

## Protein resources and aquafeed development in the Sultanate of Oman

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### موارد البروتين وتطوير أعلاف الأحياء المائية في سلطنة عمان

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**ABSTRACT.** The continued growth of intensive aquaculture is dependent on the development of sustainable protein sources to replace conventional fish meals in aquafeeds. Practical alternatives are plant-derived protein, protein from micro-organisms and protein from under-utilized marine resources. The challenges are to find alternative ingredients with high protein, suitable amino acid content, high palatability and absence of anti-nutritional factors. There is considerable biotechnology-based research in this area, including genetic modification of plant-based proteins, use of probiotics to enhance digestibility and the renewed application of fermentation technologies to produce single cell proteins. Research in Oman is focused on the utilization of marine protein resources. Fisheries by-catch and processing waste have been evaluated as liquid hydrolysates and as meals for inclusion in aquafeeds and new research is planned on the utilization of meso-pelagic fish (myctophids), which occur in abundance in the Arabian Sea and the Sea of Oman. Initial studies have been conducted on the biochemical composition of the lantern fish, *Benthosema pterotum*, which revealed favorable protein, amino acid and long-chain PUFA content. Potential limiting factors were high levels of saturated lipids and the heavy metals arsenic and cadmium. These results will be discussed within a general review of marine resources and aquafeed development in Oman.

**Keywords:** aquaculture, Oman, protein, aquafeed

**المستخلص:** يعتمد استمرار نمو الاستزراع السمكي المكثف على تطوير مصادر البروتين المستدامة ليحل محل مسحوق السمك التقليدي في الأعلاف. وتكمن البدائل في مشتقات البروتين النباتي، والبروتين المشتق من الكائنات الدقيقة والبروتين الناتج عن الموارد البحرية غير المستغلة. وتكمن التحديات في العثور على المكونات البديلة التي تحتوي على نسب عالية من البروتين، ومستويات الأحماض الأمينية المناسبة، وذات الاستساغة العالية، وليس لديها العوامل المضادة للتغذية. هنالك أبحاث قائمة بشكل كبير على التكنولوجيا الحيوية في هذا المجال، بما في ذلك التعديل الوراثي للبروتينات ذات الأصل النباتي، واستخدام معينات حيوية لتعزيز الهضم وكذلك التطبيقات المتعددة في تكنولوجيات التخمر لإنتاج بروتينات ذات خلية واحدة. وتركز الأبحاث في سلطنة عمان على الاستفادة من موارد البروتين البحرية. وقد تم تقييم الصيد الجانبي ومعالجة المخلفات من التصنيع السمكي على شكل بروتينات سائلة كوجبات لإدراجها في أعلاف الأحياء المائية. كما أن البحوث الجديدة المخطط إجرائها ستكون على استخدام الأسماك البحرية المتوسطة السطحية كسمك الفنار، والتي تتواجد بكثرة في بحر العرب وبحر عمان. وقد أجريت دراسات أولية على التكوين الكيميائي الحيوي لأسماك الفنار، والتي كشفت عن مستويات مواتية من البروتين، والأحماض الأمينية وسلسلة طويلة من محتوى الأحماض الدهنية أومغا-3 غير المشبعة. إلا العوامل المحتملة التي قد تحد من جودتها هي المستويات العالية من الدهون المشبعة والمعادن الثقيلة والزرنيخ والكادميوم. وستناقش هذه النتائج من خلال استعراض عام للموارد البحرية وتطوير أعلاف الأحياء المائية في سلطنة عمان.

**الكلمات المفتاحية:** الاستزراع السمكي، عمان، البروتين، أعلاف الأحياء المائية.

## Introduction

Aquaculture, the farming of fish, shellfish and seaweeds continues to be the fastest growing sector of global agri-food business. Aquaculture production has increased and diversified whilst output from the capture fishery has remained stable or declined over the same period. In 2011 global aquaculture production was 63.6 million tonnes, whilst the capture fishery landed an estimated 90.4 million tonnes of fish and shellfish for direct human consumption and 23.2 million tonnes of fish for reduction to fish meal (FAO, 2012). The growth in aquaculture production has

been paralleled by the growth of the aquafeed industry. Global aquafeed production was an estimated 40 million tonnes, a doubling of the 2003 requirements. The dependence of the aquafeed industry on finite quantities of fish meal and marine oils has brought into question the continued growth of the aquaculture industry and has stimulated a major research effort to find alternative, renewable supplies of protein and oils, which can be utilized in aquafeeds.

