EFFECT OF VARYING LEVELS OF DIETARY RUMEN UNDEGRADABLE PROTEIN ON DRY MATTER INTAKE, NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF CROSSBRED CATTLE HEIFERS

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ABSTRACT

Thirty six crossbred dairy cattle heifers (average weight 200±4 kg) were used in completely randomized design to examine effect of varying levels of ruminally undegradable protein on nutrient intake, nutrient digestibility, nitrogen (N) balance, blood metabolites, blood chemistry and growth performance. Heifers were divided into four groups. Four iso-nitrogenous and iso-caloric diets (RUP25, RUP35, RUP45 and RUP55) were formulated. The RUP25 diet was formulated to contain RUP 25% of CP. The RUP35, RUP45 and RUP55 diets supplied RUP 35, 45 and 55% of CP, respectively. This experiment lasted for 70 days. The heifers fed ad libitum. The intake of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) increased ($P<0.05$) with increasing level of dietary RUP. Digestibility of DM, NDF and ADF was greater ($P<0.05$) in heifers fed RUP25 diet than those fed RUP35, RUP45 and RUP55 diets. A linear increase ($P< 0.05$) in N balance was noted in heifers fed increasing level of dietary RUP. Urinary N excretion, blood urea N (BUN) and creatinine decreased ($P< 0.05$) linearly in heifers with increasing level of dietary RUP. Blood pH, blood glucose and blood chemistry remained unaltered across all treatments. In conclusion, N retention can be enhanced, weigh gain can be increased and FCR can be improved by increasing RUP in the diet of growing crossbred cattle heifers and RUP55 diet is optimum regarding DM intake, N retention, weight gain and FCR. The information developed in this study can help in cost effective dairy production through attaining early puberty in heifers.

Keywords: *Rumen Undegradable Protein, Crossbred Heifers & Growth Performance*

INTRODUCTION

Growing heifers require metabolizable protein for the tissue synthesis. Metabolizable protein is provided by ruminally degradable protein (RDP) and RUP which are fractions of dietary CP (Sultan, Javaid, Nadeem, Mustafa & Akhtar, 2009; Javaid, Sharif, Hamad, Rehman & Zamir, 2017). The RDP meet the requirements of rumen microbes and is considered essential for rumen microbial growth. The RDP improves ruminal fermentation and ensures the supply of microbial protein to host animal (Das, Kundu, Kumar & Datt, 2014; Javaid et al., 2017). Microbial protein is unable to provide metabolizable protein requirement of fast growing ruminants (Sultan et al., 2009). The RUP is supplemented when microbial protein alone is insufficient to meet ruminant metabolizable protein requirements (Kalscheur, Baldwin, Glenn & Kohn, 2006). The RUP provides additional supply of circulating amino acids (AA) which are utilized to meet ruminant fast growing requirements (Butler, 1998).

Supplementation of RUP to ruminants results to improve protein utilization (Nisa, Javaid, Sarwar & Shahzad, 2008; Javaid, Shahzad, Nisa & Sarwar, 2011). Increasing the RUP content in the diet increased N retention and weight gain in growing calves and goats (Pattanaik, Sastry, Katiyar & Lal, 2003; Paengkoum, Liang, Jelan & Basery, 2004; Sultan et al., 2009). Imbalance of RDP and RUP in ruminant diet can compromise microbial protein synthesis, ruminal digestion and energy as well as protein availability resulting to affect the productive potential of ruminant animals (Santos, Santos, Theurer & Hubber, 1998; NRC, 2001; Reynal & Broderick, 2005; Javaid et al., 2017). High level of dietary RDP causes excessive ammonia production in rumen and ultimately increases the BUN concentrations (Dhali, Mishra, Mehla & Sirohi, 2006; Javaid et al., 2017) which results to depress DM intake and decrease the efficiency of protein utilization in ruminants (Nisa et al., 2008; Javaid et al., 2011). There is little information on the literature regarding the effect of RUP on N retention and growth performance of crossbred dairy cattle heifers. Therefore the present study was planned to determine the effect of varying level of dietary RUP on DM intake, nutrient digestibility, N balance, blood metabolites, blood chemistry and the growth performance in growing crossbred dairy cattle heifers.

