

## Effect of Protein Sources of Isonitrogenous Whole Crop Barley Silage Based Diets on Performance and Blood Metabolites of Early Lactating Holstein Cows

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**Abstract:** The effect of the source of crude protein in isonitrogenous diets on early lactating cow responses was investigated. Three isonitrogenous diets (crude protein: 16.7%) with similar rumen degradable protein, but with different source of protein (Fish Meal (FM) + Canola Meal (CM) as 42.8 + 82.4 g kg<sup>-1</sup> DM (FM/CM) or Soybean Meal (SM) + Cotton Seed Meal (CSM) as 48.8 + 91 g kg<sup>-1</sup> DM (SM/CSM1) or 84 + 42.8 g kg<sup>-1</sup> DM (SM/CSM2)) were provided. The diets were fed to 24 early lactating Holstein cows (8 animals per each treatment), 10±3 days in milk and 37.34 kg day<sup>-1</sup> milk yield, for 7 weeks, using a completely randomized design with repeat measures of data in time. Daily Dry Matter Intake (DMI), milk yield and milk composition were recorded. Blood glucose and Blood Urea Nitrogen (BUN) were measured in weeks 3 and 6 of the experiment and some blood metabolites and enzymes including insulin, Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyrovic Transeaminase (SGPT) were measured at the last week of the experiment. There was no significant effect on DMI (FM/CM = 20.32, SM/CSM1 = 21.04 and SM/CSM2 = 20.82, SEM = 0.59 kg day<sup>-1</sup>) (p>0.05). However, the effect of time was significant (linear and quadratic) (p<0.0001). Milk yield was similar among treatments. Milk composition (percentage and yield) was similar for all treatments, although time effect on milk protein and fat percentage was significant (linear and quadratic) (p<0.05). Plasma concentration of insulin was similar in cows fed the experimental diets (FM/CM = 9.16, SM/CSM1 = 7.90 and SM/CSM2 = 12.41, SEM = 1.34 micro U mL<sup>-1</sup>). Blood urea nitrogen concentration was not differed among treatments (p>0.05). Serum glutamic oxaloacetic transaminase was significantly affected by the dietary protein sources (FM/CM = 74.62, SM/CSM1 = 91.12 and SM/CSM2 = 83.50 mg dL<sup>-1</sup>, SEM = 6.36) (p<0.05), in 4 h after the morning feeding. It was concluded that protein sources with similar rumen degradability might not causing significant impact on performance and some blood metabolites in early lactating Holstein cows.

**Key words:** Cow, milk, protein, silage, DMI, blood metabolites

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### INTRODUCTION

High producing dairy cows in early lactation require 18-19% Crude Protein (CP) in the diet to support milk production (NRC, 2001). Because profits are related to reproductive performance as well as milk production, existence of a negative relationship between excessive dietary protein (>19%) and fertility could be an important consideration (Carroll *et al.*, 1994). Excessive Rumen Degradable Protein (RDP) (>63% of total CP) has been reported to decrease fertility (Canfield *et al.*, 1990). Fish Meal (FM) is used in dairy cow ration as a source of Rumen Un-degradable Protein (RUP) (Heravi *et al.*, 2008). Of the various protein supplements

that are high in RUP, FM processes a good balance of lysine and methionine (Santos *et al.*, 1998). Supplementation of FM at fewer than 4% of the dietary Dry Matter (DM) might stimulate increased milk production without decreasing milk fat content (Broderick, 1992). However, when Soybean Meal (SBM) was replaced by high RUP supplements, microbial protein synthesis and flow to the duodenum decreased and the flow of non ammonia non microbial nitrogen in the duodenum increased (Clark *et al.*, 1992). However, Danesh *et al.* (2008) demonstrated that the ruminal and post-ruminal protein disappearances of oil seed meals were impacted by the sources. Recent studies (Broderick, 2003; Olmos *et al.*, 2006) have shown that

