

## Effects of Dietary Crude Protein Level on Growth Performance and Blood Parameters of Holstein Heifers and Steers Fed Ad Libitum

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**Abstract:** Two levels of dietary CP on growth performance, feed efficiency and blood parameters of Holstein heifers and steers were examined. Experimental diets consisted of 12 and 16% CP along with 2.5 Mcal kg<sup>-1</sup> of ME (DM basis). Ratios of CP: ME were 49 and 64 g/Mcal for 12 and 16% CP containing diets, respectively. Eight animals were assigned to each experimental diet in a 2X2 factorial arrangement of completely randomized design for 8 wk period. Diets containing 48% corn silage and 52% concentrate (DM basis) were fed twice daily in a total mixed ration. Intake of DM was higher for 12% CP (11.0 kg d<sup>-1</sup>) compared to 16% CP containing diet (7.7 kg d<sup>-1</sup>) across the groups. There was a higher BW gain for steers (1.27 kg d<sup>-1</sup>) compared to heifers (0.99 kg d<sup>-1</sup>) but no significant dietary CP level difference was observed for BW. Feed efficiency was higher for 16% CP (0.17) compared to 12% CP containing diet (0.11). There was no significant difference detected for serum urea N (8.6 mg dL<sup>-1</sup>) and creatinine (1.1 mg dL<sup>-1</sup>) concentrations between treatment groups. Data indicates that heifers during the growth period responded to 12% dietary CP better than 16% CP based on higher BW gain (1.16 kg d<sup>-1</sup>) and lower serum urea N concentration (8.3 mg dL<sup>-1</sup>). In steers, no difference in BW gain but higher feed efficiency (0.17 vs. 0.11) made 16% CP containing diet more preferable than 12% CP during the growth period.

**Key words:** Growth, heifer, steer, protein, urea N

### INTRODUCTION

The main purpose of heifer raising programs is to obtain appropriate age and weight for their future reproductive performance. In this respect, protein and energy feeding requirements must be provided for the earliest heat sign without any delay. Data indicates that heifers should be provided with RUP based CP sources during the first 6 mo of age followed by RDP based CP sources after that<sup>[1]</sup>. In addition, during the regular heifer growth periods (0.8 kg d<sup>-1</sup> BW gain) dietary CP levels should not exceed 12%<sup>[1]</sup>. However it has been suggested that this level of CP should be higher than 12% during accelerated heifer growth periods<sup>[2,3]</sup>. Hoffman *et al.*,<sup>[2]</sup> tested the effects of dietary CP level on accelerated heifer growth performance and found 0.3 kg higher average daily gain when heifers were fed with 16.3% dietary CP compared to 15.3% at 10 mo of age and 313 kg initial BW. In steers, NRC<sup>[4]</sup> requirements indicate that dietary CP level should not be lower than 14% along with higher RUP level compared to RDP during the first 4-6 mo of age. Later, this level of CP should be minimum 12% along with 50% RDP<sup>[4]</sup>. Brown *et al.*,<sup>[5]</sup> indicated that growing steers provided with 13% dietary CP compared to 12%

consumed 1 kg higher DM and concluded that decreasing the CP level in the diet might have caused reduction in metabolizable protein requirement of these animals. Another aspect of protein nutrition in ruminants is serum urea N concentration. Hoffman *et al.*,<sup>[6]</sup> and Abeni *et al.*,<sup>[7]</sup> found a linear increase in serum urea N concentration as dietary CP level is increased linearly.

In this study, our objectives were to compare the growth performance, feed efficiency and blood parameters of growing Holstein heifers and steers fed with two levels of dietary CP ad libitum.

### MATERIALS AND METHODS

Thirty-two Holstein heifers and steers with BW changing between 284 and 293 kg were randomly assigned to one of four experimental diets. Mean initial BW was 285, 284, 290 and 293 kg for 12% CP receiving heifer (12 H) and steer (12 S) and 16% CP receiving heifer (16 H) and steer (16 S) groups, respectively. Mean initial age of animals was 14 and 15 mo for heifers and steers, respectively. Experimental diets (Table 1) were isocaloric and differed in CP level (12 and 16%). Animals were fed with total mixed ration twice daily for ad libitum intake and

