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Effect of varying levels of heat and formaldehyde treatment of sardine fishmeal on nitrogen solubility, *in vitro* ammonia release and protein fractions

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ABSTRACT

The study was conducted to determine the effect of different levels of heat and formaldehyde treatment on nitrogen solubility, *in vitro* ammonia release and protein fractions of sardine fish meal. Heat treatment of sardine fish meal was carried out at 110, 120, 130, 140 and 150°C for 30, 60 and 120 min. A significantly lowest nitrogen solubility was observed at 150°C for 120 min in all the 4 solvents tested. A significantly lowest ammonia concentration was observed in heat treated sardine fish meal at 150°C at 120 min. A significantly higher $B_2 + B_3 – C$ (undegrdable but digestible protein) was obtained at 130°C for 120 min and there was no significant increase beyond this temperature and time. Formaldehyde treatment was examined at 0.5, 1.0, 1.5 and 2.0 g formaldehyde per 100 g crude protein. The nitrogen solubility and *in vitro* ammonia concentration was reduced significantly at 1% formaldehyde treatment. Sardine fish meal protein fractions A, B_1, B_2 and B_3 showed significant change at 1% formaldehyde treatment. On a holistic view it could be considered that heat treatment at 130°C for 120 min and formaldehyde treatment at 1% level were optimal for protection sardine fish meal protein.

Key words: Ammonia release, Fishmeal, Protected protein, Protein fractions, Solubility

Ruminal crude protein degradation of fish meal ranges from 30 to 70% (Boucher *et al.* 2009). Sardine fish meal is an attractive protein source for its superior amino acid make up and higher essential amino acid index for livestock (Ambasankar and Balakrishnan 2005) and hence protection of sardine fish meal protein assumes significant importance. Many methods were in vogue to protect the protein from ruminal degradation (Mohamed and Chaudhry 2008). In this study, an attempt was made to protect the quality protein in sardine fish meal by heat and formaldehyde treatment with a view to optimize the temperature and time combination of heat treatment and the level of formaldehyde. Further, a successful protection technique is one which makes the protein undegradable in the rumen and available at the intestinal site for absorption. The protection techniques were evaluated in terms of nitrogen solubility, in vitro ammonia release and change in protein fractions.

MATERIALS AND METHODS

Heat treatment

Heat treatment of sardine fish meal was carried out as per Sampath *et al*. (1997). Sardine fish meal was uniformly spread in a forced draft oven as 2 cm deep layers on trays that were placed in oven temperature of 110, 120, 130, 140 and 150°C for 30, 60 and 120 min. After each specified period of heating, the meal in the tray was allowed to cool to room temperature and then stored in air-tight container for subsequent studies.

Formaldehyde treatment

Formaldehyde treatment for protection of sardine fish meal was adopted from the method developed by Ashes *et al.* (1995). Formaldehyde solution (40% w/v) was sprayed on the sardine fish meal samples to have a final concentration of 0.5, 1.0, 1.5 and 2.0 g formaldehyde per 100 g crude protein. The sprayed samples were thoroughly mixed and stored in plastic containers for reaction to complete (Sengar and Mudgal 1983). Containers were opened after 7 days and Sardine meal was poured on to plastic sheets to a depth of 3–5 cm and allowed to air equilibrate for 72 h. After equilibration it was stored in air tight containers until use.

Evaluation of protection of protein

Effect of protection of protein was evaluated for its nitrogen solubility in 4 different solvents, viz. phosphate buffer, McDougall buffer, Autoclaved rumen fluid (ARF)

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and autoclaved RUSITEC fermenter fluid. The pH of the solvents is maintained at 7.0. Phosphate buffer and McDougall buffer were prepared as per McDougall (1948). The rumen fluid collected from caprine maintained on grazing was filtered through cheese cloth and autoclaved for 1 h at 1.05 kg/cm^2 steam (102°C) pressure to prepare autoclaved rumen fluid. The fluid was then cooled to 39°C and filtered through glass wool to remove clumped solids. The rumen culture maintained in the fermenter of the RUSITEC was collected from the fermenter after seven days of adaptation in the RUSITEC fermenter. The duration, pressure and filtration techniques were similar to the preparation of autoclaved rumen fluid. Nitrogen solubility in the four solvents were estimated as per the procedure described by Mehra *et al.* (1998). About 1.0 g of ground sardine fish meal and the heat/formaldehyde treated sardine fish meal in triplicates was taken in a conical flask (150 ml capacity) and 50 ml of the respective solvent was added. These flasks were incubated for 2 h at 39°C in a constant shaking water bath. After 2 h, the flasks were removed and the contents were filtered through whatman No.41 filter paper. The filtrate was collected and measured. A known amount of filtrate was digested and distilled using Kjeltec to know the soluble N content of the sample.

