Indian Journal of Animal Sciences **81** (1): 84–86, January 2011

Influence of protected sardine oil on *in vitro* rumen fermentation and nutrient digestibility of complete diet

K AMBASANKAR[1] and V BALAKRISHNAN[2]

Tamil Nadu University of Veterinary and Animal Sciences, Chennai, Tamil Nadu 600 007 India

Received: 26 November 2009; Accepted: 12 October 2010

Key words: Fish oil, Nutrient digestibility, Protected fat, Rumen fermentation

Omega 3 polyunsaturated fatty acid (n–3 PUFA) especially eicosa pentaenoic acid (EPA) and docosa hexaenoic acid (DHA) can play a very important role in reducing the cardio vascular disorders. Fish oil obtained from Indian oil sardine is an excellent source of these fatty acids (Ambasankar and Balakrishnan 2006). Inclusion of these n–3 fatty acids in ruminant diet results in extensive ruminal biohydrogenation and can greatly disrupt fermentation in the rumen causing reduced digestibility of non-lipid energy sources especially the fibre portion of the diet. In this context, ways and means to prevent their degradation in the rumen, but get delivered post ruminally for enhancing effective transfer into the milk and meat is an important step. The method of protecting fat/oil supplementation from rumen degradation varies according to the source. Hence, this research work was carried out to examine the influence of various forms of protected sardine oil in complete diet on rumen fermentation and nutrient digestibility.

Sardine fish oil was collected from 5 sardine oil-producing units in the West Coast of India and was protected from ruminal degradation by 3 methods, viz. calcium soap of sardine oil, formaldehyde treated protein encapsulated sardine oil (FTPE), and fatty acyl amide of sardine oil. Calcium soaps of sardine oil were prepared by precipitation method (Sampelayo *et al*. 2002), FTPE of sardine oil as per Sutton *et al*. (1983), and the fatty acyl amide of sardine oil was synthesized as per Loor *et al.* (2002).

Four complete diets were formulated with 50% fodder (hybrid *Pennisetum glaucum* × *Pennisetum purpureum*), 27% rice bran, 20% maize and 3% any one of fat sources, viz. sardine oil or calcium soap of sardine oil or FTPE sardine oil or fatty acyl amide of sardine oil. Mineral and vitamin mixtures were added to provide adequate level. The complete rations were formulated to meet the nutrient requirement as per ICAR (1985).

The 4 experimental rations were subjected to proximate composition (AOAC 1997) and fibre fractions in triplicate. All the 4 complete rations were evaluated using Hohenheim gas production test as per Menke and Steingass (1988) to evaluate their fermentation characteristics. In this study, individual ingredients were separately ground to pass through 1 mm sieve. They were then loaded into the syringe one after the other to make up 500 mg at the proportion mentioned earlier for all the rations. The syringes were pre-warmed at 40°C prior to incubation and were incubated at 39°C in shaking water bath, specially designed to accommodate 100 syringes. Incubation of syringes was carried out in triplicate. Two runs were made in this experiment, thus there were 6 measurements for each parameter studied.

The *in vitro* apparent digestibility at 48 h of incubation was determined as per Blummel *et al*. (1997). True degradability was calculated as the weight of substrate incubated minus the weight of the residue after NDS. Digestibility of fiber fractions (NDF, ADF, hemicellulose and cellulose) was arrived from the difference between fibre fraction present in unincubated sample and residue after 48 h incubation. Total gas production was recorded at different incubation hours. The net gas volume at each incubation hour was calculated by subtracting the mean gas volume of blank from mean gas volume of syringe containing the sample. The total gas was partitioned as CO_2 and CH_4 using saturated KOH solution. Ammonia nitrogen was estimated at 48 h of incubation.

Chemical composition and nutrient digestibility of complete diet: The per cent chemical composition, *in vitro* true and apparent dry matter digestibility, crude protein, neutral detergent fiber, acid detergent fiber, hemicellulose and cellulose digestibility of complete diets containing sardine oil and various forms of protected sardine oil are presented in Table 1. A significant difference (P<0.05) in true and apparent DMD and microbial biomass production in diets

Present address: [1]Senior Scientist (e mail: ambasankar @ciba.res.in), Central Institute of Brackishwater Aquaculture, Chennai 600 028.

[2]Professor and Head, Department of Animal Nutrition, Madras Veterinary College, Chennai.