**Table 1: Ingredient and nutrient composition of the experimental diets**

Ingredient	(% of DM)	
	12% CP	16% CP
<b>Forage</b>		
Corn silage <sup>1</sup>	48.0	48.0
Concentrate mix <sup>2</sup>		
Shelled corn	13.0	13.0
Barley	13.0	13.0
Cotton seed meal	13.0	13.0
Corn gluten feed	9.5	8.0
Molasses	2.5	2.5
Urea	-	1.5
Limestone	0.6	0.6
Sodium chloride	0.2	0.2
Mineral-vitamin mix <sup>3</sup>	0.2	0.2

Nutrient	Experimental diet <sup>4</sup>			
	(% of DM)			
	12 H	12 S	16 H	16 S
OM	90.9	91.5	90.7	90.9
CP	12.4	12.5	16.2	16.2
CF	18.7	18.5	18.5	18.2
ADF	22.6	22.3	22.2	22.0
Ether extract	3.4	3.4	3.1	3.2
Ash	9.1	8.5	9.3	9.1
NFE <sup>5</sup>	56.4	57.1	52.9	53.3
NE <sub>m</sub> (Mcal kg <sup>-1</sup> ) <sup>6</sup>	1.50	1.53	1.50	1.51
NE <sub>g</sub> (Mcal kg <sup>-1</sup> ) <sup>7</sup>	0.86	0.89	0.85	0.87
ME (Mcal kg <sup>-1</sup> ) <sup>8</sup>	2.50	2.55	2.50	2.52
CP:ME (g Mcal <sup>-1</sup> ) <sup>9</sup>	49.6	49.0	64.8	64.3

<sup>1</sup>Contained 30.4% DM, 7.5% CP, 33.1% ADF, 1.38 Mcal kg<sup>-1</sup> NE<sub>m</sub>, 0.8 Mcal kg<sup>-1</sup> NE<sub>g</sub>. <sup>2</sup>Contained 17.1% CP, 14.2% ADF, 1.67 Mcal kg<sup>-1</sup> NE<sub>m</sub>, 1.28 Mcal kg<sup>-1</sup> NE<sub>g</sub>. <sup>3</sup>Contained 10% S, 7.5% K, 5% Mg, 3% Mn, 3% Zn, 2% Fe, 0.5% Cu, 0.025% I, 0.015% Se, 0.004% Co, 2665 IU/g vitamin A, 900 IU g<sup>-1</sup> vitamin D, 3.52 IU g<sup>-1</sup> vitamin E. <sup>4</sup>12 H= 12% CP receiving heifer, 12 S= 12% CP receiving steer, 16 H= 16% CP receiving heifer, 16 S= 16% CP receiving steer. <sup>5</sup>Nitrogen free extract= 100-(CF+CP+EE+ash) <sup>6</sup>NE<sub>m</sub> (Mcal kg<sup>-1</sup>) = (0.655\*DE-0.185)/0.4536, where DE (Mcal/lb)= 0.0229\* CP+0.0349 \* EE+0.0091\*CF+0.00017\*NFE<sup>2</sup>+0.005\*NFE-0.068<sup>10</sup> <sup>7</sup>NE<sub>g</sub> (Mcal kg<sup>-1</sup>)= (0.815\*DE-0.0497\*DE-0.625)/0.4536<sup>10</sup> <sup>8</sup>Calculated as Ne<sub>m</sub>/0.6<sup>3</sup> <sup>9</sup>Calculated as (%CP\*10)/ME<sup>3</sup>

housed in a ounter-slope total confinement facility for 8 wk experimental period. Total mixed diets were composed of corn silage and shelled corn: barley: cotton seed meal based concentrate (Table 1). Feed material was sampled weekly for DM, OM, CP, EE, ash<sup>[8]</sup> and ADF<sup>[9]</sup> analyses. Body weight was recorded weekly at 0800.

Blood sampling was carried out weekly and taken out from each animal within each experimental diet. Twenty mL of blood sample was taken from coxigeal vein of the each animal using a vacutainer tube and centrifuged at 3500 rpm for 15 min. Then 1 mL of serum was transferred into another tube and stored at -20°C for urea N and creatinine analyses. Serum urea N analysis was done by using urease glutamate dehydrogenase enzyme technique<sup>[11]</sup> and samples were read in a spectrophotometer at 340 nm. Serum creatinine analysis was done by Jaffe reaction<sup>[12]</sup> and samples were read in a spectrophotometer at 510 nm.

*In vitro* DM digestibilities of treatment diets were also tested according to the method described by Tilley and Terry<sup>[13]</sup>.