The procedure adopted by Walli *et al.* (2000) was used for estimating the *in vitro* ammonia release. Sardine fish meal (control) as well as heat/formaldehyde treated sardine fish meal of 0.5 g of dried sample in triplicates was incubated in Tilley and Terry tube after adding 40 ml of McDougall artificial saliva and 10 ml of strained rumen liquor. The 0 h samples were taken as control for subtraction. Anaerobic incubations were carried out for all the samples for 2 and 6 h. At the end of respective incubation period, fermentation was terminated by the addition of few drops of concentrated H_2SO_4. Ammonia was estimated using ion selective ammonia electrode using EA 940 model meter. The different protein fractions A, B_1, B_2, B_3 and C were estimated in triplicate in sardine meal and heat/formaldehyde treated sardine meal as per Lictra *et al.* (1996) and NRC (2001). Fraction A is non-protein nitrogen (NPN), B is true protein, and C is unavailable true protein or bound protein. Fraction B is further divided into 3 fractions (B_1, B_2, and B_3) that are believed to have different rates of ruminal degradation. Fractions A and B_1 are soluble in borate phosphate buffer and are rapidly degraded in the rumen. Fraction B_2 is fermented in the rumen at lower rates than buffer-soluble fractions, and some fraction B_2 escapes to the lower gut. Fraction B_3 is believed to be more slowly degraded in the rumen than the fractions B_1 and B_2 because of its association with the cell wall; a larger proportion of B_3 is thus believed to escape the rumen. Fraction C is the acid detergent insoluble protein (ADIP), and is highly resistant to breakdown by microbial and mammalian enzymes, and it is assumed to be unavailable for the animal (Tham *et al.* 2008). The data were analyzed statistically (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Nitrogen solubility

The nitrogen solubility of sardine fish meal as affected by heat treatments in various solvents is presented in Table 1. In McDougall buffer the lowest nitrogen solubility was observed at 150°C at 120 min and it was significantly ($P<0.01$) different from the rest. The progressive decline in nitrogen solubility was observed with incremental increase in heat as well as duration. The results indicate that maximum temperature (150°C) tried for 120 min lead to reduced nitrogen solubility to the extent of 44.01% in the sardine fish meal. Whereas in phosphate buffer significantly ($P<0.01$) lowest nitrogen solubility was observed at 150°C for both 60 and 120 min, when compared to the rest. Though similar trend of reduction in solubility with increase in temperature and duration was observed in this solvent also, a clear trend of steep fall ($P<0.01$) in nitrogen solubility was observed only from 140°C at 60 mins onwards.

A striking similarity between the values of ARF, with autoclaved RUSITEC fermenter fluid was noticed with respect to nitrogen solubility in all the time and duration of heat treatment. Mondal *et al*. (2008) reported higher values of nitrogen solubility of fish meal in phosphate buffer Whereas Mehra *et al.* (1998) reported that the N solubility of fish meal was significantly ($P<0.01$) more in phosphate buffer compared to McDougall's and ARF. Reduction in nitrogen solubility can be considered to be a useful measure of converting the readily soluble fraction of protein in to insoluble but degradable protein fraction (De Jonge *et al*. 2010, Mahala and Gomaa 2007). However, it is not confirmative by itself as the fate of converted soluble nitrogen is questionable. It is quite possible that the converted soluble nitrogen could have become undegradable and it is in this context a cautious approach is needed with respect to nitrogen solubility.

In contrast to results of heat treatment, uniform response was noticed among the 4 solvents at 4 levels of formaldehyde treatment. The nitrogen solubility was reduced significantly ($P<0.01$) in all 4 solvents at 1% formaldehyde treatment. No statistically significant ($P<0.01$) reduction in nitrogen solubility was observed on further increasing the level of formaldehyde beyond 1%.