The annual global production of fishmeal is around 5 million tonnes, which is derived from 22 million tonnes of raw materials. 75% comes from small fish, mostly small pelagic species, such as menhaden, anchovy, sardines and sand eels, whilst the remainder is derived from the by-products of fish processed for human consumption (Shephard and Jackson, 2012). Global production of fishmeal is relatively stable, unless disrupted by the warm waters of an El Nino. However prospects

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for increasing fishmeal supplies from established fisheries are limited and alternative supplies of protein from unexploited and underutilized marine resources, plants and terrestrial animals are being linked to sustainable growth of the aquafeed industry.

The aquaculture industry in Oman is in its earliest stages and all aquafeeds are currently imported from international suppliers. There has however been a program of research into marine protein resources conducted by Sultan Qaboos University and the Ministry of Aquaculture and Fisheries Wealth. To the present this work has focused on the potential production of hydrolysates and fish meals manufactured from fishery by-catch and processing waste; potentially useful raw materials which are otherwise discarded. Some recent attention has also focused on the future potential to harvest and process lantern fish (Myctophidae) from the Arabian Sea and the Sea of Oman

## Fish silage

A practical method of processing fish processing waste and by-catch is through the production of acid silages (hydrolysates). These are prepared by acidifying minced raw materials, which results in rapid hydrolysis caused by the fish digestive enzymes present in the raw material. The resulting viscous liquids can then be used directly as a wet ingredient in aquafeeds or can be dried into a stable powder. In studies in Oman, acid silages were prepared by first mincing and then acidifying either whole sardines or fishery waste using propionic acid. The resulting mixture (pH < 4) liquefies, as proteins are reduced amino acids, polypeptides and peptides. The resulting liquid is most commonly co-dried, with finely ground cereal and used as a dry ingredient in formulated aquafeeds. In early studies in Oman fish silage was concentrated and co-dried with wheat bran (3:1) in solar cabinet driers for testing as an aquafeed ingredient in feeds formulated for the blue tilapia, *Oreochromis aureus*. Analysis of the manufactured sardine silage showed that its amino acid content and profile exceeded the recommended nutritional requirements for tilapia and was highly digestible (Goddard and Al-Yahyai, 2001).

The chemical and nutritional properties of dried sardine silage were examined as a potential protein supplement for aquafeeds (Goddard *et al.* 2003). The Indian oil sardine, *Sardinella longiceps*, accounts for 95% of the total landings of small pelagic fish and quantities considerably in excess of local requirements for direct human consumption are landed. Traditionally, surplus catches are dried on the beach and sold as cattle and camel food; practices which result in problems of rancidity and contamination. Procedures for the manufacture and drying of silage were examined as an alternative method for processing surplus catch into a protein-rich aquafeed ingredient. Methods were developed to take advantage

of ambient temperature conditions in a hot, arid region.

Feeding experiments using diets containing 0%, 10%, 20% and 30% co-dried silage resulted in growth and feed conversion efficiencies comparable with commercial grade fish meal, when incorporated into practical diets. Significantly lower weight gain and higher feed conversion ratios were recorded in the treatment group fed 40% co-dried silage (Goddard *et al.* 2003). No significant differences between the treatment groups were found following proximate analysis of whole carcasses. The results showed that co-dried silage could be incorporated in formulated feeds for juvenile *Oreochromis aureus* up to inclusion levels of 30%, without impairing, growth or feed conversion.

## Silage drying

In order to further examine the practicality of manufacturing dried fish silage in Oman, drying experiments were conducted in a commercial poly-tunnel (Goddard and Perret 2005). Triplicate samples of each mixture were placed in stainless steel dishes (85 mm diameter, 25 mm depth) on a 1 m high table in the center of a 20 x 9 m poly-tunnel located at the Agricultural Experiment Station of Sultan Qaboos University (23° 35' N, 58° 11' E). The poly-tunnel was oriented North-South and was covered with a single 0.2 mm UV-inhibited polyethylene film. The experiments were conducted in June, which is generally the hottest month of year in the A'Seeb area, Oman. The containers of mixtures were spread randomly on a 1-m high, perforated metal bench which allowed air to flow around the dishes. The samples were weighed in situ at regular interval over the 4-day drying period. Readings at 5 min intervals, averaged every hour, were recorded from twelve temperature and relative humidity sensors connected to a Delta-T logger. The data logger was connected to a PC that was used to download recorded data from the logger. Ten sensors were positioned along the poly-tunnel's longitudinal and perpendicular axis to verify the presence of temperature and humidity gradients while the remaining two sensors were installed outside of the greenhouse. Solar radiation was also measured every hour during the 4-day period using a pyranometer.

