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ADNAN ALI SHQUEIR for the degree of Doctor of Philosophy

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D. C. Church

The potential use of liquefied fish as a feed ingredient for ruminants was studied in in vivo and in vitro digestion as well as in performance trials. For the in vivo digestion trials, liquefied fish (LF) prepared from whole Pacific Whiting was used to replace varying levels of the crude protein (CP) provided by urea in molasses-urea liquid supplements (LS). Two digestion trials were conducted with 20 yearling, crossbred wethers randomly allotted to four diets with 5 animals/treatment. Diets were formulated to contain 15% CP (as fed). Trial I: lambs were maintained on 647 g/day of grass hay and 353 g/day of LS (molasses-urea and LF; 30% CP, as fed). LF replaced 0, 5, 10 and 15% of CP from urea in the LS. Trial II: lambs were maintained on 665 g/day of grass hay and 335 g/day of LS (as in trial I). LF replaced 0, 2.5, 5 and 7.5% of CP from urea in the LS. Results, trial I: digestibility of CP was increased (5.5%, $P<0.10$) as was digestible acid detergent fiber (ADF, $P<0.05$) and gross energy (GE; 9.6%, $P<0.01$); N retention (NR) was also improved (34%, $P<0.05$) by supplementing with LF. Trial II: digestion coefficients were increased ($P<0.05$) for digestible dry matter (DM; 6.0%), organic matter (5.7%), ADF (14.5%) and GE (5.2%)

as was NR (31.1%) as the level of LF increased in the LS. LF seemed to be stable when stored under ambient conditions.

Two rumen-fistulated wethers were utilized for the in vivo trial to study changes in certain rumen parameters after the wethers were fed supplements containing LF, cottonseed meal (CSM) or hydrolyzed feather meal (FM) plus 900 g of grass hay. These supplements supplied 50% of the total CP in each treatment. Treatment means were different in pH, acetic/propionic acid (A/P) ratio and molar percentage of acetic (Ac), propionic (Pro), isobutyric (iB) and valeric (V) acids. LF increased ammonia N (NH_4-N, $P<0.01$) compared to CSM and FM treatments.

In vitro trials were conducted to determine the optimum level of LF in LS on digestibility of grass hay and wood cellulose (Solkafloc). Treatments were formulated to contain 14% CP (dry basis) with LF added to replace 0, 2.5, 5, 7.5, 10 and 15% of urea N in the LS. CSM and FM were used as positive controls. Digestible DM was increased ($P<0.01$) by increasing LF level. NH_4-N ($P<0.01$) and total volatile fatty acids (TVFA, $P<0.05$) were higher for the LF than for the FM treatment. When Solkafloc was used as the substrate, there were no differences ($P>0.05$) among LF treatments for the various parameters, but LF treatments increased DMD, NH_4, A/P ratio and molar percentage of Ac, Pro and iB acids compared to the controls ($P<0.05$). Protein solubility and pepsin digestibility of LF were higher ($P<0.01$) than those of CSM, FM or soybean meal.

Forty-eight single-reared wethers were used in a performance trial with 15 lambs/treatment. The treatment diets were formulated to contain

13% CP with either no protein supplement, CSM, urea or LF. After being fed ad libitum for 43 days, lambs were slaughtered and carcass data were collected. During the trial fecal grab samples were obtained and digestibility of the diets was estimated using lignin as an internal indicator. LF supplementation resulted in an increase in CP and ether extract digestibilities ($P<0.05$). N-free extract digestibility was higher for lambs fed LF compared to lambs fed urea or CSM ($P<0.01$). There were no differences in ADF and DM digestibilities, daily live and carcass gain, total feed intake, feed efficiency, yield and quality carcass grades or cost per kg of live weight and carcass gain among the lambs fed the four diets. The dressing percentage was higher for lambs fed LF compared to the other three diets ($P<0.10$). There were no differnces ($P>0.10$) in organoleptic evaluation of Longissimus muscles for tenderness, flavor or overall desirability among lambs fed the four diets. CSM increased ($P<0.05$) meat juiciness over LF and urea, and urea decreased ($P<0.05$) the aroma score compared to the other diets.

EVALUATION OF LIQUEFIED FISH AS RUMINANTS' FEED

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Professor of Animal Science
in charge of major

Redacted for privacy

Head of Department of Animal Science

Redacted for privacy

Dean of Graduate School

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EVALUATION OF LIQUEFIED FISH AS RUMINANTS' FEED

INTRODUCTION

One of the major problems facing the seafood processing industry today is that of complying with effluent disposal guidelines imposed by today's more stringent pollution control laws. Thus, increasing emphasis has been placed upon developing methods of transforming seafood processing waste into marketable by-products (Crawford, 1976). In 1970, 545,000 metric tons of solid wastes were generated by the fishing industry in the United States, and only 50% of this was recovered as feeds for livestock or furbearing animals (Soderquist et al., 1970).

At a time when the world supply of high quality protein animal feeds is likely to become less and more expensive, it is essential that suitable marine materials are not wasted. Liquefaction or ensiling processes offer means for the efficient utilization of most types of fish waste that presently might be discarded.

A variety of research studies have been carried out with ruminants fed seafood wastes. These have included crab meal (Patton et al., 1975), chitin (Ortega and Church, 1979), fish protein concentrates (Guilloteau et al., 1975) and fish solubles (Huber, 1972). Animal response has been variable, depending on the source of the product and how it has been used.

The purpose of this investigation is to evaluate liquefied fish as ruminants' feed.

CHAPTER I: The Effects of Liquefied Fish Supplementation of Molasses-Urea Liquid Supplements on Digestibility of Grass Hay by Sheep.

SUMMARY

The objectives of this study were to evaluate the effects of feeding liquefied fish (LF) in liquid supplements on in vivo digestibility of dietary components and the ability of this product to withstand the ambient conditions without losing its nutrient value. LF prepared from whole hake was used to replace varying levels of the crude protein (CP) provided by urea in molasses-urea liquid supplements (LS). LF contained 25±% dry matter (DM) and 56.1 CP (dry basis). Two digestion trials were conducted with 20 yearling, crossbred wethers randomly allotted to four treatments with 5 animals/treatment. Animals were "backgrounded" for 14 days followed by a 7-day period of restricted intake. Daily feeding was divided into two equal portions; sheep were fed at 0800 and 1600 hours. Treatments were formulated to contain 15% CP (as fed). Trial I: Lambs were maintained on 647 g/day of grass hay and 353 g/day of LS (molasses-urea and LF; 30% CP, as fed). LF replaced 0, 5, 10, and 15% of CP from urea in the LS. Trial II: Lambs were maintained on 665 g/day of grass hay and 335 g/ day of LS (molasses-urea and LF; 30% CP, as fed). LF replaced 0, 2.5, 5 and 7.5% of CP from urea in LS. Feces and urine were collected daily on an individual basis for 10 days. Aliquots (10%) of feces and urine were saved for subsequent laboratory analyses. Results, Trial I: digestibility of CP was increased (5.5%, $P<0.10$) as was digestible acid detergent fiber (ADF; $P<0.05$) and gross energy (GE; 9.6%,

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P<0.01); N retention (NR) was also improved (34%, P<0.05) as was biological value (BV; 27.7, P<0.05) by supplementing with LF. Trial II: digestion coefficients were increased (P<0.05) for digestible DM (6.0%), organic matter (OM; 5.7%), ADF (14.5%) and GE (5.2%) as was NR (31.1%) and BV (28.5%) as the level of LF increased in LS. Results indicate that digestibility was near maximum when LF replaced 7.5% of LS protein. LF seemed to be stable when stored under ambient conditions. True protein of LF was estimated to be 90+%. It had an excellent amino acid profile.

(Key words: Liquefied fish, Sheep, Digestibility, Liquid supplement, Grass hay.)

INTRODUCTION

At a time when the world supply of high quality protein animal feeds is likely to become scarcer and more expensive, it is essential that suitable marine materials are not wasted. Liquefaction or ensiling processes offer means for the efficient utilization of most types of fish waste that presently might be discarded.

Seafood processing waste (frames from filleting industry, incidental catch and underutilized species such as hake) have potential uses in animal diets. The potential tonnage available is relatively large; it is estimated that 400,000 metric tons of Pacific hake (Whiting), an underutilized species, could be harvested annually off the Pacific Coast, and that one third of this would be regarded as waste. Numerous applications have been proposed or are being used currently: Shrimp waste

as fertilizer; inclusion of solubilized fish protein in fish diets; fish scraps as mink feed or in pet foods, etc.

Liquefied fish (LF) would be expected to be superior to fish protein concentrate (FPC) because of its lower ash content and higher protein quality. The primary limiting factor is the low dry matter (DM) content (25%) which would restrict its use in animal diets. However, this product is in a form that could be used directly in preparing a liquid supplement.

A variety of research studies have been carried out with ruminants fed seafood waste. These have included chitin (Patton et al., 1975; Patton and Chandler, 1975; Ortega and Church, 1979), liquefied fish protein (Geerken, 1978; Kellems, 1980; Ortega, 1980; Shqueir et al., 1980), fish silage (Wignal and Tetterson, 1977), fish protein concentrate (Seoane and Moore, 1969; Guilloteau et al., 1975; Ternouth et al., 1975) and fish solubles (Velloso et al., 1971; Huber, 1972). Animal response has been variable, depending on the source of the product and how it was been used.

The objectives of this study were to evaluate the effects of incorporating various levels of LF in liquid supplements on in vivo digestibility of various dietary components and the ability of this product to withstand ambient conditions without losing its nutrient value.