MATERIALS AND METHODS

Thirty six growing crossbred dairy cattle heifers (average weight 200±4 kg), were used in Completely Randomized Design. Heifers were divided into four groups, nine heifers in each group. Four iso-nitrogenous and iso-caloric diets were formulated using NRC (2001) criteria for protein and energy (Table 1). The control diet had RUP 25% of CP and was termed as RUP25 diet. The RUP35, RUP45 and RUP55 diets had RUP 35, 45 and 55% of CP, respectively (Table 2). The heifers fed twice daily ad libitum. Availability of fresh water was ensured round the clock during the period of experiment. The trial lasted for 70 days. The adaptation period was the first 10 days while next 60 days were for data collection. Feed offered and orts were recorded and weighed on daily basis during the experiment. Feed samples were dried at 55°C. The heifers were weighed fortnightly. The total collection method was used to determine N balance and nutrient digestibility by method described by Nisa et al. (2008). The weight of faeces and urine from individual animal was recorded daily. The 20% of mixed fecal samples were preserved and dried at 55°C.

After collection period, each animal dried fecal sample was composited and 10% of that composited sample was used for analysis. The urine was collected in buckets attached with plastic pipe made to surround the vulva. The urine was acidified using 50% H_2SO_4. The urine was collected in a container (30 liters). The 20% of acidified urine was preserved at -20°C awaiting analysis. The preserved urine samples were composited after thawing and 10% of the composited sample was taken for analysis. Blood was collected from jugular vein in heparinized syringes at 6 h post feeding to determine pH (AOAC, 2001). From each heifer, blood samples were collected lacking anticoagulant to produce serum which was stored at -20°C awaiting analysis for BUN and blood biochemical parameters. The N balance was calculated by the difference of N consumed and the sum of fecal N plus urinary N excreted.

Chemical Analysis

Dried feed, orts and fecal samples were ground in a 1 mm through Wiley mill and analyzed for DM, CP, ash, NDF and ADF. The N was determined by Kjeldahl method (AOAC, 2001) and CP was calculated by multiplying N by 6.25. The NDF and ADF contents were determined by method described by Van Soest et al. (1991). The BUN was determined using analytical kit.

Statistical Analysis

The data, thus, collected were examined using the procedure of GLM PROC of SAS 9.2 (SAS, 2009) and comparison of means was determined using "Duncan Multiple Range test" (Duncan, 1955).

RESULTS

The DM, CP, NDF and ADF intakes were higher ($P < 0.05$) in heifers fed diets containing increasing level of RUP (Table 2). The DM, NDF and ADF digestibilities were decreased ($P<0.05$) in heifers fed diet containing increasing level of RUP. However, CP digestibility remained unaltered across all treatments. There was linear increase ($P<0.05$) in N balance in heifers fed increasing level of RUP (Table 3). Similar trend was found in N intake and fecal N excretion. Whereas, urinary N excretion decreased ($P < 0.05$) linearly with increasing the level of dietary RUP. The BUN and creatinine decreased ($P < 0.05$) linearly in heifers fed increasing level of RUP (Table 4). Blood pH and blood glucose did not change by any treatment. Similar, findings were noticed about blood chemistry of growing heifers (Table 5). Daily live weight gain increased ($P<0.05$) linearly with increasing dietary level of RUP (Table 6). Daily live weight gain was 330, 430, 530 and 730 g in heifers fed RUP25, RUP35, RUP45 and RUP55 diets, respectively. The FCR was improved ($P<0.05$) with increasing level of dietary RUP and best FCR was noticed in heifers fed RUP55 diet (Table 6). The FCR was 15.90, 13.95, 12.83 and 10.54 in heifers fed RUP25, RUP35, RUP45 and RUP55 diets, respectively.

DISCUSSION

The DM intake was increased with increase in the level of dietary RUP. The intake of CP, NDF and ADF increased because of increased DM intake. Kumar et al. (2005) reported increased DM intake (12.89 to 13.20 Kg/d) in crossbred cattle when dietary RUP was enhanced from 41 to 48% of dietary CP. Similarly, Chaturvedi and Walli (2001) also reported improved DM intake in crossbred cows when the dietary RUP enhanced from 29 to 43% of CP. Similar finding were reported by Javaid et al. (2011) who explained that DM intake was increased in buffalo bulls with increase in RUP in their diet and this increase in DM intake was due to decreased BUN concentration because high concentration of BUN depress the DM intake. Increasing level of dietary RUP, DM intake may be increased because of increased demand of energy to synchronize increased supply of AA at cellular level. However, Henson et al. (1997) reported decreased DM intake with increasing RUP level from 33.7 to 45.4 % of dietary CP. The possible reason of this negative relationship may be due to unpalatability of RUP sources.