The results from these experiments clearly showed that fish silage, mixed with cereal (wheat bran) could be dried to a stable (<10%) moisture content within 3 days (Fig.1). The potential use of poly-tunnels for large scale co-drying of fish silage with cereals in tropical regions is indicated from the present study.

The inclusion of co-dried fish silage as a major ingredient in aquafeeds could reduce dependence on expensive, imported fish meals and present an opportunity to cycle fishery waste, including by-catch, over-catch and processing waste, through aquaculture. The advantages of fish silage lie in its simple, inexpensive manufacturing technology which can be applied in remote locations or

**Table 1.** Species composition of the fish batches used to make the meals for use in the experimental diets. (After Goddard *et al.*, 2008).

Fish meal	Common name	Species
Commercial fish meal		
A	Anchovy	Unknown
By-catch		
B	Catfish	<i>Arius spp</i>
	Indian mackerel	<i>Rastrelliger kanagurta</i>
	Spangled emperor	<i>Lethrinus nebulosus</i>
	Threadfin bream	<i>Nemipterus japonicus</i>
	Moontail bigeye	<i>Priacanthus humrur</i>
	Tigertoothed croaker	<i>Otolithes rubber</i>
	Oblique-banded croaker	<i>Umbrina ronchus</i>
	Largehead hairtail	<i>Trichiurus lepturus</i>
	Bluefish	<i>Pomatomus saltatrix</i>
	Olive grunt	<i>Pomadasy olivaceous</i>
C	Chub mackerel	<i>Scomber japonicus</i>
	Indian scad	<i>Decapterus russelli</i>
D	Catfish	<i>Arius spp</i>
Processing waste		
E	Yellowfin tuna	<i>Thunnus albacores</i>
	Indian oil sardine	<i>Sardinella longiceps</i>

on-board small fishing vessels.

## Fish meals from by-catch and processing waste

Research has been conducted in Oman into the potential use of fisheries by-catch in aquafeeds through the

manufacture of conventional fish meals from selected by-catch from Oman's capture fishery. Three experimental batches of fish meal (fish meals B, C and D) were prepared in a modern, commercial fish meal factory (Rusail Fish Meal and Oil Factory, Oman) from the raw materials listed in Table 1. Approximately one ton of fish, obtained from the offshore fleet of the Oman Fisheries Company, was used to prepare each batch of meal. Fish meal E, manufactured from combined sardine and tuna processing waste, was obtained from a commercial tuna and sardine cannery and fish meal plant (Dhofar Fisheries Industries Company, Oman). Fish meal A was a commercial anchovy meal. The composition of each meal is shown in Table 2.

Five experimental feeds were formulated to contain each fish meal. Varying amounts of each meal were substituted against wheat bran whilst all other ingredients were the same in each diet. The ingredients were ground, blended in a food mixer, extruded through a 4mm die, dried at room temperature and stored at -20°C. A total of 450 fish (mean weight + SD, 5.1 + 0.3 g) were randomly assigned to 15, 80-L outdoor circular tanks. Tanks were individually aerated and supplied from a header tank at a rate of 5 L h<sup>-1</sup>. Mean ambient water temperature during the feeding experiments was 28 + 2.4 °C. Fish were fed to apparent satiation twice a day at 0700 and 1800 by slowly introducing feed until the feeding response ceased. Each of the five diets was fed to three groups, each of 30 fish and records of feed consumption were maintained for each treatment group. The parameters of growth and feed utilization calculated were specific growth rate (SGR), food conversion ratio (FCR), and protein efficiency ratio (PER) and phosphorus retention were calculated as the key parameters of

**Table 2.** Growth and feed utilisation by *Oreochromis niloticus* fed test diets containing fisheries by-catch and processing waste meals for 9 weeks <sup>1,2</sup> (after Goddard *et al.*, 2008).