with diets based on alfalfa silage and corn silage and supplemented with solvent-extracted soybean meal, maximal milk and protein production was achieved with about 16.5% CP. Relative to the composition of milk protein, Canola Meal (CM) has an excellent balance of amino acids (Piepenbrink and Schingoethe, 1998). Replacement of CM for SBM had no negative effect on milk yield (Mazhari *et al.*, 2009). A supplemental supply of amino acids from sources that escape ruminal degradation and that complement the amino acid profile of microbial protein should increase performance or decrease the amount of protein required (Owens and Bergen, 1983). We hypothesized that with using the FM as protein source of diet, adding the CM as highly degradable CP could complement the effects of FM for milk production and positive effect on some measurement of blood metabolites.

Therefore, the aim of this study was to determine the effect of diets with FM (as high RUP sources) plus canola meal (as a high RDP source) or containing Cottonseed Meal (CSM, as a high RUP source) and Soybean Meal (SB, as a high RDP source) on performance and some blood metabolites of early lactating Holstein cows fed isonitrogenous whole crop barley silage based diets.

## MATERIALS AND METHODS

**Diets and animals:** Three isonitrogenous whole crop barley silage based diets (CP: 16.7%) with similar amount of CP degradability, but with different sources of protein (FM + CM as 42.8 + 82.4 g kg<sup>-1</sup> DM (FM/CM) or SM + CSM as 48.8 + 91 g kg<sup>-1</sup> DM (SM/CSM1) or 84 + 42.8 g kg<sup>-1</sup> DM (SM/CSM2)) were provided. Dietary ingredients and chemical composition are shown in Table 1. The diets were fed to 24 early lactating Holstein cows as a total mixed ration (*ad libitum* intake) twice a day (08:00 and 17:30 h). Sixteen multiparous and eight primiparous Holstein cows averaging 10±3 days in milk and 37.37 kg day<sup>-1</sup> of milk were used in this experiment. Cows were assigned to a completely randomized design employing 3 dietary treatments (8 cows per treatment) for 7 weeks. Animals were kept in tea stalls with individual feed bins in animal house and had free access to water. Banks were cleaned out each morning and orts were collected and weighted. Cows were milked three times per day at 5:00; 12:00 and 20:00 h. Dry Matter Intake (DMI) and milk production were recorded daily. Milk samples were taken at each milking during each week and mixed daily for each cow in proportion to the amount of milk produced at the 5:00; 12:00 and 20:00 h milking. Blood samples were collected in heparinized tubes from the jugular vein of each cow at 0, 2 and 4 h after the morning feeding in weeks 3 and 6. Samples were immediately centrifuged (3500 rpm, 10 min) and the plasma were

analyzed for glucose, Blood Urea Nitrogen (BUN), insulin, Serum Glutamic Oxaloacetic Transaminas (SGOT) and Serum Glutamic Pyrovic Transaminas (SGPT).

**Analytical procedure:** Dietary ingredient DM was determined by drying the samples at 105°C for 24 h and Organic Matter (OM) by ashing at 550°C for 6 h. Fat content of each feed was determined using standard procedure (Tecator Soxhlet 1043 Extraction). The CP content was determined by micro-Kjeldahl analysis (AOAC, 1990). Calcium concentration was determined by Flame Photometry (Model 410 Flame Photometer. Wag teg) and phosphorous content of the feeds was determined by Complexometric-Titrimetric method. For measurement of Milk Urea Nitrogen (MUN), 100 µL perchloric acid 25% was added to 900 µL of each milk sample, then, centrifuged (13200 rpm, 30 min, 4°C), Thereafter, 50 µL KCO<sub>3</sub> 25% was added to 450 µL of upper part of the solution. Then, the mixture was kept in room temperature for 5 h. After that, samples were centrifuged again (13000 rpm, 30 min) and upper clear solution was collected. Amount of MUN was measured using enzymatic photometric method. Amino acid concentration of the feed ingredients was determined using a Waters ACCQ. Tag Amino Acid Analysis System (Waters Corporation, Milford and MA01757). Diet amino acid profiles are shown in Table 2. Milk samples were

