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# Rumen and Post Abomasal Disappearance of Amino Acids and Some Nutrients of Barley Grain Treated with Sodium Hydroxide, Formaldehyde or Urea in Lactating Cows

[1]M. Dehghan-Banadaky, [1]A. Nikkhah, [2]H. Amanlo,
[3]M. Danesh Mesgaran and [4]H. Mansori
[1]Department of Animal Science, Faculty of Agriculture,
University of Tehran, Karaj, Iran
[2]University of Zanjan, Iran
[3]Ferdowsi University of Mashad, Iran
[4]Anim Research Center of Heydarabad, Iran

**Abstract:** Four rumen and duodenum cannulated, Holstein lactating cows were used in a change-over design to determine the effects of NaOH, Formaldehyde or Urea treated barley on disappearance of Dry Matter (DM), Crude Protein (CP), Amino Acids (AA), NDF, ADF, hemicelluloses and starch in rumen, Post Abomasal Tract (PAT) and total tract by mobile nylon bag technique. Experimental treatments were coarse milled barley, barley treated with 3.5% NaOH, barley treated with 0.4% formaldehyde and barley treated with 3.5% urea that all chemical treated barley milled coarse before feeding. NaOH Treatment reduced concentrations of Lysine and Cystine in the barley grain. All chemical treatments decreased rumen disappearances of barley CP but only NaOH and Formaldehyde treatments also decrease total AA and some of the AA disappearances in the rumen. All chemical treatments increased DM, OM, CP, starch, NDF, ADF and hemicellulose disappearance of barley in the PAT. But only NaOH and Formaldehyde treatments increased total AA and most of AA disappearances in the PAT. Effect of chemical treatments on increase of disappearance of starch, Met and Gly in the total tract was significant ($p<0.05$). Rumen disappearance of TAA was lower than CP but PAT disappearance of TAA was more than CP and finally total tract disappearance of TAA was more than CP. Individual AA in barley disappeared at different rates in the rumen and PAT. Consequently, the proportion of digesta CP and AA entering the intestine must be considered.

**Key words:** Barley grain, amino acids, mobile nylon bag, NaOH, formaldehyde, urea

## INTRODUCTION

Cereal grains can provide the major source of energy in diets in order to meet the nutrient requirements of high producing dairy cows; however, the amount of starch that can be included in the diets of dairy cows is limited, particularly if starch is rapidly fermented, such as barley starch. Modern protein evaluation systems for ruminants describe the supply and requirement of true protein that can be absorbed from the small intestine (Van Straalen *et al*., 1997). Processing of grains changes the rate and extent nutrient degradation in the rumen. Alteration of the site of digestion requires a treatment that increases nutrient flow to the small intestine without concomitant reductions in total tract digestibility. The ruminant has specific requirements for glucose, which are met largely through gluconeogenesis (Bauer *et al*., 1995). Various techniques for processing cereal grains have been developed. The beneficial effects of treatment of barley with NaOH were similar to rolling or crushing but slower digestion, decreased fluctuations in ruminal pH and lower incidence of rumenitis (Orskov, 1977). The feeding value of barley may be improved by ammoniation (Mandell *et al*., 1988). This improved has been attributed to a decreased rate of digestion and a reduction in nitrogen and non-fiber components of the ammoniated barley escaping from the rumen. An enhanced supply of protein and amino acids to the intestine has been obtained following treatment of barley with formaldehyde, which reduces rumen degradation of protein. In cereals,

**Corresponding Author:** M. Dehghan-Banadaky, Department of Animal Science, Faculty of Agriculture, University of Tehran, Karaj, Iran

starch exists as granules encapsulated in protein which can react with aldehydes and forming covalent bonds with the free amino groups. Thus, treatment of cereal grains with aldehydes might be expected to protect the starch from rumen degradation (Hyslop *et al*., 1989; Svihus *et al*., 2005).

The objective of this experiment was to determine the effects of treatment of barley by NaOH, formaldehyde or urea on rumen and post abomasal tract digestion of dry matter, crude protein, amino acids, NDF, ADF, hemicelluloses and starch in lactating cows.

