reliable inventories of methane emissions of livestock in tropical regions of the world, including northern Australia, can only be made when methane emissions of cattle fed a range of tropical forage species are available.

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Guanidination has no influence on amino acid digestibility of proteins for broiler chickens

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A novel technique based on the guanidination of dietary proteins, to distinguish between endogenous secretions and exogenous or dietary sources of amino acids in intestinal digesta, has been applied to the measurement of ileal endogenous amino acid losses in poultry (1). Guanidination is the chemical process wherein the lysine moieties in dietary proteins are transformed to homoarginine (2-amino-6-guanidino-hexanoic acid) by reaction with *o*-methylisourea under alkaline conditions. Implicit in the use of guanidinated proteins to measure endogenous amino acid secretions is that guanidinating reaction has little or no effect on the amino acid profile of the protein and on its susceptibility to proteolysis. However, the potential racemisation of amino acid residues to D-forms during guanidination is a concern associated with the alkaline pH (10.5) employed. Proteins containing D-amino acids have been reported to show decreased digestibility in vitro (2). In the present study, the influence guanidination on the amino acid digestibility of casein, soyabean meal, cottonseed meal and canola meal for broiler chickens was examined.

The proteins were guanidinated according to the procedures described elsewhere (1) with the modification that incubation was carried out at 4 ^{0} C. The digestibility assay diets contained the test ingredient (guanidinated or unreacted forms) as the only source of protein. The proportions of dextrose and the test ingredient were varied in each diet to obtain 200 g/kg crude protein. Celite (20 g/kg) was added to all diets as a source of acid-insoluble ash which was used as an indigestible marker in the calculation of digestibility coefficients. The assay methodology has been described previously (3). The amino acid digestibility values of guanidinated and unreacted ingredients were compared using the t test.

Digestibility of amino acids in guanidinated and unreacted proteins were remarkably similar, with the exception of serine and isoleucine in casein and, glutamic acid, isoleucine and leucine in soyabean meal where small, but significant ($P < 0.05$) differences were noted. The present results confirm the assumption that guanidination has little effect on the susceptibility of guanidinated proteins to proteolysis. The possibility of some degree of destruction of amino acids in another concern associated with guanidination. Calculation of recovery of the amino acids, however, showed that guanidination does not cause significant changes in the concentrations of the acid-stable amino acids (except lysine). The recoveries of amino acids were close to 100% in the four protein sources.

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Oxygen availability and fuel utilisation during intermittent exercise

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In continuous exercise fat oxidation is lower and carbohydrate oxidation is higher in both relative and absolute terms at high (85% VO_{2max}) compared to moderate (65% VO_{2max}) exercise intensity. Sahlin (1) implicated increases in lactate, pyruvate and declining O_2 availability as potential mediators of this effect. Such suggestions are confounded by differences in circulating catecholamines and muscle fibre type activation between the two exercise intensities. Increased work and recovery duration at the same exercise intensity during intermittent exercise (IE) results in higher muscle lactate (4). Using this principle we compared fuel oxidation during two protocols of IE performed under steady state conditions and at identical exercise intensity. Near-infrared spectroscopy (NIR) was used to monitor changes in muscle O_2 availability during IE.

Subjects (n=7) completed 40 min of IE on two occasions. The IE involved running on a motorised treadmill (individual subject range 18-24 km.hr-1; 0 % grade) with a work : recovery cycle of either 6 s : 9 s or 24 s : 36 s. Arterialised capillary blood was obtained at 10 min intervals. Capillary plasma was analysed for lactate, pyruvate and NEFA. Blood samples (10 ml) were collected by venepuncture pre- and post-exercise for plasma catecholamine determination. Expired gas was collected in Douglas bags and analysed for fractions of O_2 and CO_2. Carbohydrate and fat oxidation were estimated by indirect calorimetry. A RunMan® NIR spectroscopy unit (NIM, Philadelphia, Pa) was used to non-invasively monitor changes in haemoglobin saturation of the vastis lateralis during IE.