Table 1: Ingredients (g kg<sup>-1</sup>) and chemical compositions of the experimental diets

Items	Treatments*		
	FM/CM	SM/CSM1	SM/CSM2
Alfalfa hay	236.40	236.40	236.4
Barely silage	154.40	154.40	154.4
Corn grain	152.10	152.10	152.1
Wheat bran	82.90	82.90	82.9
Barely grain	152.10	152.10	152.1
Canola meal	86.40	0.00	0.0
Soybean meal	0.00	93.30	44.9
Cottonseed meal	0.00	44.90	88.3
Fish meal	44.90	0.00	0.0
Cotton seeds	62.20	62.20	62.2
Fat perils	13.80	13.80	13.8
Dicalcium phosphate (g kg <sup>-1</sup> )	1.70	1.70	1.7
Limestone (g kg <sup>-1</sup> )	0.60	0.60	0.6
Sodium bicarbonate (g kg <sup>-1</sup> )	4.80	4.80	4.8
Mineral and vitamin premix** (g kg <sup>-1</sup> )	6.80	6.80	6.8
CP (g kg <sup>-1</sup> )	16.70	16.70	16.7
ME (Mj kg <sup>-1</sup> )	10.20	10.20	10.2
NDF (g kg <sup>-1</sup> )	30.71	32.28	31.4
ADF (g kg <sup>-1</sup> )	17.00	17.00	17.0
Calcium (g kg <sup>-1</sup> )	1.50	1.30	1.4
Phosphorus (g kg <sup>-1</sup> )	1.40	1.30	1.3

\*FM/CM: Fish Meal and Canola Meal as 42.8 and 82.4 g kg<sup>-1</sup> DM, respectively; SM/CSM1: Soybean Meal and Cottonseed Meal as 48.8 and 91 g kg<sup>-1</sup> DM, respectively; SM/CSM2: Soybean Meal and Cottonseed Meal as 84 and 42.8 g kg<sup>-1</sup> DM; \*\* Premix contained (DM basis): 190000 mg kg<sup>-1</sup> Ca, 90000 mg kg<sup>-1</sup> P, 50000 mg kg<sup>-1</sup> Na, 9000 mg kg<sup>-1</sup> Mg, 3000 mg kg<sup>-1</sup> Fe, 3000 mg kg<sup>-1</sup> Zn, 2000 mg kg<sup>-1</sup> Mn, 100 mg kg<sup>-1</sup> Co, 300 mg kg<sup>-1</sup> Cu, 100 mg kg<sup>-1</sup> I, 1 mg kg<sup>-1</sup> Se, 500000 IU kg<sup>-1</sup> vitamin A, 100000 IU kg<sup>-1</sup> vitamin D3, 100 mg kg<sup>-1</sup> vitamin E, 3000 mg kg<sup>-1</sup> antioxidant (B.H.T)

Table 2: Amino acid concentration (percent of total amino acids) of the experimental diets

Amino acids	Treatments*		
	FM/CM	SM/CSM1	SM/CSM2
Alanine	5.10	4.58	5.00
Arginine	3.31	3.80	3.56
Cysteine	6.27	3.23	3.91
Glutamic acid and glutamine	1.06	1.70	1.70
Glycine	1.00	0.80	1.10
Isoleucine	3.10	4.77	4.30
Leucin	2.86	2.86	3.00
Methionine	6.78	5.71	6.12
Phenylalanine	2.24	2.21	2.25
Serine	5.25	5.60	5.75
Threonine	5.00	5.29	5.00
Tyrosine	3.55	3.77	3.70
Valine	1.07	0.71	0.95

\*FM/CM: Fish Meal and Canola Meal as 42.8 and 82.4 g kg<sup>-1</sup> DM, respectively; SM/CSM1: Soybean Meal and Cottonseed Meal as 48.8 and 91 g kg<sup>-1</sup>, DM, respectively; SM/CSM2: Soybean Meal and Cottonseed Meal as 84 and 42.8 g kg<sup>-1</sup> DM

used to determine fat, protein, lactose and Solid Not Fat (SNF) using Milko-tester (Foss Electric, Conveyor 4000). Blood urea nitrogen and glucose were determined using enzymatic procedure (AOAC, 1990). Activity of SGOT and SGPT enzymes were measured (using IFCC method) by spectrophotometer. Serum insulin concentration was determined by radioimmunoassay (using TR-IFMA (time-resolved immuno fluorometric assay)).

