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The Effect of Formaldehyde or Sodium Hydroxide on *In situ* Rumen Degradation of Low and High Fat Sunflower Meal

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Abstract: The objective of this study was to determine *in situ* dry matter (DM) and crude protein (CP) degradability of sunflower meal containing 25 and 165 g fat kg^{-1} DM and untreated and treated with sodium hydroxide (NaOH, 40 g kg^{-1} DM) and formaldehyde (30 and 60 g kg^{-1} DM). DM and CP degradation of the samples were determined using *in situ* technique in two fistulated Holstein steers (400±12 kg, body weight) and data were analysed to estimate soluble fraction (a), potentially degradable fraction (b), degradation rate (c) and effective degradability (ED). Formaldehyde decreased (a) fraction of DM ($p<0.05$). NaOH treated high fat sunflower meal had the highest (a) fraction and the lowest of (b) fraction of DM, (0.43 and 0.31, respectively). Formaldehyde and NaOH significantly decreased degradation rate (c) of DM. Formaldehyde (30 g kg^{-1} DM) treated low fat sunflower meal had the lowest ED of DM (0.44, $k = 0.03\ h^{-1}$) ($p<0.05$). Fraction of (a) and ED of DM of low fat sunflower meal was less than high fat sunflower meal. Formaldehyde and NaOH significantly were affected fractions of a, b, c and ED of CP ($p<0.05$). Fraction of (a) of CP decreased by formaldehyde and NaOH. Treatment of sunflower meal (low and high fat) with formaldehyde at 60 g kg^{-1} DM resulted in the highest (b) fraction and the lowest of (c) fraction and ED of CP. Crude protein (b) fraction of low fat sunflower meal was more than high fat sunflower meal but there was not any significant difference for (a) fraction and ED. DM and CP disappearance after 24 h was decreased by formaldehyde and NaOH. Therefore, it is appears that formaldehyde, NaOH and fat content of sunflower meal can affect DM and CP degradability parameters.

Key words: Degradability, formaldehyde, sodium hydroxide, sunflower meal (high fat, low fat)

INTRODUCTION

The nutritive value of the sunflower meal depends on the oil extraction process, variety of sunflower and the proportion the hulls removed during processing (Schingoethe *et al*., 1977). Ruminal degradability of sunflower meal protein is often > 60% (Economides, 1998). There are various methods for treating sunflower meal to reduce degradation in the rumen. Formaldehyde treatment is most common that reacts with protein and form cross-links by methylol and methylene bridges by lysine (Ashes *et al*., 1984). Other chemicals denature the proteins; e.g., sodium hydroxide has also been used (Cozzi *et al*., 1995). Sodium hydroxide (NaOH) treatment to reduce the degradation of soybean meal protein in the rumen and increase milk yield by dairy cows (Mir *et al*., 1984). Nishino *et al*. (1994) reported that the protein protection with alkali treatment related to the formation of a cross-linked amino acid, lysinoalanine.

The objective of this study was to investigate the effects of formaldehyde and NaOH treatment on *in situ* degradation characteristics of low (25 g kg^{-1} DM) and high (165 g kg^{-1} DM) fat sunflower meal, respectively, LSM and HSM.

MATERIALS AND METHODS

Formaldehyde and NaOH treatment of sunflower meal: Sunflower meals (low and high fat) were placed into a container and mixed by 37% solution of commercial formalin at rate 30 and 60 g formaldehyde/kg DM for 30 min to ensure an even distribution and were transferred inside sealed PVC bags and shaken vigorously for 5 min and kept for 5 days at room temperature. After 5 days were spread in thin layers about 4 mm on the plastic and were air-dried. Treatment of sunflower meals with 40 g NaOH/kg DM (4% solution) was done the same, but keeping time was 48 h.