MATERIALS AND METHODS

Preparation of LF. Whole Pacific Whiting (Merluccius productus) fish plus 10% tuna viscera were ground and allowed to autolyze at 60 C. After hydrolysis was completed, enzymes in the hydrolysate were

inactivated by pasteurizing at 77 C. Bones were removed by screening the hydrolysate. Ethoxyquin (.15%) was used as an antioxidant and potassium sorbate (.2%) as a fungistatic agent. Feedgrade phosphoric acid (85%) was added to reduce pH from an initial value of 6.67 to 3.42. Two in vivo digestion trials were conducted with 20 yearling, crossbred, wether lambs averaging 34.5 kg, randomly allotted to four treatments with five animals/treatment. Animals were "backgrounded" for 14 days, followed by 7 days of restricted intake and a 10-day collection period when animals were housed in digestion crates.

Daily feeding was divided into two equal portions, and animals were fed at 0800 and 1600 hours. Diets were formulated to contain 15% crude protein (CP, as fed). Composition of the LS and feeding regimes used in trials I and II are given in table 1. Nutrient composition of grass hay and LS used in the trials are given in table 2. Total feces and urine were collected daily on an individual animal basis, measured and a 10% aliquot removed and stored in a cooler at 4 C for later analyses. Five ml of phosphoric acid (85%) were added to the urine collection buckets every day to prevent loss of N as ammonia. Feces were dried at 65C, and LS were freeze-dried to determine dry matter. Total DM, organic matter (OM), CP, acid detergent fiber (ADF), ether extract (EE) and ash in feeds and feces were determined by methods described by AOAC (1975). Gross energy in the diets and feces were determined with a Parr adiabatic oxygen bomb calorimeter. N content of urine was also determined. N-free extract (NFE) was calculated by difference. N retention was calculated per unit of metabolic body weight (kg/kg $BW^{.75}$). Digestion coefficients

for diet components were calculated by methods described by Schneider and Flatt (1975).

In trial I lambs were maintained on 647 g/day of grass hay (7.4% CP, dry basis) and 353 g/day of LS (30% CP, as fed). LF replaced 0, 5, 10, and 15% of CP from urea in the LS. In trial II lambs were maintained on 665 g/day grass hay (6.9% CP, dry basis) and 335 g/day LS (30% CP, as fed). LF replaced 0, 2.5, 5, and 7.5% of CP from urea in the LS.

Stability of LF was monitored by taking monthly pH readings and determining lipid oxidation using a modified 2-thiobarbituric acid (TBA) method as described by Yu and Sinnhuber (1966). Mineral and amino acid contents (table 4) of LF were determined using methods described by Chaplin and Dixon (1974) and Spackman et al. (1958), respectively.

Data were analysed statistically by analysis of variance of a completely randomized design. Differences between means were tested for significance by the use of protected LSD procedures (Snedecor and Cochran, 1967).

RESULTS

The digestion coefficients for nutrients for trial I and II are summarized in table 3. In trial I, there was no significant effect of LF on digestible DM, OM, EE or NFE. Crude protein digestibility (CPD) increased ($P<0.10$) as did ADFD ($P<0.05$) and GED ($P<0.01$), and NR and biological value (BV) improved ($P<0.05$) when LF was supplemented at the 15% level. This increase was 5.5, 9, 27.7, and 34% for CP, ADF, BV and NR, respectively. In trial II, digestion coefficients increased ($P<0.05$)

for digestible DM (6.0%), OM (5.7%), ADF (14.5%) and GE (5.2%), and NR ($P<0.01$) and BV ($P<0.05$) were increased 31.1 and 28.5%, respectively, by supplementation with LF. When LF-7.5 was supplemented, there was a substantial increase in digestibility of DM, OM, and ADF, but much less response at the lower levels of LF in this trial.

The effects of increasing LF substitution of urea-N in LS on GED, ADFD, and NR (kg/kg $BW^{.75}$) for trials I and II can be best expressed in the linear equations as follow:

	Trial I	Trial II
GED, %	56.6 + 2.40 LF level	66.3 + .35 LF level
	R^2 = .71	R^2 = .13
ADFD, %	47.9 + 1.35 LF level	46.3 + .81 LF level
	R^2 = .33	R^2 = .18
NR, kg/kg $BW^{.75}$	.39 + .34 LF level	.48 + .19 LF level
	R^2 = .41	R^2 = .22

In general, NR was increased as the level of LF increased in the LS ($P<0.05$). NR (% of N fed) was increased by adding LF ($P<0.05$). Repeatability between trials I and II was quite good, with the minor differences possibly related to the difference in grass hay used in the two trials.

Summary of pH and TBA changes over a 7-month period are presented in figure 1. The LF seemed to be stable when stored under ambient conditions. No outward indication of deterioration over the 7-month period was observed.

With regard to analysis of LF (table 4), the Ca content was lower than expected, but this indicates that the removal of bones was quite complete. P was high as would be expected, since phosphoric acid was used to stabilize the product. The other mineral elements were at levels considered safe for animal consumption. The balance of amino acids was found to be good. The various batches were found to be good sources of lysine and methionine, which are normally the most limiting amino acids in diets for monogastric species. The true protein content of LF was high (90+% of CP). Based on these analyses the protein from LF should be superior to plant and most animal protein sources.

DISCUSSION

A variety of research studies with ruminants have been carried out with seafood wastes. The addition of fish solubles to liquid N supplements has resulted in a greater increase in cattle gains than would be predicted from the increase in dietary protein (Huber, 1972). Similar results were reported by Velloso et al. (1971) when fish solubles were fed to cattle. However, in a study with cattle, Ortega (1980) reported that the digestibility of DM and GE were negatively affected ($P<0.05$) when LF (whole Pacific Whiting) provided 10% of the CP of a molasses-urea LS which was fed with wheat straw. In our results reported here, LF significantly improved NR and GED compared to the basal LS with no LF.

In studies with cattle, NR was reduced on diets with 20% crab meal (Patton et al., 1975), and in vitro DM digestibility and total volatile

fatty acid concentrations were lower ($P<0.05$), but NH_4-N was similar for soybean meal or chitin treatment (Ortega and Church, 1979). Jones et al. (1976) determined that preformed protein in LS for cattle improved performance; similar results were reported by Kellems (1980). When four levels of fish meal (0, 33, 66, and 100%) replaced soybean meal in the concentrate, fish meal level had no effect on nutrient digestibility, except for CP; increasing the fish meal level decreased ($P<0.01$) CP digestibility, ruminal NH_4 levels 2 and 4 hr post-feeding and total volatile fatty acid concentration 2 hr post-feeding (Seoane and Moore, 1969). This was not the case in our data reported here in which inclusion of LF in LS significantly improved digestible CP, ADF, and GE in trial I, and DMD, OMD, ADFD, and GED in trial II.

In studies with sheep, when half of the protein was replaced with fish protein, a reduction in intake and gains were noted (Theriez et al., 1975). Similar results were reported by Sleiman and Huber (1971). Geerken (1978) reported a butyrate pattern of fermentation when molasses, hay and LF meal were fed to sheep (43, 16, and 39 mol for acetate, propionate and butyrate, respectively). The results reported herein indicated that inclusion of LF in molasses-urea LS improved nutrient utilization by sheep fed moderate quality hay.

In a performance trial, Shqueir (1981) observed that LF fed as 7% (as fed) of total diet had no effect on DMD compared to cottonseed meal-fed lambs ($P>0.05$). When fish protein was fed to lambs 1 to 3 weeks of age, there was possibly insufficient proteolytic activity in the abomasum, along with possible reduced pancreatic proteolytic activity in the small intestine (Guilloteau et al., 1975; Ternouth et al., 1975).

The digestibility of the protein fraction of FPC normally ranges from 80-90% (Raven, 1972; Gorril et al., 1975) when used in milk replacers for calves. However, Shqueir (1981) reported that an in vitro pepsin digestibility of LF protein was 96% (dry basis).

The following studies were reported for stabilizing fish products. Temmerman (1977) reported that an exogenous source of proteolytic enzyme (albacore tuna viscera, Thunnas alalunga) yielded a linear increase in the rate of proteolytic hydrolysis of ground whole Pacific Whiting up to 40% in the reaction mixture at 55 C. Furthermore, acidification of Pacific Whiting with 85% H_3PO_4 greatly accelerated proteolytic hydrolysis, yielding an optimum pH between 3.6 and 3.7. When samples of fish waste or whole fish were acidified to ca. pH 3.25 with H_3PO_4 (85%) and with the addition of K sorbate at the 0.2% level, acid-stabilized autolysates were stable to microbial growth through 8 months of ambient temperature (Culbertson, 1978). This is in agreement with our results reported here in which LF treated with H_3PO_4 (85%) and K sorbate (0.2%) was stable at ambient conditions without any indication of deterioration over the 7-month period.

The effect of sorbate on yeast and molds is well known, and sorbate is used in a wide variety of food products (Luck et al., 1976). K sorbate at concentrations of 0.1 to 1% acts as a mild antimicrobial agent (Chung, 1979). Furthermore, bacteria under enzymatic stress appeared to be especially vulnerable to the inhibitory effect of K sorbate, and mild heating or freezing have enhanced the effectiveness of this inhibitor.

Conclusions. LF has positive effects on nutrient utilization when incorporated in a LS and fed to ovines receiving grass hay, and there

were no apparent problems with regard to consumption of it by sheep. LF, when acidified with the addition of K sorbate, appears to be quite stable at ambient temperatures. The amino acid content and distribution of LF should be superior to plant and most animal protein sources.