The data on DM intake, final BW, daily BW gain, feed efficiency and blood parameters were subjected to statistical analysis for 2X2 factorial arrangement of completely randomized design<sup>[14]</sup>. Due to the small variation in initial ages of animals, age was considered as covariate in statistical analysis. Differences were declared at p<0.05. *In vitro* DM digestibility results were analyzed with ONE-WAY ANOVA test and treatment mean differences were tested with Duncan test.

**RESULTS**

Effect of dietary CP level on animal performance data is presented in Table 2. No significant difference was observed for DM intake between heifers and steers receiving 16% CP containing diet. However there was 1.4 kg d<sup>-1</sup> higher DM intake difference for steers compared to heifers received 12% CP containing diet. Significant DM intake difference was also observed between treatment diets as being higher for 12% CP (11.0 kg d<sup>-1</sup>) compared to 16% CP containing diet (7.7 kg d<sup>-1</sup>). In addition, similar DM intake trend was observed as % of BW for 12% CP containing diet. No significant CP intake difference was observed either between animal groups or between experimental diets. Only steers receiving 12% CP compared to 16% CP containing diet consumed numerically higher intake of CP. Heifers receiving 12% CP containing diet had 14 kg higher final BW than 16% CP containing diet. Similarly, there was a higher daily BW gain in heifers receiving 12% CP containing diet compared to 16%. However no significant daily BW gain difference was observed for steers receiving both 12 and 16% CP containing diets. In general, daily BW gain was higher for steers than heifers in both diets. Higher feed efficiency was observed for 16% CP compared to 12% CP containing diet. This was especially more pronounced for steers receiving 16% CP containing diet.

Serum urea N concentration was higher for 16% CP containing diet received by both heifers and steers. In addition heifers had higher concentration of serum urea N than steers. There was no effect of CP level on serum creatinine concentration of heifers and steers. However steers had higher concentration of serum creatinine than heifers in both treatment diets.

*In vitro* DM digestibilities of treatment diets are presented in Fig. 1. Heifers receiving 16% CP containing diet had the highest *in vitro* DM digestibility than other treatments. In average, both heifers and steers receiving 16% CP containing diet had 4% higher *in vitro* DM digestibility than 12% CP containing diet. In addition, heifers had 4% higher *in vitro* DM digestibility in average than steers ( Fig. 1).

Table 2: Effects of dietary CP level on intakes of DM and CP, daily BW gain, feed efficiency, serum urea N and creatinine concentrations of heifers and steers

Item	Experimental diet <sup>1</sup>				Effect (p-value) <sup>2</sup>			
	12 H	12 S	16 H	16 S	SEM <sup>3</sup>	A	CPL	AxCPL
DM intake (kg/d)	10.3	11.7	7.8	7.6	0.4	NS	<0.05	<0.1
DM intake (% of BW)	2.81	3.62	2.39	2.04	0.11	NS	<0.05	<0.1
CP intake (kg/d)	1.27	1.46	1.27	1.23	0.07	NS	NS	NS
CP intake (% of BW)	0.35	0.45	0.39	0.33	0.02	NS	NS	NS
Final BW (kg)	350	356	336	363	4	<0.1	NS	<0.1
BW gain (kg/d)	1.16	1.28	0.82	1.26	0.05	<0.1	NS	<0.1
Feed efficiency	0.11	0.11	0.11	0.17	0.01	NS	<0.1	<0.1
Serum urea N (mg dL <sup>-1</sup> )	8.3	7.0	10.5	8.5	0.2	<0.1	<0.05	NS
Serum creatinine (mg dL <sup>-1</sup> )	1.0	1.2	1.0	1.2	0.04	<0.1	NS	NS

12 H= 12% CP receiving heifer, 12 S= 12% CP receiving steer, 16 H= 16% CP receiving heifer, 16 S= 16% CP receiving steer <sup>2</sup>A= Animal (heifer vs. steer), CPL= CP level, AxCP= Animal x CP level interaction <sup>3</sup>SEM= Standard error of mean

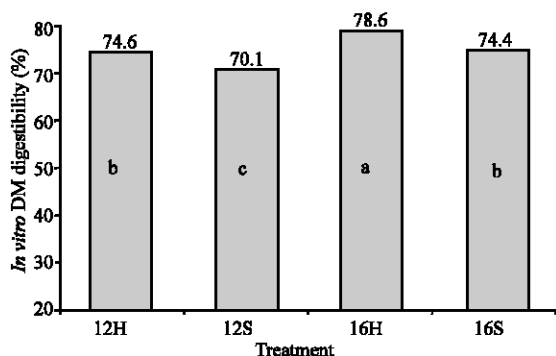


Fig. 1: *In vitro* DM digestibilities of treatment diets (12 H= 12% CP receiving heifer, 12 S= 12% CP receiving steer, 16 H= 16% CP receiving heifer, 16 S= 16% CP receiving steer). Different letters on each bar indicate a difference (p<0.05; pooled SEM= 0.9)