Moreover, decreased DM intake with animal protein as RUP source may be because of reduced ruminal NH_3-N level which might have decreased proliferation of rumen microbes resulting decreased digestibility and finally decreased nutrient intake (Faverdin et al., 1999). However, another study reported no change in DM intake in buffaloes fed diet containing increasing level (31 to 61% of CP) of RUP (Jabbar et al., 2013). The decreased DM, NDF and ADF digestibility might be because of increased DM, NDF and ADF intakes by heifers fed diets high in RUP. This reduced digestibility could occur because of increased passage rate due to enhanced DM intake. Keery and Amos (1993) documented that when dietary RUP was increased from 35 to 44 % of dietary CP then NDF digestibility reduced from 52.8 to 43.6 %. The reduction reason of digestibility at higher dietary RUP might be because of lower concentration of ruminal NH_3-N which might have reduction of fiber degrading enzyme by decrease in bacterial activity in the rumen of crossbred heifers. The low ruminal NH_3-N inhibits microbial activity and decreases rate of fiber digestion.

Santos et al. (1998) suggested that the use of RUP in the diets often resulted in a decreased RDP resulting to decrease microbial protein synthesis. Decrease in microbial activity can cause a depression in nutrient digestion (Javaid et al., 2017). Unaltered CP digestibility in the current study could be because either diets possessed adequate dietary RDP to maintain rumen fermentation, providing maximum ruminal NH_3-N to rumen microbes for digestion (Salisbury et al., 2004; Swanson et al., 2000) or heifers fed diets high in RUP recycled sufficient N to compensate for RDP deficiency, possibly consuming the enhanced AA supply which reaches the small intestine as a recyclable N source. The results of present study supported by Jabbar et al. (2013) who reported that CP digestibility did not change in early lactating buffaloes fed increasing level of RUP (from 31 to 61% of CP). The N intake and N balance were higher in growing heifers fed diets high in RUP. This higher N balance in animals fed diets high in RUP was due to their reduced urinary N excretion. Pattanaik et al. (2003) documented higher N retention by feeding low degradable protein diet than those fed high degradable protein diet.

Paengkoum et al. (2004) documented similar findings by describing that N retention increased linearly as the dietary RUP level increased in goats. In current study, the urinary N secretion reduced linearly with swelling level of dietary RUP. It is reported that the linear decrease in urinary N excretion with increasing dietary RUP in dairy animals is also reported by other scientists (Kalscheur et al., 2006; Nisa et al., 2008). Similar findings were reported by other researchers (Pattanaik et al., 2003; Davidson et al., 2003; Reynal and Broderick, 2005; Castillo et al., 2001). Contrary to results of present study, Swanson et al. (2000) reported increased urinary N output with enhanced dietary RUP in whether fed low-quality grass hay. Another study detected unchanged urinary N or fecal output in ruminants fed diets supplemented with low or high RUP when they consumed poor-quality grass hay mixture (Salisbury et al., 2004). Reduction of degradability of proteins supplement to ruminants fed low-quality forages has the potential to enhance N recycling while maintaining a positive N balance and excreting less N (Atkinson et al., 2007). The higher BUN values in heifers fed RUP25 diet were because of higher dietary RDP concentration in this diet. The high dietary RDP might have released excessive ruminal $NH_{3,}$-N exceeding the capturing capacity of ruminal microbes.