TABLE 1. LIQUID SUPPLEMENT COMPOSITION AND FEEDING REGIME FOR IN VIVO DIGESTION TRIALS I AND II[a]

		Liquefied fish levels, %[b]							
		Trial I				Trial II			
Ingredient	IFN	0	5	10	15	0	2.5	5	7.5
Molasses	4-04-696	77.1	77.6	68.1	58.5	77.1	82.5	77.6	72.9
Urea	5-05-070	9.7	9.0	8.7	8.2	9.7	9.1	9.0	8.9
Water		9.7	----	----	----	9.7	----	----	----
Liquefied fish		----	9.9	20.2	30.8	----	4.9	9.9	14.9
Cottonseed meal	5-02-048	----	----	----	----	----	----	----	----
Sulfur (flour)		.4	.4	.4	.4	.4	.4	.4	.4
Limestone	6-02-632	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Phosphoric acid (85%)		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Monosodium phosphate	6-04-288	1.0	1.0	.5	----	1.0	1.0	1.0	.8
Kelflo[c]		.1	.1	.1	.1	.1	.1	.1	.1
Vitamin A[d]		+	+	+	+	+	+	+	+

Feeding regime, g[e]	Trial I	Trial II
Grass hay	647	665
Liquid supplement	353	335

[a]Compositions of liquid supplements on an as-fed basis.

[b]Precentage of crude protein from urea replaced by liquefied fish.

[c]Suspending aid produced by Kelco, Division of Merck & Co., Inc.

[d]3300 IU/kg liquid supplement.

[e]As fed basis

TABLE 2. NUTRIENT CONTENTS OF GRASS HAY AND LIQUID SUPPLEMENTS, TRIAL I AND II.

Item	Diets, % dry basis									
	Trial I					Trial II				
	Grass hay	LF-0 basal	LF-5	LF-10	LF-15	Grass hay	LF-0 basal	LF-2.5	LF-5	LF-7.5
Dry matter	92.6	62.6	64.6	57.2	52.7	93.0	62.6	64.1	63.6	63.3
Organic matter	92.7	85.9	85.7	85.2	85.1	91.7	85.9	85.5	85.7	85.3
Crude protein	7.4	47.9	46.4	52.4	56.9	6.9	47.9	46.8	46.4	47.4
Ether extract	2.2	.4	.8	1.0	1.8	1.9	.4	.7	.8	1.0
Acid detergent fiber	38.0	.3	.2	.2	.2	35.0	.3	.3	.2	.2
N-free extract	45.1	37.3	38.3	31.6	26.2	47.9	37.3	37.7	38.3	37.2
Ash	7.3	14.1	14.3	14.8	14.9	8.3	14.1	14.5	14.3	14.2
Gross energy, kcal/g	4.3	3.3	3.4	3.6	3.8	4.2	3.3	3.3	3.4	3.5

Table 3. APPARENT DIGESTIBILITY COEFFICIENTS AND N RETENTION DATA FOR EACH DIET, TRIALS I AND II

Item	Diets, trial I[a]					Diets, Trial II[b]				
	LF-0 basal	LF-5	LF-10	LF-15	SEM	LF-0 basal	LF-2.5	LF-5	LF-7.5	SEM
Dry matter, %	64.9	65.1	65.5	65.6	± .47	65.2^{f}	65.9^{f}	66.3^{f}	69.1^{g}	±.78
Organic matter, %	67.2	67.4	67.6	68.0	± .50	67.1^{f}	67.5^{f}	67.8^{f}	70.9^{g}	±.84
Crude protein, %	72.8^{c}	78.6^{d}	78.7^{d}	80.0^{d}	± 1.07	78.5	78.6	80.1	80.7	±.85
Acid detergent fiber,%	48.3^{f}	$51.86^{f,g}$	52.6^{g}	52.7^{g}	± 1.00	46.6^{f}	47.1^{f}	$49.4^{f,g}$	53.4^{g}	±1.38
Ash, %	44.0	42.5	41.4	40.6	± 1.79	52.6^{h}	50.6^{i}	47.9^{i}	53.1^{h}	±.9
Ether extract, %	51.4	55.6	57.8	58.9	± 2.74	57.8	60.3	62.0	64.7	±2.10
N-free extract, %	73.0	73.1	73.7	74.9	± .73	73.8^{c}	74.1^{c}	74.2^{c}	77.0^{d}	±.8
Gross energy, %	59.6^{h}	60.4^{h}	64.3^{i}	66.3^{i}	.79	66.6^{f}	66.5^{f}	$67.4^{f,g}$	69.7^{g}	±.83
N consumed/day, g	24.3	24.3	24.3	24.3	---	24.3	24.3	24.3	24.3	---
Fecal N excreted/day, g	5.2	5.1	4.9	4.9	± .16	5.2	5.2	4.8	4.7	±.20
Urinary N excreted/day, g	12.8^{e}	$11.9^{d,e}$	$11.1^{c,d}$	10.2^{c}	± .57	12.7^{c}	11.2^{d}	11.0^{c}	10.8^{c}	±.34
Biological value	33.8^{f}	$38.0^{f,g}$	$43.9^{f,g}$	47.6^{g}	± 3.52	33.6^{f}	41.4^{g}	43.4^{g}	44.8^{g}	1.72
N retention, % of N fed	27.2^{f}	29.9^{f}	$32.3^{f,g}$	38.1^{g}	± 2.13	26.5^{h}	34.1^{i}	34.6^{i}	35.7^{i}	±1.48
N retention, kg/kg BW	$.5^{f,g}$	$.5^{f,g}$	$.6^{f,g}$	$.7^{g}$	± .04	$.5^{h}$	$.6^{i}$	$.6^{i}$	$.6^{i}$	±.03

[a]Liquefied fish substitute 0, 5, 10 and 15% of crude protein from urea in liquid supplement, dry matter basis.

[b]Liquefied fish substitute 0, 2.5, 5 and 7.5% of crude protein from urea in liquid supplement, dry matter basis.

[c,d,e]Means in the same row within each trial with different superscripts are different ($P<.10$).

[f,g]Means in the same row within each trial with different superscripts are different ($P<.05$).

[h,i]Means in the same row within each trial with different superscripts are different ($P<.01$).

Table 4. NUTRIENT CONTENTS, MINERAL AND AMINO ACID ANALYSES OF LIQUEFIED FISH[a,b]

Dry matter	Crude protein	Ether extract	Ash	N free extract	Gross energy, kcal/g
%					
24.7	56.1	20.4	13.7	9.8	5.4

Mineral analysis

P	K	Na	Ca	Mg	Fe	Zn	Al	Sr	Cu	Mn	Cr	B
%					Parts per million							
1.05	.35	.13	.007	.006	19.77	12.69	6.59	3.62	1.39	.64	.27	.09

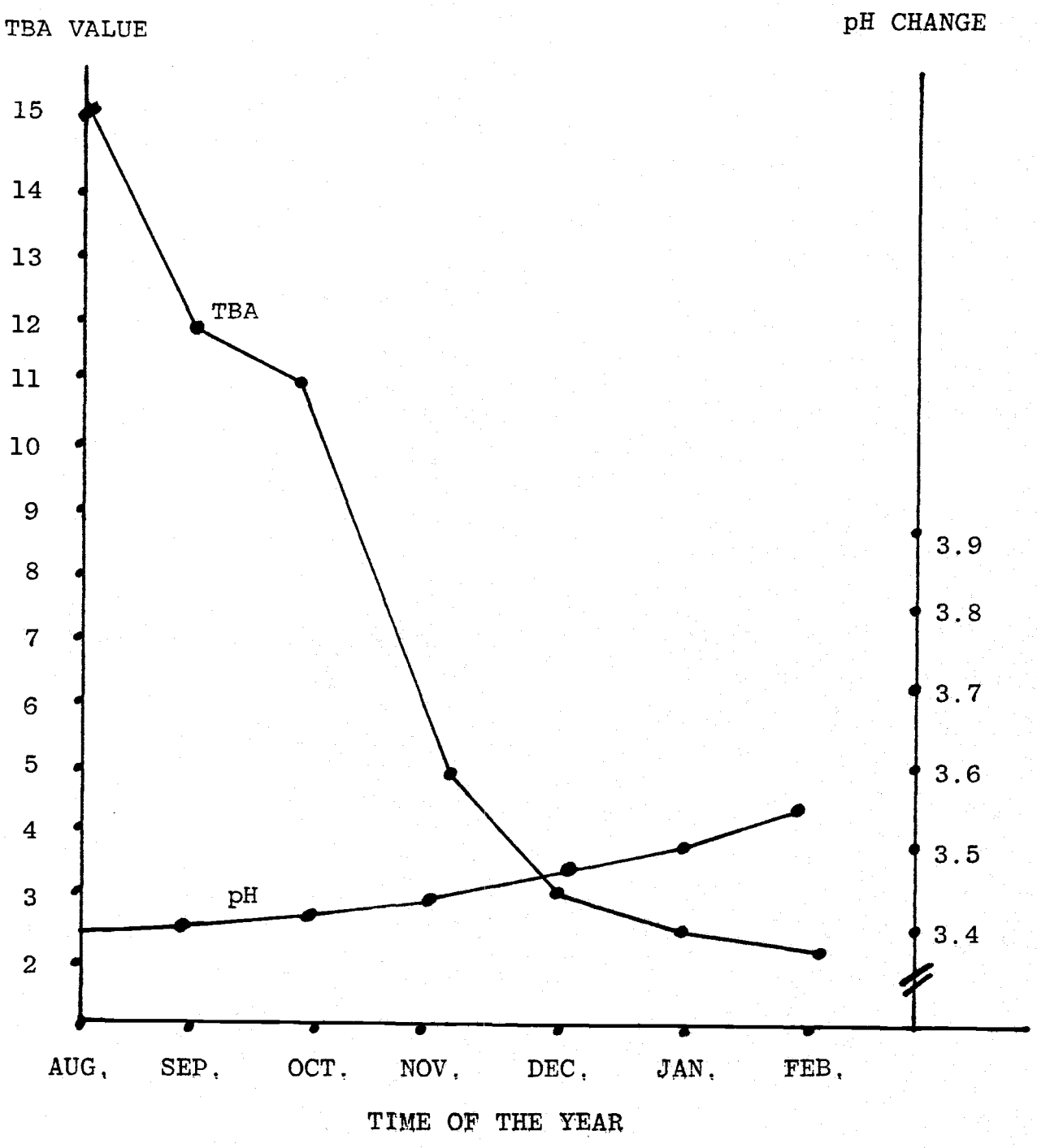
Amino acid analysis, % of total protein

Lysine	8.4	Threonine	3.7	Glycine	7.9
Histidine	1.7	Serine	4.0	Alanine	6.6
Arginine	7.0	Glutamic acid	15.2	Valine	5.2
Aspartic acid	10.4	Proline	3.6	Methionine	3.1
Isoleucine	4.5	Leucine	8.0	Tyrosine	3.2
Phenylalanine	4.1				