**Statistical analysis:** Statistical analysis was performed as a repeated measurement Analysis of Variance (ANOVA) using MIXED procedure of SAS. The model was:

$$Y_{ijk} = \mu + T_i + A_{ij} + D_k + (T \times D)_{ik} + \epsilon_{ijk}$$

Where,

- Y<sub>ijk</sub> = Dependent variable
- μ = The overall mean
- T<sub>i</sub> = Treatment effect
- A<sub>ij</sub> = Cow in treatment
- D<sub>k</sub> = Time effect
- (T × D)<sub>ik</sub> = Treatment × time
- ε<sub>ijk</sub> = Error

## RESULTS AND DISCUSSION

**Intake and lactation performance:** Dry matter intake, milk production and milk composition are shown in Table 3. Dry matter intake was unaffected by the treatments (FM/CM = 20.32, SM/CSM1 = 21.04 and SM/CSM2 = 20.82, SEM = 0.59, kg day<sup>-1</sup>), while time effect on DMI was linearly and quadratic significant (p < 0.0001). Milk yield and composition was similar among treatments, but cows fed FM/CM had the highest feed efficiency than the others (1.86 vs. 1.76 and 1.78, SEM = 0.06, for FM/CM, SM/CSM1 and SM/CSM2, respectively). Santos *et al.*

(1998) analyzed knowledge on the implication of protein supplements and protein nutrition of lactating dairy cows in a 12 years study review. In these comparisons, intake of DM was not significantly different between cows fed SBM and Menhaden FM. However, in several comparisons, DMI of FM diets was numerically lower than that of SBM diets. Salmon FM decreased the DMI when fed at >5% of the diet DM. They reported that this negative effect of Salmon FM is probably because of its high content of unsaturated fat. Milk yield was significantly increased by FM in 8 of the 32 comparisons.

Using FM/CM diet caused to increase a non significant in milk production (37.69 vs. 36.99 and 37.08, SEM = 1.65, kg day<sup>-1</sup> for FM/CM, SM/CSM1 and SM/CSM2, respectively). Several studies were conducted to compare FM to other protein sources in isonitrogenous diets. The results indicated that milk yield was not improved (p > 0.05) when FM replaced groundnut meal (Orskov *et al.*, 1981), urea (Oldham *et al.*, 1985; Gamsworthy, 1989) or corn gluten meal (Blauwiekel *et al.*, 1989). Feeding diets supplemented with either SBM or FM to cows in early lactation resulted in similar (p > 0.05) milk yield (Chmiel, 1987; Sloan *et al.*, 1988; Zerbhi *et al.*, 1988; McCarthy *et al.*, 1989). By replacing SBM with FM, increase in protein delivery to the small intestine was expected to increase milk yield (Blauwiekel *et al.*, 1989). In the experiment, all diets were designed to be similar in amount of protein and RDP and differences in milk production could not be related to the different in the amount of protein that received to small intestine. In the present study, the Amino Acid (AA) profile of the diets was not similar and FM/CM diets had better methionine/total AA ratio than SM/CSM1 and SM/CSM2 diets. Schwab *et al.* (1992a, b) pointed out the importance of both the amount and balance of Essential Amino Acid (EAA) in duodenal digesta and proposed that protein sources should be compared for percentage of Lysine (Lys) and Methionine (Met) in relation to the amount of total EAA. Assuming Lys and Met are the first two limiting AA for yields of milk and milk protein in most dairy diets (King *et al.*, 1990; Schwab *et al.*, 1992a, b). Results of previous studies showed that FM is a fairly good source of Lys, Met, but reported that the effectiveness of RUP for supplying the limiting AA for milk production appears to depend on the ability of the underlying diet to maximize microbial protein production. Among RDP protein sources, CM has excellent balance of AA (Piepenbrink and Schingoethe, 1998) therefore, blend of FM and CM supply good profiles of EAA for milk production than the other protein sources. In the present study, no differences were detected in percentage or yield of lactose, Solid Not Fat (SNF) and milk protein.