***In situ* ruminal degradability:** Dry matter and crude protein degradability of the experimental samples [ULSM (untreated LSM), SHLSM (NaOH treated LSM); F30LSM

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(30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); UHSM SHHSM (NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM)] were measured by *in situ* technique using two fistulated Holstein steers (400±12 Kg, body weight). Animals were fed twice daily, 8.8 kg of DM of a diet consisted of 40% concentrate (155 g CP/kg of DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCo3, 0.5% mineral and vitamin premix, 0.2% salt), 30% lucerne hay and 30% maize silage. Five gram (DM basis) of each milled sample (2.0 mm screen) was put in the polyester bags (10×20 cm, 52 μm pore size) and incubated in the rumen for 2, 4, 6, 8, 16, 24, 48, 72 and 96 h (n = 4). After the specific incubation periods, the bags immediately were hand-rinsed under cold tap water until clear and dried in a forced-air oven (60°C, 48 h). The bags without incubation (0 h) were washed to estimate the wash-out at zero time. Disappearance of DM and CP of material from bags with incubation time were calculated using the equation of (Orskov and McDonald, 1979):

$$P = a + b (1 - e^{-ct})$$

- P = fraction degraded in the time t,
- a = soluble fraction,
- b = potentially degradable fraction,
- c = degradation rate
- t = incubation time.

The effective degradability (k = 0.03, 0.05 and 0.08 h^{-1}) were calculated using the equation of:

$$ED = a + (bc/ (c+k)$$

where, k was the estimated rate of outflow from the rumen.

Statistical analysis: Data were subjected to analysis as a completely randomized design using the General Linear Model (GLM) procedure of SAS. After significant ($p<0.05$) F-test, means Duncan's multiple range test was used to compare treatment means.

RESULTS AND DISCUSSION

The degradability parameters and ED of DM are shown in Table 1. Effect of treatment of formaldehyde and NaOH on fractions of a, b, c and ED of DM was significant ($p<0.05$). SHHSM had the highest of (a) fraction and the lowest of (b) fraction, (0.43 and 0.31, respectively). Fraction (a) of SHHSM was significantly higher than SHLSM. Formaldehyde treatment caused to decrease (a) fraction. Nishimuta *et al*. (1974) reported the same result. Formaldehyde and NaOH increased (b) fraction that confirm the result of (Canale *et al*., 1990) for alfalfa treated with NaOH. Effect of formaldehyde on increase (b) fraction was more than NaOH, also (b) fraction of F60LSM and F30LSM was significantly higher than F60HSM and F30HSM. Formaldehyde and NaOH significantly decreased (c) fraction and ED of DM. The lowest of (c) fraction and ED in 3 outflow rate was for F30LSM and F60HSM and the highest of them was for HSM and LSM. The effect of 60 level of formaldehyde on decrease ED was more than 30 level and NaOH. NaOH treatment decreased (c) fraction that was in consistent with (O'mara *et al*., 1997). Canale *et al*. (1990) showed decrease of rate of DM disappearance likely resulted from decreased neutral detergent soluble. If soluble DM was leached, fibre would increase and the remaining DM fraction would be slower to digest. Formaldehyde treatment decreased (c) fraction but Rodehutscord *et al*. (1999) reported opposite results. The effect of formaldehyde on decrease of (c) fraction was more than NaOH. ED of DM of formaldehyde and NaOH treated samples was significantly lower than untreated samples. In agreement with it Rodehutscord *et al*. (1999) reported ED of DM (k = 0.03 h^{-1}) was 0.84 and 0.81 for untreated and formaldehyde treated samples that indicates a reduced fermentation in the rumen.

In this study, NaOH treatment decreased of disapearance of DM after 24 h incubation in rumen (Fig. 1) that was confirmed by findings (Mcniven, 1995). Increased acid-detergent lignin and hemicellulose content resulted in much less improvement in DM digestibility *in vitro* (Jackson, 1977). But Berger *et al*. (1979) concluded solubilization of hemicellulose with NaOH caused to improvement in DM digestibility. Formaldehyde treatment caused to decrease disappearance of DM after 24 h incubation in rumen (Fig. 1) and the effect of 60 level was more than 30 level and NaOH. Thomas *et al*. (1979) reported a reduction in DM degradability of meals and seed by formaldehyde (10-20 g kg^{-1} CP). But 0.3 and 0.4% formaldehyde had no effect on DM digestibility of soy bean and rapeseed meals (Madsen, 1982). DM disappearance of LSM was less than HSM. Fraction of (a) and ED of LSM was less than HSM but factions of (b) and (c) were more than HSM. This indicates, EE content of sunflower meal was not effective on DM disappearance that do not prove suggestion of Withney *et al*. (2000) that showed linear decline for DM disappearance by inclusion soybean oil in the diet.