## MATERIALS AND METHODS

**Barley treatments:** Dietary treatments were 1: coarse milled barley (3 mm particle size), 2: barley treated with 3.5% NaOH (on barley DM basis), 3: barley treated with 0.4% formaldehyde and barley treated with 3.5% urea. For all chemical treatments, whole barley grain mixed well with calculated amount of chemical material and 220 L of water per 1 tone of barley and silage on airless big nylon bags. After 30 days all treated barley were allowed to aerate and dry before they were mechanically processed (coarse milled, 3 mm particle size) same as control treatment. The barley grain used for all treatments was from the same source. The barley grain that used in this experiment was an Iranian cultivar (Valfagr).

**Cows and feeding:** Four Holstein lactating cows were fitted with rumen fistula and T-shaped cannula in the proximal duodenum and were used in a balance change-over design with 4 treatments and 4 periods (28 days). Cows averaged 75 DIM (±12 SD) and 21 (±3.5 SD) kg $d^{-1}$ milk yield when they were assigned to treatments. All cows were fed a Total Mixed Ration (TMR) consisting of 45.5% forage (DM basis), comprised of 25.29% alfalfa hay and 20.24% corn silage (DM basis). The composition of the concentrate (DM basis) was as follows: 27.21% barley, 12.56% wheat bran, 3.04% soybean meal, 9.31% cottonseed meal, 0.34% salt, 0.67% sodium bicarbonate, 0.56% calcium carbonate and 0.78% mineral and vitamin mixture. Diets were formulated to be isoenergetic (NEl basis) and isonitrogenous. Cows were fed for *ad libitum* intake. The TMR was fed twice daily with half at 07:00 and other half at 19:00 to maintain a relatively stable rumen environment. Cows were housed and milked in individual tie stalls in research barn of Tehran University, Karaj, Iran.

***In situ* rumen incubation:** A standard procedure for small nylon bags was used to estimate ruminal disappearance of nutrients (de Boer *et al*., 1987). Nylon bags (5 * 3.5 cm; 47 μm pore size) were filled with 1 g of dry and ground (mill to pass a 2 mm screen) barley and were closed using heat seal. Each barley sample was incubated in 18 replicates in the rumen for 12 h in each period. Nylon bags were suspended in the rumen in a polyester mesh bag (25×40 cm; 3 mm pore size). The nylon bags were then removed from the mesh bag and immediately wash by cold water to arrest fermentation then placed in a conventional washing machine. Washings were repeated until the rinse water remained clear. Samples were then dried in an oven at 60°C for 48 h. Replicates were pooled and ground through a 0.5 mm screen before analyses.

***In situ* intestinal incubation:** Nutrient disappearance during passage through the intestine was determined using the mobile nylon bag technique (DeBoer *et al*., 1987). Nine replicate samples were incubated in the rumen of each cow for 12 h before being inserted into the intestine. Bags were inserted every 20 min into the duodenum through the T-shaped cannulas. Vanhatalo *et al*. (1995) showed that acid-pepsin treatment of ruminally incubated bags prior to insertion into the duodenum is not necessary (Omara *et al*., 1997; Harseted and Prestloken, 2000; Taghizadeh *et al*., 2005). Duodenal bags were collected from the feces and machine washed until the rinse water remained clear. Residual DM was conducted on the pooled residues from the mobile nylon bag after drying at 60°C for 48 h.

**Chemical analyses:** Analyses for DM and Ash were carried out according to AOAC (2000) methods. Determinations of CP were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Neutral detergent fiber and ADF were measured according to the method of Goering and Van Soest (1970) includes Ash. Starch determined according to enzymatic method by Plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA) Spectrophotometer (Mccleary *et al*., 1994). For amino acid analyses, approximately 0.1 g of sample was weighed out into screw-capped test tubes, mixed with 3 mL of 6 N HCL, flushed with nitrogen and then hydrolyzed in an oven at 110°C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid and centrifuged at 2500 rpm for 15 min at room temperature. The samples were analyzed according to the method of Sedwick *et al*. (1991) using a Varian 5000 HPLC 2367 system (Varian Associates, Sunnyvale, CA) with a reversed-phase column. The amino acids in the samples were derivatized with an o-phthaldialdehyde reagent solution and detected spectrofluorometrically. Cysteine and methionine were determined as cysteic acid and methionine sulphone, respectively, obtained by oxidation

with performic acid before 6 M HCl hydrolysis (Hagen *et al*., 1989) also alkaline analysis used for Tryptophan measurement (Sedwick *et al*., 1991).

