Effect of Dietary Supplementation of Rumen Undegradable Protein on Productive Performance of Early Lactating Buffaloes

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Abstract: An experiment was conducted to study the effect of dietary supplementation of rumen un-degradable protein on productive performance of early lactating buffaloes for the duration of 90 days from February 5, 2014, to May 5, 2014. A total of twenty one lactating Murrah buffaloes in their early to mid-lactation were randomly selected and divided into three groups. Experimental diets were formulated into three- low (30.56% of dietary Crude Protein), medium (43.54% of dietary crude protein) and high (50.04% of dietary CP), iso-nitrogenous (16% CP) and iso-caloric (72% total digestible nutrients) rumen un-digestible protein (RUP) levels. A significant difference (p<0.05) in body weight gain of buffaloes was recorded in animals of high RUP group in comparison to medium and low RUP groups. Milk yield in the medium RUP group (43.54%) was significantly higher than the high and the low RUP group (p<0.05). The mean serum total protein, blood urea nitrogen concentrations were significantly lower in both the high and the medium RUP groups than in the low RUP group (p<0.05). However, blood glucose level was significantly higher in high RUP group than in low and medium RUP group (p<0.05) whereas milk fat, solid not fat, lactose, protein and electrical conductivity showed no significant differences among the three treatment groups (p>0.05). Hence, it can be concluded that the diet containing a medium level of dietary CP as RUP improved the productive performance of early lactating buffaloes. Thus, diet containing the medium level of dietary CP as RUP should be given to improve the productive performance of early lactating buffaloes under the climatic settings of Nepal.

Keywords: Buffalo feeding, Productivity, Dietary Protein, Nutritional Alterations.

INTRODUCTION

Livestock sector constitutes an important component of the national agriculture system in Nepal and contributes to around 25.68% to agriculture gross domestic product [1]. Buffalo farming is an emerging activity in Nepal that plays a significant role to increase the local rural economy, through milk and meat production [2]. Buffaloes are the traditional provider of milk, meat, hides, manure, draft power and are also the reserve capital for the farming families. They are mainly raised by the smallholder farmers across all the physic-graphic agro-ecological zones of the country [2]. The majority of the population of buffaloes in Nepal is kept by poor farmers having limited resources and access to other economic opportunities. These buffaloes are also an integral part of human food as well as economy for smallholders.

Total lactating buffalo population in Nepal is 13, 69,797 and total annual milk production from buffalo is 11, 88,433 Metric Ton [3]. This indicates a very low annual productivity of milk per buffalo. The productivity of buffalo is a matter of concern in Nepal like other Asiatic countries. Buffalo production is a complementary part of dairy production systems in Nepal like India and Pakistan. Most of the buffaloes are raised along with cattle in a mixed farming system where animals are usually fed on low-quality roughages, agricultural crop residues with high fibre content, low-quality proteins and low level of fermentable carbohydrates which ultimately leads to poor growth, production and reproduction. The inadequate feed resources and imbalanced conventional feeding practices have been the major cause of low growth rate and productivity of buffaloes. Most of the livestock in Nepal are fed with rice straw especially at the time of fodder deficiency. Along with this, some native grass species are used during pasture sufficient period. Sometimes, corn stover, wheat straw, wheat bran, rice polish, mustard oil cakes, homemade concentrate mixture and tree fodder leaves, supplement the rice straw as the main diet of livestock. These conventional feedstuffs are usually very low in both rumen digestible protein (RDP) and rumen un-degradable protein (RUP). Though these locally available feed resources can provide a certain amount

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of nutrients needed by animals, most fail to provide adequate nutrients for growth and maintenance of animals. The ability to provide adequate protein to dairy cows depends on the moderate balance between the availability of nitrogen for bacterial growth in the rumen and productive functions [4].

RDP is necessary for ruminal fermentation and synthesis of microbial proteins. Microbial protein is sufficient enough only to meet requirements of low producing buffaloes but unable to meet metabolic protein demand of rapidly growing calves and high yielding buffaloes. But, too little concentration of rumen degradable protein (RDP), found in conventional feedstuffs in Nepal, reduces the microbial growth in the rumen and decreases the microbial protein synthesis which is not sufficient enough to meet the demand of high yielding and early lactating buffalo. Therefore, the required additional dietary protein sources or so-called rumen un-degradable protein (RUP) that can escape from a microbial attack in the rumen is necessary for the high milk-producing buffalo. Rumen un-degradable protein in the diet is important to supply post-ruminal proteins and amino-acids to complement the microbial proteins and to support increased requirements of yielding ruminants especially in early lactation [5] and is a limiting factor for productivity of dairy cows [6]. Various researches have shown that diet high in RUP results in better nitrogen retention, protein utilization, and weight gain [6, 7].