[a]Dry matter basis

[b]Prepared from Pacific Whiting (*Merluccius productus*) plus 10% tuna viscera (*Thunnus alalunga*) with bone removed and stabilized with Phosphoric acid (85%).

FIGURE 1: CHANGES IN PH AND TBA VALUES



CHAPTER II: Effects of Liquefied Fish in Liquid Supplements, Cottonseed Meal and Feather Meal on In Vivo and In Vitro Rumen Parameters of Sheep and on In Vitro Protein Solubility

SUMMARY

Two rumen-fistulated, crossbred wethers were utilized to study changes in rumen pH, ammonia-nitrogen (NH_4-N) and volatile fatty acid (VFA) concentration after the wethers were fed supplements containing liquefied fish (LF), cottonseed meal (CSM) and hydrolyzed feather meal (FM). These supplements supplied 50% of the total crude protein (CP) in each treatment. Supplements were fed twice daily along with grass hay. Each treatment consisted of a 12-day adjustment period and a 3-day collection period. Treatment means were different ($P<0.05$) in rumen pH, acetic/propionic acid (A/P) ratio and molar percentage of acetic (AC), propionic (Pro), isobutyric (iB) and valeric (V) acids. Rumen pH was minimal 3-4 hr after feeding. LF increased NH_4-N ($P<0.01$) compared to CSM and FM treatments. In vitro trials were conducted in which varying levels of LF were substituted for urea-N in a liquid supplement (LS). Trials were conducted to determine optimum level of LF in LS on digestibility of grass hay and wood cellulose (Solkafloc). Treatments were formulated to contain 14% CP (dry basis) with LF added to replace 0, 2.5, 5, 7.5, 10, and 15% of urea-N in LS. CSM and FM were used as positive controls. Treatment means for dry matter digestibility (DMD), NH_4-N, A/P ratio, and molar percentage of Pro and V acids were different ($P<0.01$). DMD was increased ($P<0.01$) by increasing LF level. NH_4-N

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(P<0.01) and total volatile fatty acid concentration (TVFA; P<0.05) were higher for LF than for the FM treatment. When Solkafloc was used as the substrate, there were no differences (P>0.05) among LF treatments for the various parameters, but LF treatments increased (P<0.05) DMD, NH_4, A/P ratio and molar percentage of Ac, Pro, and iB acids compared to the controls. Protein solubility and pepsin digestibility of LF were higher (P<0.01) than those of FM, CSM or soybean meal.
(Key words: Sheep, Liquefied fish, Cottonseed meal, Feather meal, Liquid supplement, Volatile fatty acid, Ammonia-nitrogen, Soybean meal, Pepsin digestibility.)

INTRODUCTION

One of the major problems facing the seafood processing industry today is that of complying with effluent disposal guidelines imposed by today's more stringent pollution control laws. Thus, increasing emphasis has been placed upon developing methods of transforming seafood processing waste into saleable by-products (Crawford, 1976).

Projected protein needs for livestock and human consumption are high. Conventional sources of protein will not likely meet these needs of the human population. Liquefied fish (LF) or waste from processing plants could serve as an alternative source of protein for livestock.

The addition of urea to molasses has improved utilization of the nutritional components of low quality roughages such as straw, but once enough urea has been added to the diet to bring animals into a small positive N balance, additional urea is primarily excreted in the urine

without promoting N deposition in the tissues (Coombe and Tribe, 1963; Ernst et al., 1975). Limited information (Coombe and Tribe, 1963; Seoane and Moore, 1969; Velloso et al., 1971; Jones et al., 1976; Kellems, 1980; Ortega, 1980, and Shqueir et al., 1980) suggests that addition of preformed proteins to liquid supplements (LS) generally results in improved animal performance when poor quality roughage is the major dietary component. Thus, the objectives of this study were to further evaluate the effects of LF on rumen parameters, in vivo and in vitro, and to relate protein solubility to N utilization.

MATERIALS AND METHODS

In Vivo Trials. Two rumen-fistulated, crossbred wethers weighing 44-47 Kg were utilized to study changes in rumen pH, ammonia-N (NH_4-N) and volatile fatty acids (VFA) when different protein supplements were fed; these were LF, cottonseed meal (CSM) and hydrolyzed feather meal (FM). The sheep were maintained on grass hay and alfalfa prior to the start of studies. Sheep were placed in separate metabolism crates designed to allow easy access to the animals. All animals had access to water and trace mineralized salt blocks ad libitum. Grass hay served as a negative control. Experimental diets and feeding regime are shown in table 1. Chemical analyses of the diets involved in the study are presented in table 2. Animals were fed twice daily. When protein supplements were fed, the treatment time consisted of a 12-day adjustment period followed by a 3-day collection period. Since Theurer et al. (1981) determined that ruminal, postruminal and total digestibilities of organic

matter, starch and protein did not differ between the 2- and 6-day collection period in fistulated steers.

Samples of approximately 100 ml of rumen fluid were taken from the center of the rumen by gentle suction through a perforated tube every hour for 4 hr post-evening feeding. After pH measurements were made, 1 ml of 25% meta-phosphoric acid was added/5 ml rumen fluid and the samples were chilled. Samples were then centrifuged at 12,500 rpm for 20 min and the supernatant fluid was stored (-20 C) until analyzed.

Nutrient contents of the diets were determined using procedures given by AOAC (1975). Gross energy in the diets was determined in an adiabatic Parr oxygen bomb calorimeter. Ammonia-nitrogen (NH_4-N) was determined using a method described by Hawk et al. (1954). The pH of the samples was taken within one min of removal using a digital pH-meter. VFA in the rumen fluid were determined by the method described by Baumgardt (1964) using gas-liquid chromatography.

In Vitro Trials. Varying levels of LF were substituted for urea-N in a 30% crude protein (CP) molasses-urea based LS. CSM and FM, which differ in solubility, served as positive controls. The composition of LS is given in table 3. Table 4 gives the chemical composition of grass hay, CSM, FM, and LS.

Trials were conducted to determine the optimum level of supplementation of LF in a LS for digestibility of a grass hay and wood cellulose (Solkafloc) substrates. Treatments were formulated to contain 14% CP (dry basis) with LF added to replace 0, 2.5, 5, 7.5, 10, and 15% of urea-N in the LS.

Rumen fluid was obtained from a rumen-fistulated steer maintained on grass hay ad libitum and LS containing both CSM and FM. Forty ml of a 3:1 mixture of McDougall's buffer (McDougall, 1948) to rumen fluid were added to 1 g of substrate, flushed with CO_2 and incubated at 39 C for 24 hours. After incubation, each sample was filtered through a preweighed Gooch crucible and dried at 100 C for 24 hr to determine dry matter disappearance (DMD). Liquid samples obtained from filtering were used for NH_4-N and VFA determination using methods described previously in the in vivo section.

Protein solubility of LF, CSM, FM, and soybean meal (SBM) were determined using a mineral mixture diluted to 10% with distilled water (Wohlt et al., 1972). Pepsin digestibilities of the above protein sources were determined with AOAC (1975) methods.

Data from the in vivo study were analyzed statistically by the use of analysis of variance for a nested design, and from the in vitro trials as a completely randomized design. Differences between means were tested for significance by the protected LSD procedure (Snedecor and Cochran, 1967).

RESULTS

In Vivo Trials. The effects of protein source on rumen parameters are presented in table 5. Treatment means were different ($P<0.05$) in rumen pH which was lower for lambs fed LF when compared to the negative control ($P<0.05$), but was not different from lambs fed CSM or FM ($P>0.05$). Rumen pH reached minimal levels 3 to 4 hr after feeding. LF increased

NH_4-N compared to the other treatments ($P<0.01$); CSM and FM treatments were intermediate in NH_4-N between LF and the negative control, but there was no difference in NH_4-N between CSM and FM treatments. NH_4-N reached peak values 2 hr after feeding.

There was only a slight increase in total VFA concentration ($P>0.10$) when lambs were fed the protein supplements compared to the control. Peak values for VFA occurred 1 to 3 hr after feeding. There were no differences in TVFA, acetic/propionic acid (A/P) ratio and molar percentages of acetic (Ac), propionic (Pro), and valeric (V) acids between LF and CSM or FM treatments ($P>0.05$). Molar percentage of Ac was lower for the control compared to the CSM and FM treatments ($P<0.05$), and isobutyric (iB) was higher for the FM treatment compared to the other treatments ($P<0.05$). Feeding CSM decreased isovaleric (iV) acid concentration compared to LF or FM treatments ($P<0.01$), but it was not different from the control.