**Calculations and statistical analyses:** The percent disappearance of the DM, CP, starch, NDF, ADF, hemicellulose and AA at 12 h incubation in the rumen was calculated as the difference between the feed and the portion remaining after incubation in the rumen. Disappearance in the PAT was calculated by the difference between the rumen residue after 12 h of incubation and the portion remaining in samples recovered from feces. Variance analyses were performed with the GLM procedure in the Statistical Analysis System (SAS Institute, 1989), using the following model:

$$Y_{ijk} = \mu + P_i + T_j + C_k + R_l + e_{ijkl}$$

where $\mu$ = overall mean, $P_i$ = fixed effect of period ($\mu$ = 1 to 4), $T_j$ = fixed effect of treatment (j = 1 to 4), $C_k$ = random effect of cow (k = 1 to 4), $R_l$ = residual effect of previous period (l = 1 to 3) and $e_{ijkl}$ = residual errors.

The Duncan's multiple range test was conducted to comparison of means between treatments. All statements of significance are based on the probability level of 0.05.

## RESULTS

Barley treated with 3.5% NaOH tended to solidify into a hard mass that had to be broken up before milling. NaOH treatment decreased the content of Cys and Lys of barley (Table 1).

Table 1: Nutrient composition in control and treated barley grain (% of DM)[1]

| | Treated barley by | | | |
|---|---|---|---|---|
| Variable[2] | Control | NaOH | HCHO | Urea |
| DM | 89.30 | 88.60 | 88.90 | 88.20 |
| OM | 97.02 | 95.85 | 97.44 | 97.43 |
| ASH | 2.98 | 4.15 | 2.56 | 2.57 |
| CP | 10.20 | 10.51 | 10.76 | 15.01 |
| NDF | 22.15 | 23.26 | 22.72 | 21.55 |
| ADF | 7.41 | 7.76 | 7.96 | 7.16 |
| Hemicellulose[3] | 14.74 | 15.50 | 14.71 | 14.93 |
| Starch | 61.20 | 60.80 | 61.10 | 61.11 |
| TAA | 8.91 | 8.12 | 9.10 | 9.06 |
| EAA | 3.48 | 3.10 | 3.47 | 3.58 |
| Met | 0.07 | 0.06 | 0.05 | 0.05 |
| Tri | 0.14 | 0.15 | 0.15 | 0.14 |
| His | 0.12 | 0.14 | 0.06 | 0.12 |
| Thr | 0.29 | 0.23 | 0.14 | 0.16 |
| Arg | 0.46 | 0.34 | 0.67 | 0.66 |
| Val | 0.53 | 0.53 | 0.47 | 0.52 |
| Phe | 0.48 | 0.46 | 0.58 | 0.46 |
| Ile | 0.39 | 0.38 | 0.37 | 0.48 |
| Leu | 0.66 | 0.63 | 0.64 | 0.60 |
| Lys | 0.34 | 0.18 | 0.34 | 0.39 |
| NEAA | 5.43 | 5.02 | 5.63 | 5.48 |
| Pro | 0.75 | 0.79 | 0.71 | 0.71 |
| Asp | 0.73 | 0.64 | 0.61 | 0.74 |
| Glu | 2.48 | 2.31 | 2.54 | 2.57 |
| Ala | 0.39 | 0.38 | 0.51 | 0.44 |
| Tyr | 0.22 | 0.19 | 0.25 | 0.24 |
| Ser | 0.37 | 0.31 | 0.50 | 0.34 |
| Cys | 0.12 | 0.05 | 0.12 | 0.09 |
| Gly | 0.37 | 0.35 | 0.39 | 0.35 |

[1]Experimental diets 1-4 contained untreated barley, barley treated with 3.5% NaOH, 0.4% Formaldehyde (HCHO) and 3.5% Urea (DM basis), respectively, [2]Three samples analyzed for each variable, [3]Hemicellulose calculated as NDF-ADF

**Ruminal disappearance:** The chemical treatments of barley grain decreased rumen disappearance of CP ($p<0.05$) but NaOH treatment had more effect on rumen disappearance of CP rather than other chemical treatments. NaOH and formaldehyde treatments decreased disappearances of total AA, essential AA (EAA) and nonessential AA (NEAA) of barley ($p<0.05$) but treatment of barley by urea did not affect these parameters. Rumen disappearance of most of individual AA decreased by NaOH and formaldehyde treatments except Pro and Tri but only some of AA affected by urea treatment of barley (Table 2). The other nutrients did not significantly affect by chemical treatments ($p>0.05$).