Measurement	Protocol		P value
	6s : 9s[1]	24s : 36s[1]	
VO_2 (l.min-1)	3.02 ± 0.10	2.79 ± 0.13	P=0.051
Respiratory exchange ratio	0.88 ± 0.02	0.96 ± 0.02	P<0.001
Carbohydrate (moles.ATP.min-1)	0.47 ± 0.05	0.67 ± 0.06	P<0.01
Fat (moles.ATP.min-1)	0.31 ± 0.03	0.10 ± 0.04	P<0.001
Lactate (mM)	3.30 ± 0.73	5.40 ± 1.27	P<0.05
Pyruvate (μM)	147 ± 31	219 ± 41	P<0.05
NEFA (μM)[2]	1202 ± 319	823 ± 143	P=0.315
Nor Adrenaline (mM)[2]	10.95 ± 1.87	11.15 ± 1.53	P=0.906
Adrenaline (mM)[2]	1.03 ± 0.35	1.40 ± 0.15	P=0.282
Nadir %Hgb saturation	60.33 ± 4.71	48.85 ± 6.88	P<0.05

[1] mean ± sem for final measurement, [2] n=4

Despite similar VO_2 and catecholamine responses, 24 s work : 36 s recovery resulted in 42% higher carbohydrate and 67% lower fat oxidation compared to 6 s : 9 s. Diminished muscle O_2 saturation and higher plasma lactate and pyruvate concentrations were observed during the 24 s : 36 s protocol.

Although plasma NEFA tended to be lower during 24 s : 36 s, reduced availability of NEFA is unlikely to account for the decline in fat oxidation. We hypothesise that limited muscle O_2 availability during 24 s : 36 s results in elevated muscle lactate and pyruvate concentrations which in turn are linked to the suppression of fat oxidation.

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Effects of yeast culture on apparent nutrient digestibility, changes of the intestinal micro flora and growth performance in broiler chicks

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A live yeast culture supplement containing *Saccharomyces cerevisiae* live yeast cells has received increasing attention as feed additives in poultry to increase their productivity. Yeast culture may alter the intestinal microbial population by stimulating the proliferation of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria.* In ruminants, it appeared to change morphological populations of ruminal bacteria and increase numbers of ruminal protozoa (1). Supplementing the diet with yeast culture to layer breeders appeared an increase in hatchability (2). Recently, Kumar and Dingle (3) have shown that although there is no statistically significant differences between treatments, yeast culture inclusion to the diets slightly increased in body weight of broiler chicks. However, information on the effect of various levels of yeast culture addition on broiler chicks is limited. Thus, this study was conducted to determine the effect of supplemental yeast culture on growth performance, apparent nutrient digestibility, nitrogen retention and changes of intestinal microflora in broiler chicks.

Four hundred and thirty-two, one day-old broiler chicks of Maniker strain (216 males and 216 females) were divided into 36 pens of 12 chicks each. Each pen was assigned to one of the 6 treatments with 6 replications (3 pens of males and 3 pens of females) according to a 2 x 3 factorial arrangement, consisting of the 0 %, 1 % and 2 % of YC. The two levels of nutrition diets were the high nutrition (3200 Kcal ME/kg, Starter 23 % CP, Finisher 20 % CP), and low nutrition (2800 Kcal ME/kg, Starter 20 % CP, Finisher 17 % CP), respectively. A metabolism trial was conducted and the intestinal flora evaluated.

Weight gain and feed conversion rate were significantly ($P<0.01$) improved when fed the high nutrition diet than when fed the low nutrition diet. Male birds showed better growth performance than females. Although there were no statistically significant between treatments, supplementation of YC tended to increase the total number of intestinal flora, *Lactobacillus*, but decrease the number of *E. coli* and *Streptococci.* However, the addition of YC had not affected the growth performance, apparent nutrient digestibility and nitrogen retention. These results indicate that supplemental YC did not affect the growth performance in broiler chicks.

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Roasting lupins and narbon beans does not improve lamb growth.

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Reduction of the fermentability of legume protein is expected to increase the supply of amino acids (AAs) for absorption in the small intestine. If the diet is balanced for other nutrients, growth should be maximised, unless roasting is associated with reduction of nutritive value due to a decline in small intestinal digestion, absorption or intermediary metabolism of products of roasting. From the array of treatments in the preceeding paper (1), we chose roasting at 130C for 45 minutes, suspecting that hotter or longer treatments which produced the steeper response might be doing so through a qualitatively different reaction associated with deleterious effects on nutritive value. Lupin (L) and narbon beans (NB) were roasted in a laboratory oven and fed in a 2 (grain legumes) x 2 (raw vs roasted) experiment in isonitrogenous rations of 70% concentrate (raw or roasted legume + barley) and 30% roughage (lucerne + oaten chaff).Groups of six lambs (Polled Dorset x (Border Leicester x Merino)) per ration, 5 months old, average weight 33.4 kg were penned individually and fed 90% ad libitum for 9 weeks before slaughter. Despite slow introduction of diets, initial growth was poor due to ruminal acidosis. Feed intake and growth during the final five weeks, carcass weight, fat thickness and feed conversion ratio are reported.