In vitro *ammonia release*

A significantly ($P<0.01$) lowest ammonia concentration at 2 h was observed in heat treated sardine fish meal at 150°C for both 120 min as well as 60 min. Similar to nitrogen solubility a pattern of gradual decrease in *in vitro* ammonia concentration up to 140°C at 60 minutes and there after a much more rapid decline in ammonia production was observed at 2 h. At 6 h of incubation the mean ammonia concentration was significantly ($P<0.01$) lowest in heat-

Table 1. Nitrogen solubility (%) and the *in vitro* ammonia (mg/100 ml) release as affected by various treatments

Treatment		Nitrogen solubility (%)				*In vitro* ammonia release (mg %)	
Temp	Time	McDougall buffer	Phosphate buffer	Autoclaved rumen fluid	RUSITEC fluid	2 h	6 h
Control**		15.54 0.01	14.95±0.01	14.86±0.02	14.81±0.01	9.03±0.04	5.32±0.03
Heat treatment*							
110°C	30 min	14.51^{i}±0.02	13.76^{g}±0.06	13.85^{h}±0.03	13.78±0.02	8.81^{i}±0.06	4.90^{j}±0.02
110°C	60 min.	13.82^{h}±0.01	13.72^{g}±0.05	13.74^{h}±0.03	13.73^{hi}±0.03	8.71^{hg}±0.06	4.81^{ij}±0.03
110°C	120 min	13.23^{g}±0.02	13.62^{fg}±0.02	13.61^{gh}±0.02	13.63^{h}±0.01	8.64^{hi}±0.02	4.70^{i}±0.02
120°C	30 min	13.49^{f}±0.01	13.80^{g}±0.06	13.82^{oh}±0.06	13.82^{i}±0.05	8.56^{gh}±0.03	4.51^{h}±0.02
120°C	60 min	13.01^{f}±0.21	13.41^{f}±0.24	13.49^{fg}±0.05	13.46^{gh}±0.04	8.45^{fg}±0.01	4.32^{g}±0.03
120°C	120 min	12.84^{e}±0.01	13.26^{ef}±0.03	13.21^{ef}±0.03	13.21^{fg}±0.03	8.37^{fg}±0.03	4.40^{gh}±0.03
130°C	30 min	10.96^{d}±0.03	13.15^{ef}±0.03	13.14^{e}±0.05	13.16^{fg}±0.03	8.26^{ef}±0.01	4.10^{f}±0.02
130°C	60 min	10.94^{d}±0.05	13.10^{e}±0.05	13.11^{e}±0.07	13.09^{ef}±0.05	8.01^{d}±0.01	4.10^{f}±0.03
130°C	120 min	10.76^{c}±0.10	13.00^{e}±0.06	12.97^{de}±0.05	13.01^{e}±0.03	7.81^{d}±0.04	3.81^{e}±0.04
140°C	30 min	10.90^{d}±0.01	12.36^{d}±0.07	12.44^{d}±0.03	12.39^{d}±0.02	6.93^{c}±0.07	3.60^{d}±0.03
140°C	60 min	10.92^{d}±0.06	12.30^{d}±0.01	12.30^{d}±0.01	12.31^{d}±0.03	6.80^{c}±0.08	3.40^{c}±0.01
140°C	120 min	10.86^{cd}±0.01	11.55^{c}±0.05	11.66^{c}±0.02	$11.60^{c}_{\pm}$0.02	6.41^{a}±0.06	3.11^{b}±0.02
150°C	30 min	8.90^{b}±0.03	10.18^{b}±0.05	10.23^{b}±0.05	10.27^{b}±0.01	6.53^{b}±0.15	$3.10^{b}_{\pm}$0.03
150°C	60 min	8.90^{b}±0.02	8.80^{a}±0.03	8.91^{a}±0.04	8.93^{a}±0.03	6.23^{a}±0.05	3.10^{b}±0.02
150°C	120 min	8.70^{a}±0.01	8.70^{a}±0.02	8.75^{a}±0.03	8.70^{a}±0.02	6.05^{a}±0.03	2.84^{a}±0.03
Formaldehyde	0.5	12.34^{II}±0.02	12.41^{II}±0.07	12.27^{II}±0.13	12.61^{II}±0.03	7.84^{II}±0.02	4.50^{II}±0.04
treatment* (%)	1.0	10.66^{I}±0.15	10.67^{I}±0.15	10.21^{I}±0.18	10.41^{I}±0.18	5.51^{I}±0.03	3.54^{I}±0.07
	1.5	10.44^{I}±0.22	10.34^{I}±0.22	10.11^{I}±0.11	10.32^{I}±0.21	5.46^{I}±0.04	3.23^{I}±0.06
	2.0	10.17^{I}±0.50	10.27^{I}±0.25	10.02^{I}±0.14	9.99^{I}±0.31	5.42^{I}±0.03	3.21^{I}±0.13