**PAT disappearance:** The effects of chemical treatments on PAT disappearance of all nutrients were significant. NaOH, Formaldehyde or Urea treatments increased disappearance of DM, OM, CP, NDF, hemicellulose and starch of barley in PAT. The disappearance of ash did not affected by NaOH treatments and disappearance of ADF decrease by formaldehyde treatment of barley in PAT. NaOH and formaldehyde treatments (treatment 2 and 3) increased disappearance of TAA, EAA, NEAA and individual AA (except Thr) of barley ($p< 0.05$). Treatment of barley by urea did not affect on TAA and NEAA but increased EAA disappearance of barley in PAT. Effects of urea treatment on individual AA disappearance in PAT were variable (Table 3), PAT disappearance of Thr, Val and Arg decreased but Glu, Tyr, Phe and Ile did not affect and other AA of barley increased by urea treatment.

**Total tract disappearance:** The chemical treatments of barley grain increased total tract disappearance of starch ($p<0.05$) but NaOH treatment had more effect on total tract disappearance of starch rather than other chemical treatments. NaoH treatment of barley increased total tract disappearance of Met, Gly and Phe and decrease His disappearance of barley. Formaldehyde treatment of barley increased Met and Gly and decrease His, Thr and

Table 2: Disappearance after 12 h rumen incubation of nutrients in control and treated barley grain (% of content in feed)

| | Treated barley by | | | | |
|---|---|---|---|---|---|
| Variable | Control[1] | NaOH | HCHO | Urea | SEM[2] |
| DM | 88.52 | 87.94 | 87.43 | 88.56 | 1.68 |
| OM | 88.98 | 87.08 | 87.95 | 89.17 | 1.84 |
| ASH | 81.56 | 89.18 | 74.24 | 75.15 | 7.15 |
| CP | 93.78[a] | 77.55[c] | 79.69[c] | 87.88[b] | 2.14 |
| NDF | 60.96 | 67.83 | 62.62 | 62.68 | 8.79 |
| ADF | 31.13 | 33.40 | 48.52 | 43.36 | 9.39 |
| Hemicellulose | 75.95 | 85.06 | 70.25 | 72.20 | 9.09 |
| Starch | 95.24 | 95.15 | 95.76 | 96.37 | 1.24 |
| TAA | 88.58[a] | 79.36[b] | 79.05[b] | 88.40[a] | 2.50 |
| EAA | 87.68[a] | 78.31[b] | 78.15[b] | 86.97[a] | 2.83 |
| Met | 88.74[a] | 75.82[c] | 75.57[c] | 80.80[b] | 3.71 |
| Tri | 86.92 | 86.42 | 85.07 | 87.92 | 1.89 |
| His | 94.68[a] | 76.52[c] | 93.20[a] | 86.81[b] | 3.39 |
| Thr | 94.54[a] | 91.14[b] | 85.31[c] | 85.60[c] | 1.65 |
| Arg | 73.96[b] | 65.35[c] | 77.15[b] | 86.89[a] | 2.99 |
| Val | 89.28[a] | 83.05[b] | 77.59[c] | 88.03[a] | 1.62 |
| Phe | 88.56[a] | 76.95[b] | 79.00[b] | 89.65[a] | 3.24 |
| Ile | 89.55[a] | 80.16[b] | 77.27[b] | 88.09[a] | 2.2 |
| Leu | 88.02[a] | 77.40[c] | 76.80[c] | 82.85[b] | 3.44 |
| Lys | 91.24[a] | 69.88[c] | 76.14[b] | 87.32[a] | 3.02 |
| NEAA | 89.16[a] | 80.00[b] | 76.56[b] | 89.21[a] | 2.32 |
| Pro | 86.83 | 86.70 | 82.94 | 87.77 | 3.72 |
| Asp | 90.62[a] | 82.12[c] | 77.54[d] | 87.33[b] | 1.84 |
| Glu | 89.79[a] | 79.86[b] | 80.02[b] | 91.32[a] | 2.40 |
| Ala | 87.83[a] | 78.31[b] | 76.63[b] | 87.49[a] | 2.69 |
| Tyr | 91.53[a] | 73.41[c] | 82.13[b] | 91.20[a] | 5.06 |
| Ser | 88.77[a] | 73.88[c] | 78.73[b] | 87.89[a] | 3.33 |
| Cys | 96.91[a] | 84.03[b] | 86.96[b] | 85.84[b] | 1.99 |
| Gly | 84.56[b] | 72.22[b] | 77.25[b] | 84.28[a] | 2.03 |