Table 1. Per cent chemical composition and *in vitro* digestibility of nutrient in complete diets containing sardine oil and various forms of protected sardine oil at 48 hours of incubation along with the respective microbial biomass production (mean±SE)

Treatment Nutrient	Sardine oil	Calcium soap of sardine oil	FTPE sardine oil	Fatty acyl amide of sardine oil
Chemical composition (mean*±SE)				
Dry matter	90.31±1.03	91.06±1.10	90.07±1.01	90.11±0.89
Crude protein	9.15±0.95	9.01±0.78	9.97±0.83	9.23±0.76
Ether extract	5.10±0.02	5.97±0.03	5.91±0.03	5.01±0.04
Crude fibre	21.11±1.02	20.98±0.97	210.3±0.09	21.14±0.85
Total ash	5.65±0.32	6.41±0.09	5.61±0.14	5.47±0.11
NFE	58.99±1.23	57.63±0.08	57.48±0.93	58.85±0.97
Neutral detergent fibre	40.15±1.32	41.17±0.89	40.65±0.78	41.17±0.66
Acid detergent fibre	21.56±0.85	22.15±0.78	21.23±0.56	20.98±0.53
Hemicellulose	18.59±0.34	19.02±0.43	19.42±0.43	20.19±0.24
Cellulose	17.91±0.35	18.45±0.42	17.03±0.32	16.95±0.11
In vitro digestibility of nutrient in complete rations (mean**±SE)				
Apparent DMD	63.17^{a}±0.87	64.35^{b}±1.21	64.65^{b}±1.71	65.63^{c}±1.67
True DMD	66.53^{a}±0.91	68.96^{b}±1.31	68.41^{b}±0.97	71.79^{c}±1.91
Microbial biomass	3.36^{a}±0.09	4.61^{a}±0.11	4.76^{a}±0.13	6.16^{b}±0.21
Crude protein	54.42±0.73	58.11±1.37	59.16±1.57	58.61±0.95
Neutral detergent fibre	56.13±1.11	55.17±1.41	56.93±1.57	57.61±0.95
Acid detergent fibre	47.15±0.93	48.53±1.71	46.13±1.56	47.13±0.78
Hemicellulose	50.32±0.86	50.97±0.91	51.09±1.17	49.96±1.11
Cellulose	52.11^{a}±0.79	53.25^{a}±0.86	54.91^{ab}±1.01	56.01^{b}±1.07

*Mean of 3 observations; **mean of 6 observations; means bearing different superscripts within in a row differ significantly (P<0.05).

containing sardine oil and various forms of protected sardine oil were observed. However, Loor *et al.* (2002) reported a nonsignificant difference in dry matter digestibility in cow fed with canola oil, canolamide and mixture of oil and amide. Significantly, highest (P<0.05) true and apparent digestibility in fatty acyl amide of sardine oil indicates that the negative effects of free oil is reduced by converting into fatty acyl amide.

The crude protein, neutral detergent fibre, and acid detergent fibre digestibility showed no significant difference among various treatments. A significant (P<0.05) increase in the cellulose digestibility was observed in fatty acyl amide of sardine oil. The increased cellulose digestibility in the diet containing fatty acyl amide of sardine oil showed that fatty acyl amide had effectively bound sardine oil and thereby reduced the negative effect of unsaturated oil on fibre digestibility.

Rumen fermentation characteristics: The cumulative gas production and CO_2: CH_4 ratio of sardine oil and different forms of protected sardine oil are given in Table 2. The complete diet containing FTPE of sardine oil had significantly (P<0.05) lowest gas production. While no

Table 2. Cumulative gas produced (ml) 500 mg of complete diets formulated to test the efficacy of protecting sardine oil at various hours of incubation along with the CO_2: CH_4 (mean*±SE)

Treatment	Incubation (h)				
	8	12	24	36	48
Cumulative gas produced (ml) per 500 mg of complete diets					
Sardine oil	27.00^{ab}±1.20	48.00±1.60	75.00±2.10	90.00±2.30	112.00^{a}±2.70
Calcium soap of sardine oil	31.00^{b}±1.10	50.00±1.65	79.00±1.83	95.00±1.78	120.00^{b}±1.91
FTPE of sardine oil	25.00^{a}±0.66	50.00±0.93	76.00±1.50	91.00±2.10	116.00^{ab}±2.60
Fatty acyl amides of sardine oil	29.00 ^{b}±2.10	49.00±1.30	77.00±1.80	93.00±2.10	117.00^{b}±2.13
CO_2: CH_4^{NS}					
Sardine oil	1.46±0.07	1.50±0.09	1.59±0.18	1.57±0.19	1.59±0.19
Calcium soap of sardine oil	1.43±0.06	1.49±0.07	1.48±0.17	1.61±0.18	1.54±0.21
FTPE sardine oil	1.35±0.06	1.57±0.08	1.53±0.19	1.47±0.21	1.61±0.17
Sardine oil amide	1.51±0.07	1.41±0.11	1.51±0.60	1.59±0.23	1.53±0.14