Table 3: Feed intake, milk production and milk composition of early lactating Holstein cows fed diets containing different sources of protein

Items	Treatments*			Treatment effect		Time effect (p-value)	
	FM/CM	SM/CSM1	SM/CSM2	SEM	p-value	Linearly	Quadratic
Dry matter intake (kg day <sup>-1</sup> )	20.32	21.04	20.82	0.59	0.17	0.01	0.010
Milk yield (kg day <sup>-1</sup> )	37.69	36.99	37.08	1.65	0.94	0.01	0.010
Feed efficiency	1.860	1.760	1.780	0.06	0.65	0.95	0.760
Milk fat (g kg <sup>-1</sup> )	25.80	28.02	28.77	1.17	0.18	0.01	0.010
Milk protein (g kg <sup>-1</sup> )	29.58	29.52	29.70	5.20	0.88	0.01	0.010
Milk lactose (g kg <sup>-1</sup> )	42.75	42.86	42.40	7.30	0.73	0.58	0.830
Milk solid not fat (g kg <sup>-1</sup> )	79.37	79.90	78.40	13.7	0.83	0.67	0.390
Milk urea nitrogen (mg dL <sup>-1</sup> )	10.98	11.12	11.01	0.26	0.94	0.05	0.136

Table 4: Various blood metabolites of early lactating Holstein cows fed diets containing different sources of protein

Items	Time of sampling (week)	Treatments*			Treatment effect		Time effect (p-value)	
		FM/CM	SM/CSM1	SM/CSM2	SEM	p-value	Linearly	Quadratic
Blood glucose (mg dL <sup>-1</sup> )	3	59.20	59.3	62.8	2.58	0.53	0.01	0.08
	6	61.70	63.5	66.2	1.82	0.23	0.58	0.39
Blood urea nitrogen (mg dL <sup>-1</sup> )	3	16.50	17.7	16.3	0.82	0.43	0.03	0.51
	6	14.60	17.6	16.4	1.08	0.17	0.01	0.38
Insulin (micro U mL <sup>-1</sup> )	6	9.16	12.4	7.9	1.34	0.10	0.01	0.09
Serum glutamic oxaloacetic transaminase (mg dL <sup>-1</sup> )	6	74.60	91.1	83.5	6.36	0.23	0.13	0.56
Serum glutamic pyrovic transeaminase (mg dL <sup>-1</sup> )	6	25.80	25.7	31.3	2.78	0.31	0.36	0.60

\*FM/CM: Fish Meal and Canola Meal as 42.8 and 82.4 g kg<sup>-1</sup> DM, respectively; SM/CSM1: Soybean Meal and Cottonseed Meal as 48.8 and 91 g kg<sup>-1</sup> DM, respectively; SM/CSM2: Soybean Meal and Cottonseed Meal as 84 and 42.8 g kg<sup>-1</sup> DM; SEM: Standard Error of Mean

Although, milk fat yield was not significantly different, milk fat percentage was numerically lower in FM/CM treatment than the other cows (FM/CM = 25.6, SM/CSM1 = 27.5 and SM/CSM2 = 28.7, SEM = 1.1, g kg<sup>-1</sup>). The detrimental effect of fish meal on milk fat secretion has been attributed to impairments in ruminal fermentation and/or post absorptive metabolism of lipids elicited by polyunsaturated fatty acids in fish oil (Ipharraguerre and Clark, 2005). Milk Urea Nitrogen in cows fed FM/CM and SM/CSM2 tended to be decrease (FM/CM = 10.98 and SM/CSM2 = 11.01 and SM/CSM1 = 11.12, SEM: 0.26, mg dL<sup>-1</sup>) although, no significant differences was observed. A strong correlation exists among dietary CP, BUN and MUN (Baker *et al.*, 1995; Rosler *et al.*, 1993).