In Vitro Trials. A summary is given in table 6 of the effects of protein sources on rumen parameters when the substrate was grass hay. Treatment means for DMD, NH_4-N, A/P ratio and molar percentage of Pro and V acids were different ($P<0.01$). DMD was increased by increasing LF levels, with a significant increase when the higher levels of LF were added.

The effects of increasing LF substitution of urea-N in LS on DMD and NH_4-N can be best expressed by the liner equations,

$$\text{DMD \%} = 58.16 + 1.48 \text{ LF level}, \quad R^2 = .65$$

$$NH_4\text{-N(mg/dl)} = 36.02 + 1.39 \text{ LF level}, \quad R^2 = .85$$

NH_4-N concentration was increased (P>0.01) by increasing LF level; LF-15 produced the highest value, but LF treatments were not different from the CSM treatment (P>0.01), while FM decreased NH_4-N concentration (P<0.01).

Differences among treatment means were significant (P<0.05) in total VFA and molar percentages of Ac, B, and iV acids. The LF-15 treatment increased total VFA compared to CSM (P<0.05), and FM treatment increased total VFA compared to all other treatments (P<0.05). The changes in total VFA caused by LF levels are expressed in the linear equation,

$$\text{TVFA (m Mol/liter)} = 61.27 + 2.18 \text{ LF level},$$

$$R^2 = .46$$

Data on Pro and A/P ratios indicate that LF enhanced the production of Pro acid.

The in vitro results when wood cellulose was used as substrate are presented in table 7. There were no differences (P>0.05) among LF treatments in parameters reported here except for V acid molar percentage. LF treatments were different from controls for DMD, NH_4-N, A/P acid ratio and molar percentages of Ac, Pro, iB, and V acids (P<0.05). FM treatment resulted in a lower value (P<0.05) for total VFA concentration compared to the other treatments.

Protein solubility results are presented in table 8. LF solubility was not affected by a change in pH of the mineral solution, but CSM, FM and SBM CP solubility increased when the pH of the mineral solution was increased. There was a marked difference (P<0.01) in protein solubility

of LF compared to CSM, FM, and SBM when measured at two pH levels. Pepsin-hydrochloric acid digestibility of protein resulted in marked differences ($P<0.01$) among CP sources.

DISCUSSION

Fish proteins from various sources have been used in a number of research studies with ruminants. The use of fish solubles in liquid supplements for cattle has improved gain (Huber, 1972; Velloso et al., 1971); similar results were reported by Kellems (1980) when liquefied fish was fed to cattle. When four levels of fish meal (0, 33, 66, and 100%) replaced SBM in the concentrate, fish meal level had no effect on nutrient digestibility, except for CP; increasing fish meal level decreased ($P<0.01$) CP digestibility, ruminal NH_4 levels 2 and 4 hr post-feeding, total VFA concentration 2 hr post-feeding (Seoane and Moore, 1969). This was not the case in our in vitro results in which peak values for NH_4 and total VFA concentration were 2 to 3 hr post-feeding, and increasing LF level increased NH_4-N and total VFA concentration, respectively.

The addition of fermentation solubles (fish and distillers) to liquid N supplements has resulted in a greater increase in animal gains than would be predicted from the increase in dietary protein (Huber, 1972). Shqueir et al. (1980) reported an increase in N retention when LF in a LS was fed to sheep. When steer and heifer calves were self-fed a complete mixed ration of 16 parts ground ear corn to 1 part of a high urea (20.5%) LS or 2.5% fish solubles (substituting for urea-N in

LS), there was a slight increase in average daily gain over the control (Perry et al., 1969). In the in vitro study reported herein, LF or a combination of LF and LS resulted in higher DMD, VFA concentrations and NH_4-N when compared to CSM or FM treatments. Elias (1971) reported that in early-weaned calves, rumen liquor pH and butyric acid content were higher in calves given urea than in those of fish meal, but in the older calves, rumen liquor pH and constituents varied according to diet. This is in agreement with our results reported here, in which pH and rumen parameters were affected by the protein source.

Shqueir et al. (1980) observed an increase in DM and acid detergent fiber digestibilities when varying levels of LF in a LS were fed to sheep receiving grass hay. This is in general agreement with our in vitro findings which demonstrated improved DMD compared to the controls. However, in a study with cattle, Ortega (1980) reported that digestibility of dry matter and gross energy were negatively affected ($P<0.05$) when LF (whole hake) provided 10% of the CP of a molasses-urea LS. Geerken (1978) reported a butyrate pattern of fermentation when molasses, hay and fish meal were fed to sheep (43, 16, and 39 mol for AC, Pro, and B, respectively). This was not the case in our study reported here.

The addition of molasses to a high urea-corn diet has been shown to improve nutrient utilization and performance (Lawrence and Mugerwa, 1974) as did the addition of preformed protein to a urea-molasses based LS (McMeniman et al., 1974) or diets with poor quality roughages (Jones et al., 1976; Kellems, 1980). Boleman et al. (1975) fed a commercial molasses mix plus urea to growing steers and observed inferior performance

of cattle compared to those fed commercial molasses mix plus natural protein. Wahlberg and Cash (1979) reported no differences in daily gain when four different liquid by-products plus urea were fed to growing-finishing steers as protein supplements. Jones et al. (1976) determined that preformed protein in a molasses-urea mixture for cattle improved dry matter and cellulose digestibilities and digestible and metabolizable energy. The results reported herein indicated that inclusion of LF in molasses-urea LS improved rumen parameters and significantly improved DMD (in vitro) over the controls.

In metabolic studies with sheep, the use of LS with whey or faba bean starch improved dry matter digestibility and N retention over urea alone on a high fiber diet (Steed et al., 1979).

Protein solubility can be influenced by factors associated with the solvent such as salt-pH, ionic strength, temperature, degree of agitation and extraction time (Lehninger, 1970). Protein solubilities vary within feeds of similar type and with the specific solvent conditions. This agrees with our findings reported here. Mahadevan et al. (1979) reported that the solubility of proteins can not be equated to their degradability, therefore, in order to evaluate by-pass protein, direct measurements of protein degradability should be done. Also, when the degradation of the protein from fish meal or SBM was measured from the amount of amino acids liberated when incubated with a proteolytic enzyme prepared from Bacteroides amylophilus (which is one of the main proteolytic micro-organisms present in the rumen), soluble fish meal proteins were hydrolyzed at about twice the rate at which soluble protein from SBM was hydrolyzed. Our results tend to confirm these observations.

When Holstein cows were fed different CP levels (12-15%) with different urea levels, the utilization coefficients for soluble N and insoluble N were relatively constant across diets, but within each diet the utilization coefficient for insoluble N was greater than for soluble N (Aitchison et al., 1976). Similar results were reported by Crawford et al. (1978). In a study with lactating dairy cows fed diets in which 22 or 42% of the N was soluble, the soluble N did not affect dry matter, CP or energy consumption. However, cows fed the diets with lower N solubility gave more milk (Majdoub et al., 1978).

Satter and Roffler (1977) reported that NH_4-N concentrations above 5 mg/dl of rumen liquor are not utilized by rumen microflora for protein synthesis by cattle. In our study, NH_4-N was in the range of 4.8 to 16.2 and 3.8 to 43.0 mg/dl for in vivo and in vitro trials, respectively. However, it has not been established that all of the differences in N utilization between different protein sources are due to differential ruminal ammonia production related to solubility.

In conclusion, LF has positive effects on rumen parameters and is highly degradable in the rumen compared to CSM, FM, or SBM proteins; its protein is highly soluble in mineral buffer and pepsin-hydrochloric acid solutions.

TABLE 1. RATION COMPOSITION AND FEEDING REGIME, IN VIVO TRIAL[a]

Ingredients	IFN	Diets, % dry basis			
		Control	Cottonseed meal	Liquefied fish	Feather meal
Grass hay	1-04-067	100.0	88.0	90.4	93.7
Cottonseed meal	5-01-621		12.0	----	----
Liquefied fish			----	9.6	----
Feather meal	5-03-795		----	----	6.3
Feeding regime[b]					
Grass hay		900.0	900.0	900.0	900.0
Cottonseed meal		----	122.0	----	----
Liquefied fish		----	----	362.0	----
Feather meal		----	----	----	58.8

[a]Fifty percent of total protein in each diet is from protein supplement.

[b]Grams, as fed basis.

TABLE 2. CHEMICAL COMPOSITION OF THE DIETS, IN VIVO TRIAL[a]

Item	Diets, dry basis			
	Negative control(hay)	Cottonseed meal	Liquefied fish	Feather meal
Dry matter, %	94.0	94.1	70.3	94.1
Crude protein, %	7.1	11.8	11.8	11.8
Acid detergent fiber, %	37.0	34.3	33.5	34.8
Ether extract, %	2.0	2.0	3.7	1.9
N-free extract, %	46.5	37.1	43.0	44.2
Ash, %	7.4	7.4	8.0	7.3
Gross energy, Kcal/g	4.3	4.3	4.4	4.4

[a]Mean of all diets.