Variable	Diet					SEM <sup>1</sup>
	1	2	3	4	5	
Initial fish weight (g)	4.9	5.2	5.3	4.9	5.0	0.08
Final fish weight (g)	26.6 a	30.1 ab	31.0 ab	33.5 b	29.8 ab	0.75
Weight gain (%)	520.5 a	554.6 a	576.3 ab	658.5 b	594.1 ab	14.5
Specific growth rate (% d <sup>-1</sup> ) <sup>3</sup>	2.7 a	2.8 a	2.8 a	3.1 b	2.8 a	0.05
Feed consumption (g d <sup>-1</sup> )	0.53 a	0.53 a	0.54 ab	0.57 b	0.55 ab	0.06
Feed conversion ratio <sup>4</sup>	1.55 a	1.34 b	1.34 b	1.36 b	1.40 b	0.03
Protein conversion ratio <sup>5</sup>	1.64 a	1.87 b	1.92 b	1.81 ab	1.77 ab	0.03
Phosphorus retention <sup>6</sup>	18.33 ab	20.05 b	15.93 ab	13.94 b	15.30 b	0.08
Survival (%)	98.8	98.8	96.6	97.3	96.6	

<sup>1</sup> Pooled standard error of mean

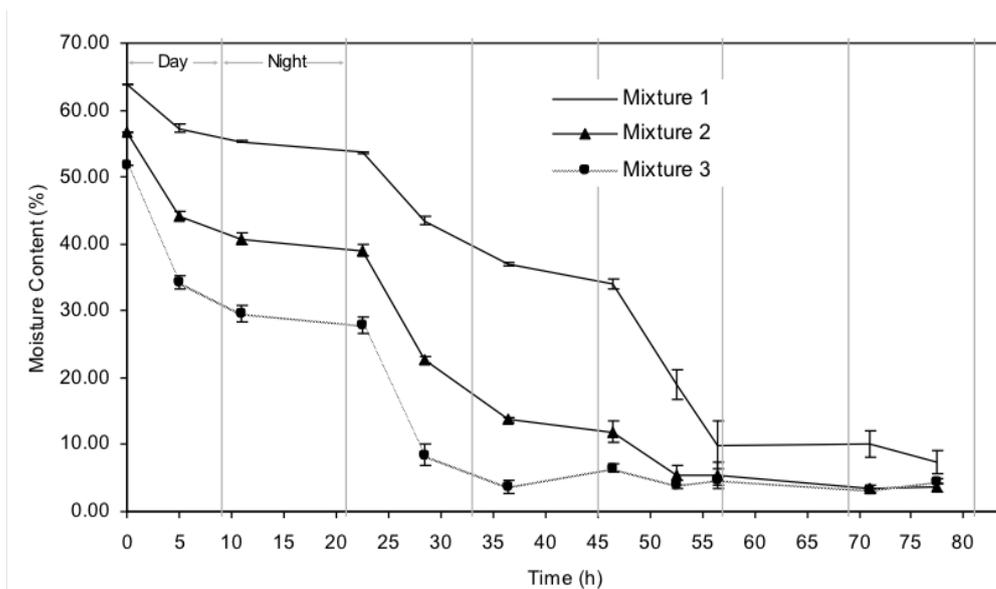
<sup>2</sup> Mean values in each row followed by the same letter were not significantly different ( $P > 0.05$ )

<sup>3</sup> Specific growth rate, SGR =  $(\ln \text{initial wt} - \ln \text{final wt})/t$

<sup>4</sup> Food conversion ratio, FCR =  $\text{g dry food fed}/\text{g wet wt gain}$

<sup>5</sup> Protein efficiency ratio, PER =  $\text{g wet wt gain}/\text{g protein fed}$ .

<sup>6</sup> Phosphorus retention =  $100 \times [\text{g phosphorus}/\text{fish END} - \text{g phosphorus}/\text{fish START}] / \text{g phosphorus in consumed feed}$



**Figure 1.** Drying curves for the three fish silage (FS) and wheat bran (WB) mixtures of the ratios 85FS : 15WB (mixture 1) 75FS : 25WB (mixture 2) and 65FS : 35WB (mixture 3). (After Goddard and Perret, 2005).

growth and feed utilization (Table 2).