**Blood metabolites:** Data of various blood metabolites are shown in Table 4. Altering protein sources had no effect on blood glucose concentration. The results for both blood glucose and BUN concentrations were investigated separately for week 3 and 6. No difference was exist among treatments at week 3 of the experimental period, but time sampling (0.0, 2 and 4 h after the morning feeding) was linearly (p = 0.001) significant. In addition, no difference was evident at week 6, although cows fed FM/CM had numerically lower blood glucose concentration than the other cows (FM/CM = 61.07 vs. SM/CSM1 = 63.58 and SM/CSM2 = 66.20, SEM = 1.82, mg dL<sup>-1</sup>). In all treatments, lowest blood glucose concentration was observed in 2 h after the morning feeding, then increased after 2 h. This decline might be

related to consumption of glucose by gastrointestinal tracts. Plasma insulin concentration at the last week of the experimental period was not significantly impacted by the treatments (FM/CM = 9.16, SM/CSM2 = 7.9 and SM/CSM1 = 12.41, SEM = 1.34, micro U mL<sup>-1</sup>). Several researchers demonstrated that an increase in the supply of AA to animal might be resulted in greater release of insulin (Kuhara *et al.*, 1992; Lemosquet *et al.*, 1997). It has been accepted that insulin is affected by the plasma concentration of glucose. In the present study, in spite of differences in blood insulin concentration, blood glucose concentration did not differ among the treatments. Blood urea nitrogen was determined at week 3 and 6 of the experimental period and results showed that it was not significantly differed among the treatments. However, at week 6, it was lower for cows fed FM/CM than the other cows (FM/CM = 14.66 vs. SM/CSM1 = 17.66 and SM/CSM2 = 16.41, SEM = 1.08, mg dL<sup>-1</sup>). Lower concentration of BUN, may be reflected to the lower DMI by this group of animals. The concentration of BUN reflects the status of dietary CP such as the percentage of CP, digestibility of CP and the energy level of ration (Rosler *et al.*, 1993). In most reports, the concentration of BUN is higher tat of MUN, which are in agreement with the results of the present study.

A number of blood constituents are useful as monitors for clinical or sub-clinical signs of metabolic disorder in high yielding cows. The elevation of SGPT and SGOT might be an indicator of accumulation of free fatty acids transported from blood to hepatocytes

(Studer *et al.*, 1993). High concentration of SGOT might also, imply damages in organs other than the liver, because SGOT exist in muscle, kidney, intestine and brain (Xu *et al.*, 1996). West (1990) suggested that arise in SGOT immediately after calving might reflect muscle damage. The two enzymes in this study did not differ across treatment rations. Although, SGOT concentration was numerically lower in cows fed FM/CM than the other cows (FM/CM = 74.6 vs. SM/CSM1 = 91.1 and SM/CSM2 = 83.5, SEM = 6.36, mg dL<sup>-1</sup>).

### CONCLUSION

It might be suggested that diets with similar amount of rumen degradable protein, but with different sources of protein (fish meal, canola meal, soybean meal and cottonseed meal) causing similar responses in early lactating Holstein cows. Results of the present study showed that milk production and DMI were not significantly influenced by the source of protein, when diets provided similar amount of RDP. In addition, various blood metabolite concentrations, as determined in the present study, indicated similar patterns in cows fed different protein sources. Therefore, it was concluded that diets with similar amount of RDP might not cause a difference in blood metabolite responses of early lactating Holstein cows fed isonitrogenous whole crop barley silage based rations.

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