TABLE 3. LIQUID SUPPLEMENT COMPOSITION, IN VITRO TRIALS[a]

Ingredients	IFN	Liquefied fish levels (%)[b]					
		0	2.5	5	7.5	10	15
Molasses	4-04-696	77.1	82.5	77.6	72.9	68.1	58.5
Urea	5-05-070	9.7	9.1	9.0	8.9	8.7	8.2
Water		8.7	----	----	----	----	----
Liquefied fish		----	4.9	9.9	14.9	20.2	30.8
Sulfur (flour)		.4	.4	.4	.4	.4	.4
Limestone	6-02-632	1.0	1.0	1.0	1.0	1.0	1.0
Phosphoric acid (85%)		1.0	1.0	1.0	1.0	1.0	1.0
Monosodium phosphate		1.0	1.0	1.0	.8	.5	----
Kelflo[c]		.1	.1	.1	.1	.1	.1
Vitamin A[d]		+	+	+	+	+	+

[a]Composition of liquid supplements on an as fed basis, formulated to contain 30% CP.

[b]Percentage of crude protein from urea replaced by liquefied fish.

[c]Suspending aid produced by Kelco, Division of Merck & Co., Inc.

[d]3300 IU/kg liquid supplement.

TABLE 4. NUTRIENT CONTENTS OF GRASS HAY, COTTONSEED MEAL FEATHER MEAL AND LIQUID SUPPLEMENTS, IN VITRO TRIALS

Item	Analysis, % dry basis								
	Grass hay	Cotton-seed meal	Feather meal	LF-0	LF-2.5	LF-5	LF-7.5	LF-10	LF-15
Dry matter	93.0	94.0	95.0	62.6	64.1	64.6	63.3	57.2	52.7
Organic matter	92.8	93.0	95.0	85.9	85.5	85.7	85.3	85.2	85.1
Crude protein	7.1	44.1	89.0	47.9	46.8	46.4	47.4	52.4	56.9
Ether extract	2.0	1.5	1.0	.4	.7	.8	1.0	1.0	1.8
Acid detergent fiber	37.0	16.0	1.0	.3	.3	.2	.2	.2	.2
N-free extract	46.5	31.4	4.0	37.3	37.7	38.3	37.2	31.6	26.2
Ash	7.2	7.0	5.0	14.1	14.5	14.3	14.2	14.8	14.9

TABLE 5. EFFECT OF PROTEIN SOURCE ON RUMEN pH, AMMONIA, A/P RATIO, TOTAL VOLATILE FATTY ACID CONCENTRATION AND VFA MOLAR RATIO, IN VIVO TRIAL[a]

Treatment	Sampling time, hr	pH	NH_3-N, mg/dl	Total VFA mM/liter	A/P ratio	Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric
						Molar %					
Negative control	1	6.5	7.0	63.0	2.5	64.0	25.3	.7	8.9	.3	.8
	2	6.3	5.4	62.7	2.6	64.3	24.9	.5	8.8	.8	.7
	3	6.1	3.8	62.5	2.7	65.4	24.4	.5	8.9	.2	.6
	4	6.1	3.0	63.8	2.8	66.5	23.7	.4	8.9	.0	.5
	$\bar{X}$	6.3[c]	4.8[d]	63.0	2.7[c]	65.1[f]	24.6[b]	.5[b]	8.9	.3[d,e]	.6[b]
Liquefied fish	1	6.0	15.5	69.6	2.4	63.3	26.7	1.0	7.4	.7	.9
	2	6.0	19.4	62.9	2.5	63.6	25.6	1.1	8.0	.5	1.2
	3	5.9	16.8	66.9	2.6	64.4	25.2	1.1	7.9	.3	1.1
	4	5.9	13.3	57.7	2.9	68.1	23.4	.6	7.2	.1	.6
	$\bar{X}$	6.0[b]	16.2[f]	64.3	2.6[b,c]	64.8[b,c]	25.2[b,c]	.9[c]	7.6	.4[e]	.9[c]
Cottonseed meal	1	6.2	10.4	63.5	2.4	63.5	26.4	.6	8.3	.4	.8
	2	6.1	12.1	64.8	2.6	63.7	24.9	.6	9.5	.3	1.0
	3	6.1	9.5	63.8	2.6	64.0	24.3	.4	9.6	.1	.9
	4	6.0	6.6	65.0	2.7	64.7	24.2	.5	9.5	.1	.7
	$\bar{X}$	6.1[b,c]	9.6[e]	64.3	2.6[b,c]	64.0[b]	25.0[b,c]	.5[b]	9.3	.2[d]	.8[c]
Feather meal	1	6.1	11.2	66.1	2.2	62.0	28.0	.8	7.9	.5	.8
	2	6.1	12.9	69.3	2.4	63.7	26.1	.9	8.0	.4	.9
	3	6.1	10.8	72.8	2.6	65.0	24.9	.6	8.3	.4	.8
	4	6.2	7.5	67.2	2.8	66.1	23.8	.6	8.4	.3	.8
	$\bar{X}$	6.1[b,c]	10.6[e]	68.8	2.5[b]	64.2[b]	25.7[c]	.7[b,c]	8.1	.4[e]	.8[c]
S E M		±.02	±.21	±1.87	±.04	±.23	±.29	±.09	±.93	±.02	±.03

[a] Two animals per observation. Three days was the collection period.

[b,b] Column means with different superscripts are different ($P<.05$).

[d,e,f] Column means with different superscripts are different ($P<.01$).

TABLE 6. EFFECT OF PROTEIN SOURCE AND GRASS HAY ON DRY MATTER, AMMONIA, A/P RATIO, TOTAL VOLATILE FATTY ACID CONCENTRATION AND VFA MOLAR RATIO, IN VITRO TRIALS[a]

Treatment	Dry matter digestibility, %	NH_4-N mg/dl	Total VFA mM/liter	A/P ratio	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric
					Molar %					
LF-0	$59.8^{f,g}$	35.4^{f}	65.5^{c}	1.8^{f}	58.6^{c}	$33.4^{f,g}$	.3	6.9^{b}	----	$.8^{e}$
LF-2.5	$61.2^{f,g}$	40.1^{f}	65.9^{c}	1.7^{e}	57.2^{b}	33.8^{g}	.6	$7.3^{b,c}$	$.1^{b}$	$.9^{e}$
LF-5	62.4^{g}	41.1^{f}	$67.1^{c,d}$	1.7^{e}	57.2^{b}	33.6^{g}	1.0	7.1^{b}	$.1^{b}$	1.0^{e}
LF-7.5	$63.5^{g,h}$	42.7^{f}	$68.4^{c,d}$	1.7^{e}	58.1^{c}	$32.9^{f,g}$	.6	$7.5^{b,c}$	$.1^{b}$	$.8^{e}$
LF-10	$66.0^{h,i}$	43.0^{f}	$69.4^{c,d}$	1.8^{f}	58.3^{c}	$33.0^{f,g}$	.3	$7.3^{b,c}$	$.1^{b}$	1.0^{e}
LF-15	67.1^{i}	43.1^{f}	77.9^{d}	1.8^{f}	58.3^{c}	31.6^{f}	.7	8.2^{d}	$.2^{b}$	1.0^{e}
Cottonseed meal	58.5^{f}	40.7^{f}	66.5^{c}	2.1^{g}	58.4^{c}	27.5^{e}	1.4	9.0^{d}	1.2^{c}	2.5^{f}
Feather meal	45.1^{e}	8.3^{e}	59.5^{b}	2.1^{g}	61.6^{d}	29.0^{e}	.4	$7.7^{b,c}$	$.3^{b}$	1.0^{e}
S E M	±.60	±3.44	±3.59	±.02	±.25	±.37	±.58	±.31	±.31	±.20

[a]Liquefied fish substitute 0, 2.5, 5, 7.5, 10 and 15% of crude protein from urea in liquid supplement on dry matter basis.

[b,c,d]Means in the same column with different superscripts are different ($P < .05$)

[e,f,g,h]Means in the same column with different superscripts are different ($P < .01$).

TABLE 7. THE EFFECT OF PROTEIN SOURCE AND SOLKAFLOC ON DRY MATTER, AMMONIA A/P RATIO, TOTAL VOLATILE FATTY ACID CONCENTRATION AND VFA MOLAR RATIO, IN VITRO TRIALS[a,b]

Treatment	Dry matter digestibility, %	NH_4-N, mg/dl	Total VFA mM/liter	A/P ratio	Acetic	Pro-	Iso-butyric	Butyric	Valeric
					Molar %				
LF-0	70.6[e]	13.2[d]	60.5[d]	1.3[c]	53.4[c]	40.4[e]	.1[c]	5.8	.3[c]
LF-2.5	73.6[e]	20.7[e]	60.9[d]	1.3[c]	53.1[c]	40.5[e]	----	6.3	.1[c]
LF-5	75.2[e]	21.3[e]	61.0[d]	1.2[c]	52.1[c]	41.7[e]	.1[c]	5.8	.3[d]
LF-7.5	75.5[e]	22.0[e]	61.8[d]	1.3[c]	52.8[c]	41.3[e]	.1[c]	5.7	.1[c]
LF-10	75.9[e]	23.1[e]	62.0[d]	1.4[c]	54.5[c]	39.3[e]	.1[c]	5.9	.2[c,d]
LF-15	74.8[e]	22.2[e]	60.9[d]	1.3[c]	53.3[c]	40.2[e]	.1[c]	5.8	.5[e]
Cottonseed meal	55.5[d]	10.0[d]	58.8[d]	1.7[d]	58.0[d]	32.9[d]	1.2[e]	6.6	.8[f]
Feather meal	25.2[c]	3.8[c]	40.1[c]	2.1[e]	61.3[d]	29.4[c]	.7[d]	6.9	1.0[g]
S E M	±4.89	±2.32	+2.01	±.08	±1.06	±.83	±.04	±.38	±.03

[a]Liquefied fish substitute 0, 2.5, 5, 7.5, 10 and 15% of crude protein from urea in liquid supplement on dry matter basis.