[1]Experimental diets 1-4 contained untreated barley, barley treated with 3.5% NaOH, 0.4% Formaldehyde and 3.5% Urea (DM basis), respectively, [2]Standard Error of Means, [3]a,b,c,d Means within a row with different superscripts differ ($p<0.05$)

Table 3: PAT disappearance of nutrients in control and treated barley grain (% of 12 h rumen residue)

| | Treated barley by | | | | |
|---|---|---|---|---|---|
| Variable | Control[1] | NaOH | HCHO | Urea | SEM[2] |
| DM | 20.22[d] | 51.71[a] | 23.31[c] | 25.57[b] | 0.16 |
| OM | 19.24[d] | 57.69[a] | 21.79[c] | 25.04[b] | 0.13 |
| ASH | 31.34[c] | 30.89[c] | 51.34[a] | 38.59[b] | 4.67 |
| CP | 62.68[c] | 86.57[a] | 64.57[b] | 65.37[b] | 0.33 |
| NDF | 5.34[d] | 37.48[a] | 9.87[c] | 14.05[b] | 4.95 |
| ADF | 6.43[bc] | 17.80[a] | 4.75[c] | 9.72[b] | 3.55 |
| Hemicellulose | 4.99[d] | 49.09[a] | 13.19[c] | 16.60[b] | 0.07 |
| Starch | 36.80[c] | 78.74[a] | 41.21[b] | 40.21[b] | 1.27 |
| TAA | 81.39[c] | 94.39[a] | 89.46[b] | 80.66[c] | 0.17 |
| EAA | 79.54[c] | 93.16[a] | 86.55[b] | 76.08[d] | 0.26 |
| Met | 42.79[d] | 88.09[a] | 76.79[c] | 78.25[b] | 0.13 |
| Tri | 86.36[d] | 93.10[a] | 89.95[b] | 87.78[c] | 0.03 |
| His | 40.27[c] | 78.98[a] | 38.20[d] | 44.81[b] | 1.25 |
| Thr | 48.78[b] | 82.36[a] | 32.36[d] | 39.20[c] | 2.02 |
| Arg | 91.14[b] | 96.72[a] | 90.92[b] | 81.75[c] | 0.25 |
| Val | 75.58[c] | 92.77[a] | 87.11[b] | 73.47[d] | 0.33 |
| Phe | 80.98[c] | 95.40[a] | 88.63[b] | 81.57[c] | 0.19 |
| Ile | 77.42[c] | 93.74[a] | 85.96[b] | 77.14[c] | 0.26 |
| Leu | 80.03[d] | 94.53[a] | 90.39[b] | 81.80[c] | 0.14 |
| Lys | 65.75[d] | 89.74[a] | 83.68[b] | 67.42[c] | 0.39 |
| NEAA | 82.73[c] | 95.20[a] | 91.27[b] | 83.78[d] | 0.11 |
| Pro | 91.04[d] | 95.88[a] | 93.86[b] | 92.45[c] | 0.02 |

Table 3: Continued

| | Treated barley by | | | | |
|---|---|---|---|---|---|
| Variable | Control[1] | NaOH | HCHO | Urea | SEM[2] |
| Asp | 68.26[d] | 91.33[a] | 81.34[c] | 70.13[b] | 0.40 |
| Glu | 89.78[c] | 97.33[a] | 95.00[b] | 89.27[c] | 0.06 |
| Ala | 72.41[d] | 92.31[a] | 88.02[b] | 76.14[c] | 0.20 |
| Tyr | 77.83[c] | 94.23[a] | 82.15[b] | 78.38[c] | 0.26 |
| Ser | 77.14[d] | 95.23[a] | 89.14[b] | 81.67[c] | 0.19 |
| Cys | 31.53[d] | 82.98[a] | 76.10[c] | 80.50[b] | 0.18 |
| Gly | 70.30[c] | 92.80[a] | 92.52[a] | 81.89[b] | 0.07 |