Characteristics of the CP degradability of samples are given in Table 2. Fractions of a, b, c and ED of CP of the samples were significantly affected by formaldehyde and NaOH ($p<0.05$). Formaldehyde and NaOH significantly decreased (a) fraction and increased (b) fraction of CP. F60LSM and F60HSM had the highest of (b) fraction.

Table 1: The degradation kinetics of DM of formaldehyde and NaOH treated sunflower meal (low and high fat)

	ED			Degradation parameters		
Samples[1]	a	b	c	k = 0.03	k = 0.05	k = 0.08
ULSM	0.32c	0.41bc	0.16a	0.66b	0.63b	0.59b
SHLSM	0.32c	0.42bc	0.09b	0.63c	0.59d	0.54d
F30LSM	0.22f	0.50b	0.06bc	0.55d	0.49f	0.43f
F60LSM	0.25e	0.53a	0.02d	0.44f	0.38h	0.34h
UHSM	0.39b	0.39bc	0.14a	0.71a	0.68a	0.64a
SHHSM	0.43a	0.31c	0.06bc	0.63c	0.59c	0.56c
F30HSM	0.31c	0.39bc	0.05dc	0.54d	0.49e	0.45e
F60HSM	0.29d	0.39bc	0.04dc	0.49e	0.45g	0.40g
SEM	0.009	0.04	0.01	0.006	0.002	0.002

[1]ULSM (untreated LSM), SHLSM (NaOH treated LSM); F30LSM (30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); UHSM (untreated HSM); SHHSM (NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM); a, soluble fraction; b, potentially degradable fraction; c, degradation rate (/h); ED, effective degradability; k, outflow rate (/h); SEM., standard error of mean; Means within each column with different letters are significantly different ($p<0.05$)

Table 2: The degradation kinetics of CP of formaldehyde and NaOH treated sunflower meal (low and high fat)

	ED			Degradation parameters		
Samples[1]	a	b	c	k = 0.03	k = 0.05	k = 0.08
ULSM	0.63a	0.32d	0.11b	0.88a	0.85b	0.81b
SHLSM	0.38b	0.56bc	0.05bc	0.73b	0.66c	0.59c
F30LSM	0.21d	0.75b	0.044bc	0.63c	0.53d	0.45e
F60LSM	0.22cd	0.79a	0.04c	0.5e	0.41f	0.34g
UHSM	0.63a	0.29d	0.28a	0.89a	0.88a	0.86a
SHHSM	0.39b	0.59bc	0.041bc	0.73b	0.66c	0.59d
F30HSM	0.25c	0.76b	0.029d	0.6d	0.51e	0.43f
F60HSM	0.23cd	0.8a	0.025d	0.50e	0.41f	0.34h
SEM	0.011	0.098	0.025	0.006	0.0008	0.0005

[1]ULSM (untreated LSM), SHLSM (NaOH treated LSM); F30LSM (30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); UHSM (untreated HSM); SHHSM (NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM); a , soluble fraction; b, potentially degradable fraction; c ,degradation rate (/h); ED, effective degradability; k, outflow rate (/h); SEM, standard error of mean; Means within each column with different letters are significantly different ($p<0.05$)