Mean of 3 observations.

*Mean having different alphabet as superscript in the same column for heat treatment and roman letter as superscript for formaldehyde treatment differ significantly (P<0.01); ** Not included in statistical evaluation.

treated Sardine meal at 150°C for 120 min. In contrast to the pattern observed at 2 h, *in vitro* ammonia release did not show any pattern or trend at 6 h even though there was decline in ammonia production as the temperature and time of heat treatment of sardine fish meal increased. In this context it is pertinent to note that the ammonia estimated was the residual ammonia and not the total ammonia released. Considerable proportion of ammonia would have been utilized by the microbes for microbial protein synthesis at 6 h compared to 2 h.

The *in vitro* ammonia (mg/100 ml) released was observed to be significantly lower (P<0.01) at 1% formaldehyde treatment in 2 and 6 h of incubation and uniform response was noticed among the four levels of formaldehyde treatment. No statistically significant (P<0.01) reduction in ammonia production was observed on increasing the level of formaldehyde beyond 1%.

Protein fractions

The per cent protein fractions of heat and formaldehyde treated sardine fish meal for the fractions A, B_1, B_2, B_3 and C are given in Table 2. Significantly (P<0.01) lowered "A" fraction was in sardine fish meal heat treated at 150°C for 120 min. Similarly "B_1" was significantly lower at 150°C for 120 min but comparable from 140°C heat treatment for 60 min onwards. There was not much difference or any clear trend in B_2 fraction of heat treated Sardine meal. The B_3 fraction showed a linear increase as the temperature and time increased except in few treatments.

The C fraction showed nonsignificant difference up to 140°C for 30 min and thereafter rapid increase (P<0.01) in C was observed. Increased C fraction was reported by Sampath *et al.* (1997) in heat treated groundnut cake at 130°C for 180 min. Nakamura *et al.* (1994) reported that NDIN and ADIN is a measure of quality of protein in heat treated feeds. Feedstuff having lower content of A and B_1 and higher content of B_2 and B_3 dietary protein fractions are preferred sources of digestible undegradable nitrogen (Sharma and Singh 1997). Hence to arrive at meaningful conclusion $B_2 + B_3 - C$ was calculated to indicate undegradable but digestible protein portion (Sniffen *et al*. 1992). A significantly higher (P<0.01) $B_2 + B_3 - C$ (undegrdable but digestible protein) was obtained at 130°C for 120 min and there was no significant increase beyond this temperature and time.

A, B_1, B_2 and B_3 fractions showed significant (P<0.01) change at 1% formaldehyde treatment. No statistically

Table 2. Per cent protein fraction in sardine fish meal as affected by various treatments of heat/formaldehyde (mean±SE on dry matter basis)