Performance of lambs during the final five weeks

	Diet[1]				
	NB	RNB	L	RL	SEM[2]
Weight at week five(kg)	33.90	34.02	31.98	33.07	1.22
Final weight (kg)	41.87	40.83	42.28	41.15	1.19
Daily weight gain (kg day^{-1})	0.23^{ab}	0.19^{a}	0.29^{b}	0.23^{ab}	0.01
Dry matter intake (kg day^{-1})	1.22	1.11	1.09	1.00	0.04
Feed Conversion Ratio (Feed:Gain)	5.63^{a}	5.81^{a}	3.83^{b}	4.89^{b}	0.27
Cold carcass weight (kg)	20.35	19.58	20.03	19.55	0.67
Fat thickness (mm)	15.33	13.50	10.50	12.67	1.02

[1]NB = raw, RNB = roasted narbon; L = raw, RL = roasted lupin [2]SEM = standard error of the mean
Means in the same row with different letter superscripts differ (P<0.05).

Roasting L and NB did not improve animal performance. Possible explanations include unpalatability of roasted L and NB, over-protection of protein resulting in either low digestibility in the small intestine (2), or of one limiting AA, producing an imbalance (3).

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Enrichment of pork with omega-3 fatty acids from fishmeal

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Awareness of the potential health benefits of increased consumption of omega-3 fatty acids has prompted the development of new options for introducing them into our diet. One such option is omega-3 enriched pork, produced by feeding flaxseed to pigs (1). This results in substantial elevation of α-linolenic acid but little increase in the longer chain omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the purported mediators of health benefits. Although the latter can be increased by dose-dependent addition of fish oil to pig rations, use of fish oil or excessive amounts of fishmeal has been avoided as it causes tainting of the pork (2). However, it appears that the tainting may be minimised by withdrawing fish oil for a suitable period before slaughter (2). Using this approach, we have now examined the retention of long chain omega-3 fatty acids in pigs fed fishmeal.

Six 9 wk old male pigs were fed a grower diet containing 20% PorcOmega fishmeal for 6 or 10 wk, reverting to an isocaloric control diet one week before being slaughtered at a commercial abbatoir. The growth rates and food conversion rates were comparable to that of age-matched pigs fed the control diet. Blood samples were taken at slaughter and one week beforehand. The latter revealed striking increases of plasma EPA and DHA in pigs eating fishmeal compared with controls. By the time of slaughter however, the increases were largely attenuated (2.8 fold for EPA and 3.4 fold for DHA) and no differences in quality of the meat were observed during butchering. Nevertheless, fatty acid analysis on lean portions taken from fresh cuts of meat showed significant retention of the long-chain omega-3 fatty acids in pork from the pigs fed fishmeal (Figures 1 and 2).

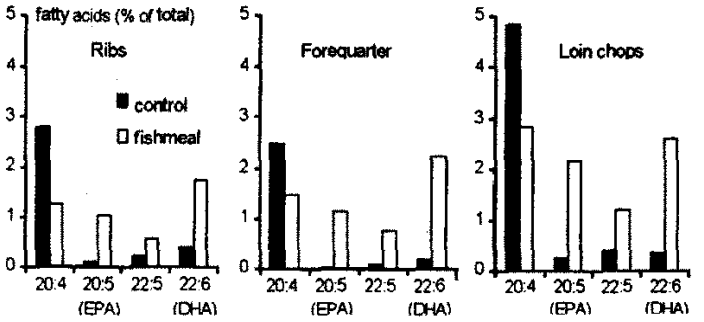

Figure 1: Relative proportions of long chain fatty acids in cuts of meat from pigs fed control (n=4) or fishmeal diet (n=3) for 10 wks

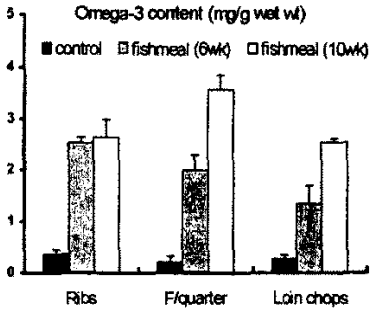

Figure 2: Omega-3 concentrations in meat from pigs fed control or fishmeal diet for 6 or 10 wks (mean±sem)

The degree of omega-3 enrichment of lean pork achieved by feeding fishmeal (up to 3.6 mg/g) not only exceeded that obtained by feeding 15% flaxseed (1) but the ratio of EPA and DHA to α-linolenic acid was at least 100 fold higher. Moreover the relative enrichment of DHA to EPA was higher than seen in pigs fed fish oil (2). Thus omega-3 enriched pork offers an alternative dietary source of EPA and DHA which compares favourably with locally available fish or fish oil preparations. This study was sponsored by Bartlett Grain Pty Ltd, who also provided PorcOmega™

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