After treating with formaldehyde, methylene bridges are reversibly formed between the protein chains that reduce the solubility of the protein (Freer and Dove, 1984). O'Mara *et al.* (1997) observed alkali treatment significantly increased CP soluble fraction. Fraction of (b) of formaldehyde treated samples was more than NaOH. Fraction of (c) of samples significantly decreased by formaldehyde and NaOH. Also, Nishino *et al.* (1995) obtained this for soy bean meal treated by 25 and 50 g NaOH kg DM. The mechanism of protein protection by NaOH has not been understood. It may be related to cross-linking reactions to produce cross-linked amino acids such as lysinoalanine and cross-links into protein that could render protein less susceptible to microbial breakdown in the rumen (Nishino *et al.*, 1994). Formaldehyde treatment reduced the (c) fraction for sunflower and rapeseed meals (Rodehutscord *et al.*, 1999). The ED of CP for LSM and HSM was more than treated samples with formaldehyde and NaOH. O'mara *et al.*

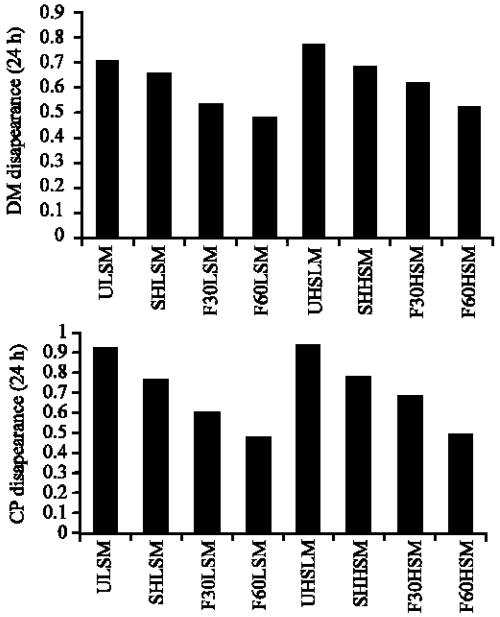

Fig. 1: Disappearance of DM and CP of different samples after 24 h ruminal incubation. ULSM (untreated LSM), SHLSM (NaOH treated LSM); F30LSM (30 g formaldehyde/kg DM treated LSM); F60LSM (60 g formaldehyde/kg DM treated LSM); UHSM (untreated HSM); SHHSM (NaOH treated HSM); F30HSM (30 g formaldehyde/kg DM treated HSM); F60HSM (60 g formaldehyde/kg DM treated HSM)

(1997) concluded alkali treatment significantly increased ED of N. The ED of dietary N ($k = 0.06\ h^{-1}$) varied from 0.42-0.74, respectively for formaldehyde treated and non-treated supplements (Coombe *et al.*, 1985).

Formaldehyde treatment caused to decrease of disappearance of CP after 24 h incubation in rumen and the effect of 60 level was more than the other (Fig. 1). 92-95% of the nitrogen in untreated sunflower meal had disappeared after 24 h incubation in the rumen is similar to the result of current study (Freer and Dove, 1984). Waltz and Stern (1989) reported depressing in protein degradation of formaldehyde-treated soy bean meal was for the chemical combination of formaldehyde with the lysine. Formaldehyde reactions with protein are formed between the active groups of amino acids, e.g. S-H, -NH2 and the carbonyl group of formaldehyde. Secondary cross-linking by methylene bridges may also be possible (Anyoniewicz *et al.*, 1992). NaOH treatment decreased disappearance of CP after 24 h incubation in rumen that proves (Mir *et al.*, 1984) (Fig. 1). High fat in sunflower meal had no effect on rumen fermentation and CP disappearance but Getachew *et al.* (2001) proposed that inclusion fat caused to decreased deaminase activity and

proteolysis. Unsaturated fatty acids have negative effects on rumen micro organisms and reduces digestibility (Palmquist and Jenkins, 1980).

CONCLUSION

It is appears that degradability parameters are influenced by formaldehyde and NaOH and fat content of sunflower meal. Formaldehyde and NaOH reduced (a) fraction and ED of CP and DM in rumen. The effect of formaldehyde on decrease (a) fraction and increase (b) fraction was more than NaOH and 60 level of formaldehyde appears to have the least of ED of DM and CP.

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