[b]Sulkafloc is a cellulose product produced by Brown Company.

[c,d,e,f,g]Means in the same column with different superscripts are different($P<.05$).

Table 8. EFFECT OF PH ON PROTEIN SOLUBILITY IN MINERAL SOLUTION AND PEPSIN-HYDROCHLORIC ACID DIGESTIBILITY OF LIQUEFIED FISH, COTTONSEED MEAL, FEATHER MEAL AND SOYBEAN MEAL

Protein Source	Crude Protein, % dry basis	Solubility in Mineral solutions %		Pepsin-hydrochloric acid, % dry basis
		PH 6.4	PH 7.8	
Liquefied fish	56.1	80.0^{c}	81.0^{c}	96.0^{d}
Cottonseed meal	44.8	16.6^{b}	31.5^{b}	60.1^{b}
Feather meal	86.6	10.4^{a}	18.9^{a}	78.2^{c}
Soybean meal	47.5	15.8^{b}	25.4^{b}	44.6^{a}
S E M		$\pm.82$	±1.08	$\pm.75$

^{a,b,c,d}Means in the same column with different superscripts are different ($P<.01$)

CHAPTER III: Feedlot Performance and Sensory Evaluation of Meat from Lambs Supplemented with Liquefied Fish

SUMMARY

The objectives of this study were to evaluate the effects of feeding liquefied fish (LF) against cottonseed meal and urea N supplements on the performance of feedlot lambs and to determine if there were any effects on the palatability of the meat. Forty-eight, single-reared lambs were randomly allotted to four treatments with three replicates of four lambs/treatment. The treatment diets were formulated to contain 13% crude protein (CP) with either no protein supplement, cottonseed meal (CSM), urea or LF. LF contained 24.7% dry matter and 56.1% CP (dry basis). During the trial fecal grab samples were collected for determining digestibility using lignin as the indicator. After being fed for 43 days, lambs were slaughtered and carcass data were collected. LF supplementation resulted in an increase ($P<0.05$) in CP and ether extract digestibilities. N-free extract digestibility was higher for lambs fed LF compared to lambs fed urea or CSM ($P<0.01$). There were no differences in acid detergent fiber and dry matter digestibilities, daily live and carcass gain, total feed intake, feed efficiency, yield and quality carcass grades or cost per kg of live weight and carcass gain among the lambs fed the four diets ($P>0.10$). The dressing percentage was higher for lambs fed LF compared to the other diets ($P<0.10$). There were no differences in organoleptic evaluation of roasted Longissimus muscles for tenderness, flavor or overall desirability among lambs fed the four

diets ($P>0.10$). CSM increased meat juiciness over LF and urea ($P<0.05$), and urea decreased the aroma score compared to the control diet ($P<0.05$). (Key Words: Liquefied Fish, Cottonseed Meal, Feedlot Lambs, Digestibility, Meat Flavor.)

INTRODUCTION

Wastes generated from the processing of seafoods have become a concern to some regulating agencies. In 1970, 545,000 metric tons of solid wastes were generated by the fishing industry in the United States, and only 50% of this was recovered as feeds for livestock or furbearing animals (Soderquist et al., 1970). The potential harvest from the sea has been estimated at 227 million metric tons annually. However, only 15% of this is currently being harvested (Timmerman, 1977).

Under the Federal Water Pollution Control Act Amendment of 1972 and the Environment Protection Agency effluent guidelines, the seafood processing industry will be required to reduce the quantity of waste that is being discharged from processing plants. These regulations are tentatively to be enforced in 1983.

There is an increased need for food to meet the needs of the ever-increasing world population. If these requirements are to be met, it is imperative to make use of all available by-products, and/or all the potential sources. Liquefied fish waste might be one important source.

A variety of research studies have been carried out with ruminants fed seafood wastes. These have included crab meal (Patton et al., 1975),

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chitin (Ortega and Church, 1979), liquefied fish protein (Kellems, 1980; Ortega, 1980), fish protein concentrates (Guilloteau et al., 1975; Ternouth et al., 1975) and fish solubles (Velloso et al., 1971; Huber, 1972). Animal response has been variable, depending on the source of the product and how it has been used.

The objectives of this study were to evaluate the effects of feeding liquefied fish (LF) against other N sources on lamb performance and to determine if there were any adverse effects on the palatability of the meat.

MATERIALS AND METHODS

Four pelleted diets were utilized in this study. Composition of the diets, their costs and their chemical analysis are presented in table 1. A control diet (60% alfalfa hay) with no protein supplement was compared against diets with supplementary N provided by LF (whole whiting, Merluccius productus), cottonseed meal (CSM) or urea. In the diets with supplementary N sources, ryegrass seed screenings were substituted for alfalfa hay in order to allow blending of diets which were isonitrogenous and approximately isocaloric. Forty-eight, single-reared, crossbred wethers averaging 34.2 kg were randomly allotted to the four diets. Each diet was fed ad libitum to three pens of four animals each. Lambs had free access to water and trace mineralized salt. Lambs were weighed bi-weekly during the trial.

Samples of diets were taken weekly, pooled and analyzed for their nutrient contents. Feces were collected directly from the rectum of

all the lambs involved during the third, fourth and fifth week of the study. Feces were freeze-dried, ground and nutrient contents of the diets and feces were determined using procedures given by AOAC (1975). With these data apparent digestibilities of diet components were calculated by the indicator method using lignin as the internal indicator (Schneider and Flatt, 1975).

Following a 43-day feeding period, lambs were slaughtered and routine carcass data were collected. Taste panel evaluations on carcasses of six lambs/treatment were conducted by the Department of Food Science and Technology, Oregon State University. Lambs racks were cooked to an internal temperature of 73.9 C and the two eye muscles (Longissimus) from the 11 to 13th rib were used by a twelve-member trained sensory panel. The roasts were evaluated for aroma, tenderness, juiciness, flavor and overall desirability on an 8-point hedonic scale with 8 being the most desirable and 1 the least.

Cost per kg of live and carcass gain were calculated using the following input costs: yardage, $0.05/hd/day; antibiotic (TM-10), $0.73/10 g; veterinary cost, $1/hd; pelleting and processing, $20/ton and table 1. Cost for the various diets are given in table 6.

Data were analyzed statistically by analysis of variance procedures for a one-way classification of a completely randomized design. A protected LSD test (Snedecor and Cochran, 1967) was used to determine levels of differences among means.

RESULTS

Diet Digestibility. Apparent average digestibilities of the diets are presented in table 2. The addition of LF had no effect on dry matter (DM) or acid detergent fiber (ADF) digestibilities ($P>0.05$). LF-fed lambs had a substantial increase in crude protein (CP) and ether extract (EE) digestibilities compared to control and urea-fed lambs ($P<0.05$). Feeding LF resulted in an increase in N-free extract digestibility compared to CSM or urea-fed lambs ($P<0.01$).

The digestion coefficients shown in table 2 are considerably lower than might be expected from a standard digestion trial. Since lignin may be digested to an appreciable extent (Fahey et al., 1979, 1980; Herrera-Saldana et al., 1981), this fact probably accounts for the low values reported here.

Lamb Growth and Feed Efficiency. The average live and carcass daily gains of the lambs fed the diets are presented in table 3. CSM-fed lambs had the highest (0.39 kg) live daily gain, while LF-fed lambs had the lowest (0.37 kg), but none of the differences in live or carcass gain were significant ($P>0.10$). The addition of LF to the diet resulted in a 3.8% reduction ($P>0.10$) in feed intake compared to the CSM diet and a 1.6% increase over the control diet. There were no differences ($P>0.10$) in feed efficiency (FE) among the four groups of lambs fed the four diets.

Carcass Traits. Data for carcass traits of lambs fed each of the four diets are presented in table 4. LF did not affect ($P>0.10$) either yield or quality grades of lambs carcasses. CSM-fed lambs tended to

have improved grades when compared to LF-fed lambs. Dressing percentages were different ($P<0.10$) among the four groups of lambs fed the four diets. LF-fed lambs had a higher dressing percentage (+1.1 and +1.5) than those fed the control and CSM, respectively.

Taste Panel Evaluation. Sensory evaluation data of the Longissimus muscle from the four treatment groups are summarized in table 5. When compared to lambs fed CSM, LF-fed lambs showed decreased juiciness ($P<0.05$); the rating on the aroma tended to be lower ($P>0.05$). The urea diet resulted in the lowest aroma value (5.6). There were no differences ($P>0.05$) in tenderness, flavor or overall desirability among the carcasses of the four groups of lambs fed the four diets.

Economic Results. Table 6 shows the costs per kg of live weight and carcass gain of lambs fed the four diets using prices in effect when the study was conducted. None of the experimental diets showed a significant effect ($P>0.10$) on cost per unit of gain. The estimated cost per kg of carcass gain favored the LF-fed lambs compared to the controls, but the most favorable cost was with the urea-fed lambs.

DISCUSSION

A variety of research studies with ruminants have been carried out with seafood wastes. Patton et al. (1975) noticed no decline in average daily gain, FE or feed intake due to the addition of up to 20% of crab (Birgus latra) meal (38% CP) to the diet of Holstein calves; however, N retention was reduced on diets with crab meal. Makdani et al. (1971) and Genskov et al. (1968) reported poor calf performance when all or a

high proportion of milk protein was substituted by fish protein. The replacement of milk protein with dried fish protein hydrolysates had no significant effects on live-weight gain, feed conversion or nutrient digestibility of milk replacers fed to lambs. Also, apparent digestibility of fish protein was not affected by the drying process (Soliman and Ørskov, 1979).