[1]Experimental diets 1-4 contained untreated barley, barley, treated with 3.5% NaOH, 0.4% Formaldehyde and 3.5 % Urea (DM basis), respectively, [2]Standard Error of Means, [3]a,b,c,d Means within a row with different superscripts differ ($p<0.05$)

Table 4: Disappearance in the total tracts of nutrients in control and treated barley grain (% of content in feed)

| | Treated barley by | | | | |
|---|---|---|---|---|---|
| Variable | Control[1] | NaOH | HCHO | Urea | SEM[2] |
| DM | 90.84 | 94.83 | 90.55 | 91.62 | 2.98 |
| OM | 91.10 | 94.75 | 90.81 | 92.11 | 2.12 |
| ASH | 87.30 | 94.89 | 87.49 | 84.67 | 4.82 |
| CP | 97.68 | 97.77 | 96.33 | 97.48 | 1.07 |
| NDF | 61.43 | 78.37 | 62.61 | 65.39 | 12.11 |
| ADF | 56.12 | 68.35 | 55.77 | 59.46 | 11.06 |
| Hemicellulose | 64.09 | 83.39 | 66.30 | 68.34 | 16.10 |
| Starch | 95.62[c] | 98.74[a] | 96.74[b] | 97.10[b] | 0.20 |
| TAA | 97.87 | 98.83 | 97.79 | 97.75 | 0.70 |
| EAA | 97.48 | 98.50 | 97.06 | 96.87 | 1.87 |
| Met | 93.21[c] | 97.08[a] | 95.40[b] | 95.81[b] | 0.27 |
| Tri | 98.22 | 99.05 | 98.45 | 98.42 | 0.73 |
| His | 96.82[a] | 95.00[b] | 94.44[b] | 92.70[c] | 0.43 |
| Thr | 97.20[a] | 98.42[a] | 90.07[b] | 91.22[b] | 1.01 |
| Arg | 97.69 | 98.85 | 97.92 | 97.60 | 0.86 |
| Val | 97.38[b] | 98.76[a] | 97.11[b] | 96.82[b] | 1.63 |
| Phe | 97.82[b] | 98.93[a] | 97.61[b] | 98.09[b] | 0.15 |
| Ile | 97.64 | 98.74 | 96.81 | 97.27 | 2.90 |
| Leu | 97.61 | 98.75 | 97.77 | 96.87 | 1.14 |
| Lys | 96.99 | 96.87 | 96.11 | 95.86 | 0.16 |
| NEAA | 98.12 | 99.03 | 98.22 | 98.25 | 0.63 |
| Pro | 98.95 | 99.45 | 98.95 | 99.07 | 0.31 |
| Asp | 97.02 | 98.43 | 96.20 | 95.81 | 2.47 |
| Glu | 98.96 | 99.46 | 99.00 | 99.07 | 0.63 |
| Ala | 96.64 | 98.31 | 97.20 | 97.01 | 1.41 |
| Tyr | 98.12[a] | 98.45[a] | 96.81[b] | 98.09[a] | 0.28 |
| Ser | 97.43 | 98.74 | 97.69 | 97.77 | 0.80 |
| Cys | 97.88 | 97.25 | 96.88 | 97.23 | 0.90 |
| Gly | 95.41[c] | 97.97[b] | 98.30[a] | 97.14[b] | 0.43 |

[1]Experimental diets 1-4 contained untreated barley, barley treated with 3.5% NaOH, 0.4% Formaldehyde and 3.5% Urea (DM basis) respectively, [2]Standard Error of Means, [3]a,b,c,d Means within a row with different superscripts differ ($p<0.05$)

Tyr PAT disappearance of barley ($p<0.05$). Urea treatment of barley increased Met and Gly and decreased His and Thr disappearance of barley in PAT (Table 4).