### DISCUSSION

It has been suggested that during heifer and steer growth periods, ad libitum feed intake is highly preferable for obtaining maximal growth<sup>[3,15]</sup>. In the present study, 2.5 and 4.1 kg d<sup>-1</sup> of higher DM intake for heifers and steers, respectively might be explained by the lower RDP content of 12% CP containing diet due to urea addition. Zimm *et al.*,<sup>[15]</sup> found higher intakes of DM and CP in growing steers when they were fed with higher RDP containing diets. In addition, Tomlinson *et al.*,<sup>[6]</sup> indicated that increasing level of RUP from 31 to 55% increased DM intake in heifers by 1.6 kg d<sup>-1</sup>. In the present study, 12% CP containing diet had 25% less RDP than 16% CP containing diet where DM intake was increased by 43% for 12% CP containing diet. Although no significant BW gain difference was found between 12 and 16% CP

containing diets in our study, Fluharty and Loerch<sup>[17]</sup> found a linear increase in BW gain of growing steers receiving dietary CP concentrations of 12, 14 and 16%, respectively. Based on the NRC<sup>[1]</sup> requirements of growing heifers, dietary CP: ME ratios should be 60 and 50 g Mcal<sup>-1</sup> during 6-12 mo and after 12 mo of age, respectively. In our study, heifers were 14 mo of age at the beginning for 12% CP containing diet in which dietary CP: ME ratio was 49.6 g Mcal<sup>-1</sup>. So that heifers receiving 12% CP containing diet gained 0.34 kg d<sup>-1</sup> higher body weight. Similarly Bagg *et al.*,<sup>[18]</sup> concluded that heifers receiving diet at 14% CP level after 12 mo of age gained higher BW than heifers receiving diet at 16% CP level with same dietary energy concentration. In addition, Hoffman *et al.*,<sup>[6]</sup> found no significant BW gain difference in growing heifers (300 kg BW) where diets contain CP level higher than 12.7%. These results indicate that growing heifers respond to diets containing 50 g Mcal<sup>-1</sup> CP: ME ratio better in terms of daily BW gain. Heifers still may gain weight above this ratio but it is necessary to check whether or not this excessive BW gain is due to body protein or fat deposition<sup>[19]</sup>.

In growing ruminants, blood urea N and creatinine concentrations are the important outcomes for knowing the protein nutrition status. Particularly urea N concentration is tended to be higher when heifers and steers are fed with the dietary CP level 40% above the requirements<sup>[20,21]</sup>. In the present study, serum urea N concentrations were in desirable range across the treatments and averaging 8.6 mg dL<sup>-1</sup>. Utlu *et al.*<sup>[22]</sup> indicated that Holstein heifers and steers during growth periods fed with regular diets should have 8-12 mg dL<sup>-1</sup> and 0.8-1.3 mg dL<sup>-1</sup> of serum urea N and creatinine concentrations, respectively. Higher amounts of CP

intake in growing heifers and steers might elevate the serum urea N and creatinine concentrations<sup>[7,23]</sup>. Elrod and Butler<sup>[23]</sup> found about two folds of increase in serum urea N concentration (14 vs. 24 mg dL<sup>-1</sup>) of heifers fed with either 13 or 19% of dietary CP, respectively. In our study, this trend was also observed for 16% CP containing diet (9.5 mg dL<sup>-1</sup>) compared to 12% CP (7.6 mg dL<sup>-1</sup>) in both heifers and steers. However the difference was not so extreme and values were in the range of literature<sup>[24]</sup>. Higher serum creatinine concentration is usually an indication of BW gain or muscle deposition in growing animals. However in our study this pattern was not correlated with BW gain of neither heifers nor steers. Although similar serum creatinine concentration was observed between the treatment diets for both heifers and steers, especially the latter have been reported for having higher concentration of serum creatinine compared to heifers during the growth period<sup>[5]</sup>.

Although *in vitro* DM digestibility of treatment diets in the present study is not a significant parameter to correlate with CP nutrition of growing heifers and steers, it was more correlated with DM intake. There was a significant *in vitro* DM digestibility reduction for 12% CP containing diet as DM intake was higher for this treatment in both animal groups (Fig. 1).

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