The results of the performance trial reported here are difficult to compare to results in the literature since most of the experimental work has been done with fish protein concentrate in milk replacers or LF in LS. The average daily gain, FE and feed intake were, in general, in agreement with Patton et al. (1975) who used crab meal in calf diets and Soliman and Ørskov (1979) who added dried fish protein to milk replacer fed to lambs.

In a study with cattle, the digestion of DM and gross energy were negatively affected ($P<0.05$) when LF (Whole Hake) provided 10% of the CP of a molasses-urea LS (Ortega, 1980). This was not the case in this study since LF improved total digestible nutrients ($P<0.05$) and DM digestibility ($P>0.05$) as compared to the CSM or urea supplements. When beef steers were fed straw supplemented with (10% of the CP in a LS) CSM, urea or LF, Kellems (1980) reported average daily gains were 0.15, 0.18, and 0.23 kg per day, respectively. Furthermore, incorporation of the LF into a LS increased consumption of the LS but did not decrease consumption of straw. In our performance trial with sheep fed diets with more energy, daily live or carcass gains were not affected by the N source, but there was a reduction ($P>0.10$) in total feed intake for lambs fed LF compared to CSM-fed lambs.

Jones et al. (1976) determined that preformed protein in LS for cattle improved performance, and similar results were reported by Kellems (1980). The use of fish solubles in LS for cattle has improved gain (Velloso et al., 1971; Huber, 1972). Wignal and Tetterson (1977) reported that weaned calves gained more weight and were in better condition when fed an acid-LF product (fish silage) as a protein source.

In a study with sheep, Shqueir et al. (1980) observed an increase in DM and ADF digestibility and an improvement in N retention when varying levels of LF in a LS were fed to sheep receiving grass hay. But in the performance trial reported here, LF had no significant effect on DMD or ADFD but improved dressing percentage ($P<0.10$). When half of the protein was replaced with fish protein, a reduction in intake and gains were noted in sheep (Theriez et al., 1975). Similar results were reported by Sleiman and Huber (1971). This is in agreement with observations in our performance trial.

When oily fish such as sparts (Clupca sprattus) and sand eels (Gonorhynchus gonorhynchus) are to be fed to animals at a significant level, it is essential to reduce the oil content of the fish silage to 20% to avoid the possibility of taint in the meat (Wignall and Tetterson, 1977). In our study there was no effect of diet on taste panel evaluation of the meat. Thus, either the LF had no effect on meat palatability or the amount in the diet was too low to be detected in the cooked meat. The LF made up only 2% (dry basis) of the diet and only 20% of the LF (dry basis) was ether extract.

In conclusion, LF appears to be a competitive protein supplement which can be used in animal feed in pelleted diets. Furthermore, there were no adverse effects on carcasses' palatability or quality when LF was the protein supplement.

Table 1. DIET COMPONENTS, COSTS AND CHEMICAL ANALYSES

Ingredient	IFN	Diets, % dry basis				Costs $/Kg
		Control	Cottonseed meal	Urea	Liquefied fish	
Grass seed screenings	4-26-071	10.0	45.6	30.1	43.8	.077
Alfalfa hay	1-00-068	60.0	16.0	36.0	20.1	.120
Barley	4-07-939	25.0	25.0	28.0	28.1	.154
Molasses	4-04-696	5.0	5.0	5.0	5.0	.143
Cottonseed meal	5-02-048	----	8.0	----	----	.266
Urea	5-05-070	----	----	.5	.5	.277
Liquefied fish		----	----	----	2.0	.242
Limestone	6-02-632	----	.4	----	.25	.046
Monosodium phosphate	6-04-288	----	----	.4	.25	.770
Antibiotic[a]		+	+	+	+	

Chemical analyses	Diets, % dry basis			
Item	Control	Cottonseed meal	Urea	Liquefied fish
Dry matter	91.9	91.9	91.7	90.6
Crude protein	13.0	13.0	13.0	13.0
Ether extract	2.1	2.2	2.1	3.0
Acid detergent fiber	21.0	16.0	18.4	16.3
Ash	8.5	7.9	8.6	8.4
N-free extract	55.4	60.9	57.9	59.2
Lignin	6.3	5.0	6.2	4.9

[a]Antibiotic TM-10 were added, 10 g/ton.

Table 2. APPARENT DIGESTIBILITY COEFFICIENTS AND TOTAL DIGESTIBLE NUTRIENTS OF THE EXPERIMENTAL DIETS[a]

Item	Diets, % dry basis				
	Control	Cottonseed meal	Urea	Liquefied fish	S E M[h]
Dry matter	51.4	52.3	48.1	52.8	±1.23
Organic matter	52.2[b]	60.0[c]	49.0[b]	54.5[b,c]	±1.41
Crude protein	55.9[c]	56.9[c,d]	51.6[b]	57.8[d]	±1.39
Ether extract	45.0[b]	52.7[c]	42.3[b]	62.0[d]	±2.19
Acid detergent fiber	24.8	19.5	22.7	22.7	± .56
N-free extract	55.4[f,g]	53.8[e,f]	46.1[e]	59.2[g]	±1.54
Total digestible nutrients	45.2[f]	46.3[f]	40.0[e]	50.4[g]	± .78

[a]Means are average of three pens fed each diet.
[b,c,d]Means in the same row with different superscripts are different (P<.05).
[e,f,g]Means in the same row with different superscripts are different (P<.01).
[h]Standard error of the mean.

Table 3. AVERAGE INITIAL WEIGHT, FINAL WEIGHT, DAILY LIVE AND CARCASS GAIN, TOTAL FEED INTAKE AND FEED EFFICIENCY[a]

Item	Diets, kg				
	Control	Cottonseed meal	Urea	Liquefied fish	S E M
Avg. Initial wt.[a]	34.3	34.1	34.0	34.5	±.74
Avg. final wt.[a]	49.6	52.0	51.2	50.1	±1.02
Daily gain					
Live Wt basis[a]	.38	.39	.38	.37	±.025
Carcass basis[b]	.18	.18	.18	.17	±.021
Total feed intake/pen[c]	383.1	404.8	393.4	389.3	±8.24
Feed efficiency[c]	6.0	6.0	5.9	6.1	±.24

[a]Means are average of twelve lambs fed each diet.

[b]Estimated by multiplying average daily gain by .47.

[c]Means are average of three pens fed each diet.

Table 4. MEAN VALUES OF CARCASS TRAITS

Item	Diets				
	Control	Cottonseed meal	Urea	Liquefied fish	S E M
Yield grade[a]	2.8	2.8	3.0	3.0	±.18
Leg conformation score[b,c]	12.3	13.5	13.0	13.0	----
Backfat thickness, cm[b]	.6	.6	.7	.7	----
Kidney and pelvic fat, %[b]	2.4	2.6	2.6	2.5	----
Quality grade[a]	11.9	12.2	12.2	11.8	±.29
Dressing, %[a]	47.9[e]	47.5[d]	48.7[f]	49.0[g]	±.11

[a]Means are average of twelve lambs fed each ration.
[b]Means are average of six lambs fed each ration.
[c]13 = low prime, 12 = high choice, 11 = average choice, 10 = low choice.
[d,e,f,g]Means in the same row with different superscripts are different (P<.10).

Table 5. MEAN VALUES OF CARCASS TASTE PANEL COMPARISON[a]

Item	Diets				S E M
	Control	Cottonseed meal	Urea	Lequefied fish	
Aroma	5.9[c]	5.8[b,c]	5.6[b]	5.7[b,c]	±.22
Tenderness	6.4	6.6	6.6	6.3	±.32
Juiciness	6.1[b,c]	6.4[d]	6.2[b,c]	6.0[b]	±.29
Flavor	6.4	6.4	6.2	6.4	±.29
Overall	6.1	6.2	6.0	6.2	±.32

[a]Means are average of six lambs fed each diet.

[b,c,d]Means in the same row with different superscripts are different ($P<0.05$).

Table 6. COST PER KILOGRAM OF LIVE AND CARCASS WEIGHT GAIN OF LAMBS FED THE FOUR DIETS

Item	Diets				
	Control	Cottonseed meal	Urea	Liquefied fish	S E M
Feed intake/lamb, kg	95.80	101.22	98.40	97.32	----
Cost/T diet, $	146.08	142.15	141.84	137.26	----
Feed cost/lamb, $	13.99	14.38	13.96	13.34	----
Veterinary cost/lamb, $	1.00	1.00	1.00	1.00	----
Yardage cost/lamb, $	2.15	2.15	2.15	2.15	----
Total cost/lamb, $	17.14	17.54	17.10	16.51	----
Total gain/lamb, kg	16.00	16.81	16.60	15.70	----
Cost/kg live gain, $	1.07	1.04	1.03	1.05	±.036
Estimated carcass gain/lamb, kg	7.52	7.92	7.81	7.40	----
Estimated cost/kg carcass gain, $	2.28	2.21	2.19	2.23	±.086

CONCLUSIONS

From the data obtained in this study the following conclusions were derived:

* Acidified LF appears to be stable at ambient temperatures.
* The amino acid content and distribution should be superior to plant and most animal protein sources.
* LF has positive effects on nutrient utilization when incorporated in LS and fed to sheep.
* LF has positive effects on rumen parameters.
* LF is highly degradable in the rumen and its protein is highly soluble in mineral buffer and pepsin-hydrochloric acid solutions.
* LF appears to be a competitive protein supplement which can be used in animal feed in pelleted diets.
* LF has no adverse effects on carcasses palatability or quality.
* There were no apparent problems with regard to consumption by sheep.

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