Each of the 5 diets tested was readily consumed during the feeding experiments and supported favorable growth in juvenile tilapia (Table 2). Whilst some mortalities occurred (1.2-3.4%) no trends were apparent and these were not attributed to any nutritional deficiencies in the diets. Fish fed Diet 4 had significantly higher levels of food intake and growth and reduced feed conversion ratios. No significant differences were observed in the moisture, protein, ash or phosphorus content of the fish at the end of the 9-week feeding period. Some significant differences in lipid content were observed however. Lipid levels in fish, which had been fed Diet 1, were significantly higher than values from fish receiving Diets 2-5, and lipid levels in fish fed Diet 2 were significantly lower than valued from fish receiving Diets 1, 3-5. The phosphorus content of the diets ranged from 1.04 – 1.69% and phosphorus retention values ranged from 15.30 - 20.05%. Phosphorus retention values were significantly higher in those fish fed Diets 1 and 2, which had the lowest phosphorus content.

The five fish meals used in the study were manufactured from different raw materials and showed a range of proximate compositions. The meals were selected on the basis of their ash and protein content, which are indicators of quality. Those manufactured from the by-catch species and from fish processing waste contained more ash and less protein than the commercial anchovy meal. Fish meals containing more than 15% ash may be categorized as high ash meals (Hardy and Barrows 2002) and typically have lower protein content. This trend was apparent in the four locally manufactured meals. High ash content is of particular concern with regard to excretion of phosphorus into the aquatic environment and

phosphorus content is an important parameter of quality in fish meals used in aquafeeds. Phosphorus content of the experimental feeds were higher than recommended dietary levels, which have been estimated at 0.7-1.0 % depending on fish size and dietary ingredients (Viola *et al.* 1986). In the present study phosphorus retention was highest in fish fed diets with the lowest phosphorus content. No significant differences were seen, however, in the values of phosphorus from whole body analysis between any of the treatment groups at the end of the feeding experiment.

Values for weight gain (SGR) were consistent with, or exceeded, published data for tilapia fingerlings (El-Saidy and Gaber 2002). Diet 4, containing catfish meal, supported the most favorable growth, exceeding the performance of fish fed a commercial anchovy meal (Diet 1). Catfish meal contained the highest levels of each essential amino acid, with the exception of methionine.

The results clearly indicate the potential to use fishery by-catch meals in practical diets for tilapia. With details of the proximate composition of by-catch species, fish meals can be made from different species or batches of species with known protein, lipid or ash content. These can then be blended to meet the specific requirements of aquafeed formulation.

Limitations on the use of fisheries by-catch and processing waste for the manufacture of silages and fish-meals for use in aquafeeds lie in the handling, maintenance of quality and logistics of using small quantities often in remote locations.

**Table 3.** Regional distribution of myctophids.

Area	Biomass (Mt)	Density (g m <sup>-2</sup> )	Density (t nm <sup>-2</sup> )
Sea of Oman (Oman)	5	73	250
Sea of Oman (Pakistan)	3	35	120
Arabian Sea	33	22	75
Gulf of Aden	5	24	83
Total	46		

## Lantern fish (Myctophidae)

Mesopelagic fish are found throughout the world's oceans and current estimates of 1000 million tonnes reveal that they dominate the world's total fish biomass, forming a deep-scattering layer at depths between 200 and 1000 m (Irigoiien *et al.* 2014). Surveys have revealed high densities in the Sea of Oman and the Arabian Sea (Gjøsaeter, 1984), where the dominant species is *Benthosema pterotum*. This is a small species of lantern fish attaining a maximum size of 30 - 50mm, over an estimated lifespan of one year or less (Hussain and Al-Khan, 1987). The regional distribution of lantern fish (Table 3) has encouraged some commercial fishing in the Sea of Oman by Iranian vessels. The trials followed a series of fishery surveys conducted between 1993 and 1998 (Valinassab, 2007). Trial catch rates (< 30 t daily per boat) were too low however to support a viable fishery and further trials were indicated to identify more efficient gear and vessel size (Valinassab *et al.*, 2007). There is also some small-scale utilization of myctophids, collected from the Arabian Sea, for fish meal manufacture in India (Sebastine *et al.*, 2011).