In current study, higher daily weight gain and better feed conversion ratio in heifers were due to extra supply of dietary RUP as well as increased DM intake. The animal provided with additional RUP provided bonus metabolizable protein for tissue deposition and a part of that RUP also served as N source for endogenous recycling. The increasing dietary RUP level increased the weight gain through increasing body tissue deposition. The current study results were not in accordance with those documented by Can et al. (2004) who observed that DM intake and feed efficiency were unchanged by increasing dietary RUP level. The present study findings are in accordance with those of Chalupa (1975) who reported increased daily weight gain of growing ruminants fed diets high in dietary RUP. It is opined that protein from microbial supply was lacking to meet AA requirement for rapid growth and maintenance of heifers, therefore, additional supply of RUP met AA requirement of growing heifers, resulting in high daily weight gain. In addition to moderating ruminal NH_3 levels immediately following supplementation, the prolonged AA deamination contained in supplemental RUP might have supported a more stable environment in rumen by providing the animal with a more sustained recyclable N source.

CONCLUSION

The results of present study reveals that DM intake, N retention, weight gain can be increased and FCR can be improved by increasing RUP in the diet of growing crossbred dairy cattle heifers. The diet containing RUP 55% of CP is considered optimum regarding DM intake, N retention, weight gain and FCR in growing crossbred dairy cattle heifers. The information developed in this study can help in cost effective dairy production through attaining early puberty in heifers.

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Annexures

Table 1 Ingredients and Chemical Composition of Experimental Diets

Ingredients (%)	**Diets[1]**			
	RUP25	RUP35	RUP45	RUP55
Barseem fodder	15.00	15.00	15.00	15.00
Oat fodder	15.00	15.00	15.00	15.00
Maize Broken	10.00	12.00	14.00	6.50
Wheat Straw	1.00	5.00	14.00	25.90
Wheat bran	34.40	16.00	0.50	0.50
Maize gluten Meal 30%	1.00	1.00	2.50	0.50
Maize gluten Meal 60%	0.10	1.00	3.00	0.10
Canola meal	3.00	6.00	1.00	0.50
Soybean Meal	1.00	6.00	9.00	11.00
AmiPro	0.00	6.00	9.00	14.00
Vegetable Oil	1.00	1.00	1.00	1.00
Cane Molasses	15.00	14.00	14.00	8.00
Mineral Mix[2]	2.00	2.00	2.00	2.00
Urea	1.50	0.00	0.00	0.00
Chemical composition (%)				
Dry matter	65.94	65.81	66.13	67.41
Crude protein	16.15	16.14	16.13	16.11
RUP % of CP	25.06	35.02	45.01	54.99
Neutral detergent fiber	23.80	22.78	27.93	33.44
Acid detergent fiber	11.38	12.28	15.59	22.92
Acid detergent lignin	2.51	3.04	3.31	4.75
Ash	9.24	8.90	7.20	7.80
RUP % of dry matter	4.07	5.68	7.26	8.86
RDP % of dry matter	12.07	10.50	8.87	7.25
Metabolizable Energy Mcal/kg	2.17	2.20	2.19	2.16

[1]RUP25, RUP35, RUP45 and RUP55 contain 25, 35, 45 and 55% rumen undegradable protein, respectively.

[2]Mineral mixture contains 22, 9.0, 0.2, 0.02, 0.6, 0.5. 0.02, 0.20 and 0.02% Ca, P, Mn, I, Fe, Cu, Co, Zn and Se, respectively.

Table 2 Dry Matter Intake and Digestibility in Growing Crossbred Dairy Heifers Fed Diets Containing different levels of Rumen Un-degradable Protein

Variable	Diets[1]				SEM[2]	Significance
	RUP25	RUP35	RUP45	RUP55		
Intake (kg/d)						
Dry matter	5.25^{d}	6.00^{c}	6.80^{b}	7.70^{a}	0.53	*
Crude protein	0.85^{c}	0.94^{c}	1.08^{b}	1.24^{a}	0.09	*
Neutral detergent fiber	1.24^{c}	1.36^{c}	1.89^{b}	2.57^{a}	0.30	*
Acid detergent fiber	0.59^{d}	0.73^{c}	1.06^{b}	1.76^{a}	0.26	*
Digestibility (%)						
Dry matter	65.21^{a}	61.05^{b}	58.71^{c}	54.61^{d}	2.22	*
Crude protein	73.55	75.5	71.24	70.62	1.12	NS
Neutral detergent fiber	63.11^{a}	62.03^{a}	59.44^{b}	59.0^{b}	1.00	*
Acid detergent fiber	47.20^{a}	48.50^{a}	41.14^{b}	40.10^{b}	2.11	*

[1]RUP25, RUP35, RUP45 and RUP55 contain 25, 35, 45 and 55% rumen undegradable protein
[2]Standard error mean. NS for Non-significant (P>0.05) & * stand for significant (P<0.05).
[a,b,c,d] Means in a row with different superscripts differ significantly (p<0.05).