*Mean of 6 measurements; means bearing different superscript within a column differ significantly (P<0.05); ^{NS} nonsignificant.

significant difference in total gas production was observed among the sardine oil and various forms of protected sardine oil containing rations at 12, 24 and 36 h of incubation. At 48 h of incubation a significantly ($P<0.05$) lower level of gas production was observed in sardine oil containing complete diet but that was comparable to FTPE of sardine oil. Whereas significantly higher ($P<0.05$) gas production was observed in sardine oil of calcium soap or fatty acyl amide containing complete diet at 48 h of incubation. This observation further supports that protection by calcium soap and fatty acyl amide depressed the negative effect on digestibility and resulting in increased gas production

Irrespective of treatments, no significant variation in CO_2: CH_4 ratio was observed at different hours of incubation. Our results indicate that sardine oil at 3% dietary dry matter was not sufficient to inhibit methane production. The ammonia nitrogen (mg/100 ml) level of complete diets were 6.11±0.07, 6.07±0.06, 6.08±0.07 and 6.12±0.06, respectively, for sardine oil, FTPE of sardine oil, calcium soap of sardine and fatty acyl amide of sardine oil, and did not differ ($P>0.05$) among themselves. This was in line with the findings of Naik *et al.* (2010). This infers that oil and protected forms of sardine oil had no significant effect on protein digestibility.

SUMMARY

The present study was carried out with the objective to examine the influence of various forms of protected sardine oil in a complete diet on rumen fermentation and nutrient digestibility**.** *In vitro* gas production studies on 4 complete diet having sardine oil, calcium soap of sardine oil, formaldehyde treated protein encapsulated sardine oil and fatty acyl amide of sardine oil at 3% levels revealed significantly higher digestibility of apparent dry matter (65.63%), true dry matter (71.79%) and cellulose (56.01%) in 3% fatty acyl amide of sardine oil based complete diet. No significant difference in ammonia nitrogen, total gas production and CO_2: CH_4 ratio was observed among the experimental diets at 12, 24 and 36 h of incubation. Significantly higher microbial biomass production (6.16%) was observed in diet containing fatty acyl amide of sardine oil. This study revealed fatty acyl amide of sardine oil based complete diet was effective in protecting the fat from degradation in rumen and improves the apparent and true DMD. However, further studies are needed to conclusively tell the improved nutritive value of the diets through this protection technique.

ACKNOWLEDGEMENT

The author is grateful to Director, CIBA, for granting study leave to the first author and Dean, Madras Veterinary College, for providing necessary facilities to carry out this work.

REFERENCES

Ambasankar K and Balakrishnan V. 2006. Indian sardine oil (*Sardinella longiceps*) as a source of Omega 3 fatty acids. *Animal Nutrition and Feed Technology* **6**: 283–87.

A O A C. 1997. *Official methods of Analysis.* 18th edn. Association of Official Analytical Chemists, Benjamin Franklin Station, Washington D C.

Blummel M, Makkar H P S, Chisanga G, Mtimuni J and Becker K. 1997. The production of dry matter intake of temperate and tropical roughages from *in vitro* digestibility/gas production of African roughage in relation to ruminant live weight gain. *Animal Feed Science and Technology* **69**: 131–41.

I C A R. 1985. *Nutrient Requirements of Livestock and Poultry*. Directorate of Information and Publications, ICAR, New Delhi.

Loor J J, Herbein J H and Jenkins T C. 2002. Nutrient digestion, biohydrogenation and fatty acid profiles in blood plasma and milk fat from lactating Holstein cows fed canola oil or canolamide. *Animal Feed Science and Technol*ogy **97**: 65–82.

Menke K H and Steingass H. 1998. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Development* **28**: 7–55.

Naik P K, Saijpaul S and Kaur K. 2010. Effect of supplementation of indigenously prepared rumen protected fat on rumen fermentation in buffaloes. *Indian Journal of Animal Sciences* **80**: 902–05.

Sampelayo S M R, Perez L, Martin J J, Extremra F G and Boza J. 2002. Effects of concentrates of protected fat rich in PUFAs on the performance of lactating Granadina goats. 1. Feed intake, nutrient digestibility, N and energy utilization for milk production. *Small Ruminant Research* **43**: 133–39.

Sutton J D, Knight R, McAllan A B and Smith R H. 1983. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. *British Journal of Nutrition* **49**: 419–32.