Specimens of *B. pterotum* were collected during the RV *Mustaqila* survey of the Oman fishery in 2007-8, freeze-dried in the Department of Marine Science and Fisheries at Sultan Qaboos University and sent to Ewos Innovation for analysis in Norway. Total protein and amino acid content shows that fish meal from lantern fish is comparable with a commercial South American anchovy meal, except for slightly lower protein and lower histidine content (Table 4). Whilst not reviewed in the present paper, parallel studies on lipid classes and fatty acid analysis of the freeze-dried fish revealed higher levels of saturated fats (40%) than are typical of commercial fish oils (20-30%). The samples also contained higher levels of phospho-lipids. n-3, long-chain PUFA values were comparable to those of South American fish oils (El-Mowafi *et al.*, 2010).

The estimated levels of heavy metals in fish meal manufactured from lantern fish showed that both cadmium and arsenic are close to current upper limits set by the European Union (Table 5). If confirmed in more extensive studies this would potentially restrict the inclusion of lantern fish meal to < 30% in a formulated aquafeed, assuming that fish meal was the only source of these metals. Persistent organic pollutants (POP) were low in

**Table 4.** Essential amino acid and total protein content of a single sample of freeze-dried lantern fish collected from the Arabian Sea compared with commercial anchovy meal (after El-Mowafi *et al.*, 2010). Values in g kg<sup>-1</sup>.

Amino Acid	Freeze dried fish	Fish meal	Anchovy meal
Arginine	36.4	37.5	37.9
Histidine	14.0	14.4	21.4
Isoleucine	24.0	24.7	30.2
Leucine	46.3	47.7	50.0
Lysine	50.4	51.9	53.3
Methionine	18.1	18.6	18.5
Phenylalanine	24.8	25.5	26.6
Threonine	27.5	28.3	28.3
Tryptophan	6.7	6.9	n/a
Valine	29.3	30.2	35.5
<b>Protein (N x 6.25)</b>	63.2	65.1	66.7

<sup>1</sup> Calculated values derived by adjustment of the freeze-dried lantern fish amino acid values to an 8% lipid and 10% moisture fish meal.

the analysed sample and were well below the upper limit for dioxins and dioxin-like compounds in feed ingredients (Table 5). Further testing for 'old' organochlorine pesticides (eg. DDT) is necessary. These preliminary results of the biochemical content of lantern fishes are of limited value since they came from a single sample. A full program of sample collections at different seasons and from different geographical areas is necessary in order to more fully evaluate their biochemical and nutrient composition and presence of contaminants.

Results from oceanographic and fishing surveys show a high myctophid biomass available and there are clear indications that the species would be highly resilient to fishing, given the short nature of their life-cycle. At present however it is not clear whether this species is a single or multiple spawner (Dalpadado, 1988) although there is some evidence that two periods of maximum spawning occur, one in March-June and one in September to November (Gjøsaeter, 1984). Prior to commercial exploitation further studies are needed to provide more details of the life-cycle, population structure and biomass of *Benthosema pterotum*. Whilst initial use of myctophids in the region has been in the manufacture of fishmeal (Valinassab, 2007) recent research in Taiwan has demonstrated the potential to manufacture protein-rich hydrolysates from the myctophid, *Benthosema pterotum* (Chai *et al.*, 2012).

## Conclusions

Marine resources exist in Oman which could contribute to the manufacture of aquafeeds. The potential to manufacture fish silages and fish meals from fisheries by-catch and processing waste has been established. Challenges

**Table 5.** Heavy metal and residue content measured in a single sample of the lantern fish, *Benthoosema pterotum*, collected from the Arabian Sea (after El-Mowafi *et al.*, 2010).

Heavy metals	Measured values in fish (mg kg <sup>-1</sup> )	Estimated values in fish meal	Maximum legal Content, EU1	Compliance
Arsenic	13.8	16.0	15.0	risk
Cadmium	1.73	2.0	2.0	risk
Lead	0.09	0.10	10.0	compliant
Mercury	0.10	0.10	0.50	compliant
Residues				
Dioxins (PCDD, PCDF and PCB's)	0.249 TEQ pg/g		4.25	compliant

lie however in the maintenance of quality in otherwise discarded materials. A necessary step in their utilization is to establish the value of these materials to both fishermen and end-users through commercial use. This may be anticipated in the future as aquaculture develops in Oman and creates increasing demand for aquafeeds. The known stocks of lantern fish present an opportunity for Oman to develop fishmeal manufacturing on an international scale. This must be preceded however by detailed studies of the resource and development of appropriate fishing methods in order to establish an economically-viable and sustainable fishery.

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