Table 3 Nitrogen Balance in Growing Crossbred Dairy Heifers Fed different levels of Rumen Un-degradable Protein

Parameters (g)	Diets[1]				SEM[2]	Significance
	RUP25	RUP35	RUP45	RUP55		
Nitrogen intake	136^{d}	153.6^{c}	174.4^{b}	198.4^{a}	13.5	*
Fecal nitrogen	36.0^{c}	37.70^{c}	50.15^{b}	58.28^{a}	5.3	*
Urinary nitrogen	112.52^{a}	102.23^{b}	87.93^{c}	81.91^{d}	6.9	*
Nitrogen balance	-12.52^{d}	13.67^{c}	36.32^{b}	58.21^{a}	15.2	*

[1]RUP25, RUP35, RUP45 and RUP55 contain 25, 35, 45 and 55% rumen Un-degradable protein.
[2]Standard Error Mean. * stand for significant (P<0.05).
[a,b,c,d] Means in a row with different superscripts differ significantly (p<0.05).

Table 4 Blood pH, Blood Urea Nitrogen, Blood Glucose and Blood Creatinine level in Growing Crossbred Heifers Fed different levels of Rumen Protein

Parameters	Diets[1]				SEM[2]	Significance
	RUP25	RUP35	RUP45	RUP55		
Blood pH	7.81	7.80	7.83	7.81	0.01	NS
Blood urea nitrogen, mg/dL	21.75^{a}	20.40^{a}	17.40^{b}	16.50^{c}	1.24	*
Blood glucose, mg/dL	73.41	73.50	73.00	73.50	0.12	NS
Creatinine, mg/dL	1.94^{a}	1.87^{a}	1.65^{b}	1.50^{c}	0.10	*

[1]RUP25, RUP35, RUP45 & RUP55 contain 25, 35, 45 & 55% rumen undegra; protein.
[2]Standard error mean. NS for non-significant (P>0.05) & *stand for (P<0.05).
[a,b,c] Means in a row with different superscripts differ significantly (p<0.05).

Table 5 Blood Chemistry of Growing Crossbred Dairy Heifers Fed different levels of Rumen Un-degradable Protein

Parameters (%)	Diets[1]				SEM[2]	Significance
	RUP25	RUP35	RUP45	RUP55		
Hemoglobin (g/dL)	13.94	13.70	14.00	14.00	0.07	NS
Neutrophils	28.50	28.40	28.70	28.72	0.08	NS
Lymphocytes	63.80	63.33	63.79	63.79	0.12	NS
Monocytes	3.45	3.36	3.33	3.50	0.04	NS
Eosinophils	5.60	5.96	5.82	5.70	0.08	NS
Basophils	1.44	1.39	1.50	1.51	0.03	NS
Platelets (k/µL)	593.51	594.44	596.57	593.26	0.75	NS

[1]RUP25, RUP35, RUP45 & RUP55 contain 25, 35, 45 & 55% rumen Un-degradable protein.

[2]Standard error mean. NS stand for non-significant (P>0.05)

Table 6 Growth Performance of Growing Crossbred Dairy Heifers Fed different levels of Rumen Un-degradable Protein

Parameters	Diets[1]				SEM[2]	Significance
	RUP25	RUP35	RUP45	RUP55		
Dry matter intake, (kg/d)	5.25^{d}	6.00^{c}	6.80^{b}	7.70^{a}	0.53	*
Daily weight gain, (g/d)	330^{d}	430^{c}	530^{b}	730^{a}	85.39	*
Feed conversion ratio	15.90^{a}	13.95^{b}	12.83^{c}	10.54^{d}	1.12	*

[1]RUP25, RUP35, RUP45 and RUP55 contain 25, 35, 45 and 55% rumen undegradable protein, respectively.

[2]Standard error mean *Significance (P<0.05)

[a,b,c,d] Means in a row with different superscripts differ significantly (p<0.05).