## DISCUSSION

**NaOH treatment:** In the present trial, increase of PAT and total tract disappearance of starch in cows fed chemical

treated barley compared with control cows suggest that chemical treatment especially NaOH, influenced the amount of starch escaping intact from the rumen although rumen disappearances of starch did not significantly decrease. An increased supply of starch to the small intestine may improve the efficiency of feed utilization by reducing losses of energy as methane or heat (McAllister and Cheng, 1992). Digestion of starch in the small intestine and the subsequent absorption of glucose may enhance N utilization through a sparing effect on gluconeogenic AA (Matras and Preston, 1989). NaOH treatment of whole barley disrupts the seed coat by partial hydrolysis of hemicellulose and lignin. However in present study NDF, ADF and hemicellulose disappearances of barely in the rumen did not affect by NaOH treatment but PAT disappearance of these increased. NaOH treatment decreased the rumen disappearances of CP and AA, also increased PAT disappearance of CP and AA, without effect on total tract digestion, that shows protein escape from rumen to intestines. Treatment of barley with NaOH reduced the nutritional quality of the barley in several ways. Amount of Lys and Cys were reduced in barley treated with NaOH, perhaps because of the formation of the cross linked AA, lysinoalanine (McNiven *et al*., 1995). Nishino *et al.* (1994) found a significant relationship between the amount of lysinoalanine and the rate of protein degradation in the rumen after treatment of soybean meal with NaOH. McNiven *et al.* (1995) also report decrease of Lys and Cys in barley treated by NaOH, they reported that total vitamin E content was not detectable in the barley treated with NaOH.

**Formaldehyde treatment:** The results of present study shown that treatment of barley grain with 0.4% formaldehyde reduced rumen disappearance of CP (from 93.78 to 79.69%) and TAA after 12 h rumen incubation and increased the disappearance of protein and TAA in the PAT, but did not significantly affect the digestion of CP and AA in the whole gastrointestinal tract. Resistance to hydration and enzyme degradation following formaldehyde treatment is due, in part, to formation of acetal derivatives between the aldehyde and hydroxyl groups of glucose and amino groups in protein molecules (Ortega-Cerrilla *et al*., 1999a,b). Formaldehyde treatment had a great protective effect against protein and AA degradation in the rumen without starch protection, similar result approximately has been observed by others (McAllister *et al*., 1990a). Degradation of starch may be linked to degradation of protein, the degree of association depending on the depth to which the starch grains are embedded in the protein. McAllister *et al.* (1990b) concluded, following electron microscopy studies of formaldehyde treated barley subjected to *in vitro* incubation with rumen fluid, that the reduced microbial degradation of barley starch following treatment was due to the protective effect of formaldehyde against protein degradation. Reducing the digestibility in the protein matrix of the endosperm inhibited access of bacteria to the embedded starch granules (McAllister *et al*., 1990a). However in present study protection afforded to the protein was not sufficient, or not associated with the appropriate endosperm proteins, to reduce the rumen degradability of starch.

**Urea treatment:** Urea treated barley had lower effect on CP and individual AA disappearance of barley in rumen and PAT than the NaOH and Formaldehyde processing method. Also there were no treatment effects on ruminal disappearance of DM, OM, ADF, NDF and hemicellulose. Ruminal starch disappearance averaged 95%, in good agreement with other studies evaluating barley (90%, Zinn, 1993; 90%, Zinn *et al*., 1996; 93%, Zinn and Barajas, 1997). Urea treatment had a trend to increase ($p>0.05$) ruminal starch digestion rather than control group. Zinn (2003) also observed greater ruminal starch digestion, when urea adds to steam-flaked barley-based finishing diet. Stimulation of microbial growth and fermentation may be causes of more starch degradation. However in present study starch disappearance from PAT and total tract also increase by urea treatment of barley that may be increase net energy value of barley but Zinn (2003) concluded that urea supplementation may not increase the net energy value of barely based finishing diets when ruminal degradable intake protein is greater than 85% of microbial protein synthesis.

## CONCLUSIONS

The data show that appropriate treatment of barley with NaOH or formaldehyde provides substantial protection of CP and individual AA from rumen digestion and increase disappearance of most of barley nutrients in PAT but on base of this experiment, NaOH treatment reduced the AA quality of the barley. Consequently formaldehyde treatment is better way to chemical treatment of barley grain. Individual AA in barley disappeared at different rates in the rumen and in the intestinal tract and the proportion of protein and AA entering the intestine must be considered.

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