Treatment		Protein fractions (%)					Change in fraction
Temp	Time	A	B_1	B_2	B_3	C	$B_2 + B_3 - C$
Control**		22.49±0.15	4.71±0.12	40.56±0.32	31.11±0.23	1.13±0.04	70.53±0.17
Heat treatment*							
110°C	30 min	23.03^{g}±0.29	5.51^{g}±0.18	39.37^{abcde}±0.58	32.16^{ab}±0.55	1.10^{a}±0.06	70.43^{ab}±0.23
110°C	60 min	22.56^{fg}±0.29	5.13^{fg}±0.17	38.98^{abcd}±0.28	32.32^{ab}±0.57	1.01^{a}±0.04	70.27^{ab}±0.28
110°C	120 min	22.91^{g}±0.12	4.65^{ef}±0.12	39.50^{bcde}±0.29	31.77^{a}±0.54	1.17^{a}±0.06	70.10^{ab}±0.32
120°C	30 min	23.12^{g}±0.29	4.72^{ef}±0.23	38.36^{ab}±0.58	32.59^{ab}±0.29	1.21^{a}±0.05	69.74^{ab}±0.26
120°C	60 min	21.97^{f}±0.58	4.31^{de}±0.23	39.13^{abcd}±0.29	33.42^{b}±0.21	1.17^{a}±0.12	71.38^{c}±0.17
120°C	120 min	20.15^{e}±0.35	4.56^{def}±0.29	38.56^{abc}±0.29	33.58^{b}±0.52	1.15^{a}±0.11	70.99^{bc}±0.31
130°C	30 min	20.11^{e}±0.01	3.93^{d}±0.12	37.92^{a}±0.58	36.91^{c}±0.27	1.13^{a}±0.04	73.70^{d}±0.41
130°C	60 min	19.53^{de}±0.29	2.97^{c}±0.01	39.91^{cdef}±0.58	36.40^{c}±0.06	1.19^{a}±0.01	75.12^{e}±0.29
130°C	120 min	18.17^{c}±0.01	2.16^{b}±0.29	40.73^{ef}±0.29	37.04^{c}±0.11	1.20^{a}±0.05	76.57^{f}±0.31
140°C	30 min	19.18^{d}±0.12	2.10^{b}±0.12	41.06f±0.28	36.45^{c}±0.57	1.21^{a}±0.01	76.30^{f}±0.17
140°C	60 min	18.13^{c}±0.12	2.05^{ab}±0.01	40.74^{ef}±0.57	37.59^{c}±0.52	1.53^{b}±0.12	76.80^{f}±0.51
140°C	120 min	17.56^{bc}±0.29	1.56^{ab}±0.06	39.38^{abcde}±0.57	39.51^{de}±0.55	1.99^{c}±0.13	76.90^{f}±0.47
150°C	30 min	18.03^{bc}±0.01	1.91^{ab}±0.12	40.13^{dbf}±0.26	37.92^{cd}±0.28	2.01^{c}±0.08	76.04^{ef}±0.41
150°C	60 min	17.17^{ab}±0.17	1.65^{ab}±0.17	39.15^{abcd}±0.25	39.51^{de}±0.26	2.58^{d}±0.11	76.08^{ef}±0.38
150°C	120 min	16.53^{a}±0.03	1.41^{a}±0.06	38.37^{abc}±0.11	40.98^{e}±0.23	2.71^{d}±0.05	76.64^{f}±0.24
Formaldehyde treatment* (%)							
	0.5	20.53^{II}±0.58	3.17^{II}±0.12	38.09^{II}±0.57	37.89^{II}±0.70	1.21±0.04	74.77^{II}±0.21
	1.0	18.21^{I}±0.29	1.56^{I}±0.06	37.56^{I}±0.23	41.64^{I}±0.26	79.04^{I}±0.31	78.13^{I}±0.17
	1.5	18.17^{I}±0.17	1.47^{I}±0.17	37.11^{I}±0.54	41.65^{I}±0.23	1.15±0.06	78.76^{I}±0.29
	2.0	18.26^{I}±0.23	1.49^{I}±0.16	36.98^{I}±0.27	42.06^{I}±0.57	1.21±0.12	79.04^{I}±0.31

Mean of 3 observations.* Mean having different alphabet as superscript in the same column for heat treatment and roman letter as superscript for formaldehyde treatment differ significantly (P<0.01).** Not included in statistical evaluation.

significant (P<0.01) reduction or improvement was observed on increasing the level of formaldehyde beyond 1%. However the B_2 fraction was significantly (P<0.01) better in 0.50% formaldehyde treated sardine fish meal than the rest. There was no significant (P>0.01) difference in C fraction in various treatments of formaldehyde.

The digestible undegradable portion ($B_2 + B_3 - C$) was calculated and it was significantly (P<0.01) better in 1% formaldehyde treatment. Thus it can be deduced that 1% formaldehyde treatment to sardine fish meal will be beneficial in increasing digestible undegradable protein. Based on the results of nitrogen solubility, *in vitro* ammonia release and change in protein fractions on a holistic view heat treatment at 130°C for 120 min and formaldehyde treatment at 1% level were chosen as the desirable treatments for protection of sardine fish meal protein

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