Early lactation in buffaloes is the most crucial for high milk-producing animals. Inappropriate feeding of dairy buffaloes during lactation may lead to physiological, metabolic and nutritional alterations with poor productive and reproductive performance as also observed by [8] in Murrah buffaloes. During the lactation, the majority of usually have a negative energy balance because of low feed intake compared to requirement needed for growth, maintenance and production. To compensate this problem, farmers usually provide high grain diets during early lactation that further exacerbate the condition by precipitating metabolic acidosis [9]. Moreover, they try to provide high-quality RDP that result in excess endogenous urea concentration in blood, milk and urea that leads to poor lactation yield and poor fertility [10]. Though there has held several types of research on the effects of various levels of RUP on productive performance and ovarian function in dairy cattle, information on various levels of RUP of lactating buffalo is limited. Thus, this study was designed to evaluate the effects of dietary supplementation of different levels of rumen un-degradable protein (RUP) on productive performance of early lactating buffaloes in prevailing dietary conditions and available feeding resources for dairy buffaloes in Nepal.

MATERIALS AND METHODS

Experimental Site

This research was carried out from February 5, 2014, to May 5, 2014, at buffalo production unit of Pokhara, District Kaski. This buffalo production unit is located at an altitude ranging from 640 to 770 meters

Figure 1: Map of Nepal showing the study site.

above sea level and at a distance of 200 km west from Kathmandu (Figure **1**).

The mean monthly temperature, rainfall and relative humidity of Pokhara valley are recorded at Pokhara Airport by the Department of Hydrology and Meteorology. The mean monthly minimum temperature in Pokhara ranges from 7.3 °C to 22.2°C while the mean monthly maximum temperature ranges from 20.2°C to 31°C. Kaski district receives maximum rainfall in Nepal. About 65.5 % (2280.7 mm) of the total 3480.3 mm rainfall in Pokhara valley occurs during the first trimester (July to October) of the year 2010/11 (DHM, 2011). Source of water in the farm is a deep tube well and this groundwater is used by the animals of the research area. The water from the deep tube well is found to be of good quality with a slightly higher amount of iron content and is safe for animal consumption.

Selection of Experimental Animals

Twenty-one Murrah buffaloes [body weight, 516.9 ± 35.19 kg; milk yield, 7.84 ± 0.89 kg/d; parity, 3.9 ± 1.15; days in milk, 112.23 ± 22.34; (mean ± s.d.)] were used. The higher producing buffaloes in the stage of early to mid-lactation from the herd were selected for the trial.

Experimental Design

A randomized complete block design (RCBD) was used. Just before the experimental period, the buffaloes were blocked according to milk production (of the previous 15 days) and within each block were randomly divided into three groups. These three groups were randomly allocated to three experimental treatments. There were altogether three treatments and seven replications**.**

Experimental Diets

The experimental diet was formulated into three different RUP level, low (30.56% of dietary CP), medium (43.54% of dietary CP) and high (50.04% of dietary CP) in iso-caloric (72% TDN) and iso-nitrogenous (16% CP) formulations (Table **1**).

Corn gluten meal was used as a source of rumen un-degradable protein in the experimental diets. Each treatment groups were also provided an equal amount

Table 1: Chemical Composition of Three Different Experimental Diets (gram/100gram)

Ingredients	Low RUP	Med RUP	High RUP
Maize	40	35	32
De-oiled rice bran	14.5	20.5	24
Rice Polish	10	18	22
Mustard Cake	27	9	-
Corn gluten meal	-	9	13.5
Molasses	5	5	5
Di-calcium phosphate	0.25	0.25	0.25
Shell grit	1.75	1.75	1.75
Minamil	0.5	0.5	0.5
Salt	1	1	1
Total	100	100	100
Calculated nutrient value			
Crude protein (%)	16.16	16.15	16.16
Undegradable protein (% CP)	30.56	43.54	50.04
TDN(%)	72.41	72.35	72.2
Analyzed nutrient value			
Crude protein (%)	16.18	16.37	16.78
Crude fiber (%)	5.8	5.4	5.0
Dry matter (%)	92.4	91.0	92.0
Ether extract (%)	4.4	4.3	4.3
Total ash (%)	9.1	9.3	9.2

of seasonal grasses (Oat and Berseem) and silage. The RUP level was calculated using the reported value for the ingredients in the book entitled Nutrient Requirement of Dairy Cattle, second revised edition, NRC, 2001.

Corn gluten meal was purchased from one of the commercial feed company of Nepal and other feed ingredients were purchased from the local feed supplier of Pokhara valley. Feed was manufactured at the feed factory of livestock development farm. Three iso-caloric and iso-nitrogenous feed with three different levels of RUP as a percentage of CP was formulated using Double Pearson's Square method (the Pearson square or box method of balancing rations is a simple procedure that has been used for many years. It is of greatest value when only two ingredients are to be mixed). Buffaloes were individually fed twice daily at 4:30 and 13:00 respectively.

Measurement of Production Parameters

Bodyweight of buffaloes was measured at the beginning (day 1st) and at the end of the experimental period (day 90th). Buffaloes were milked twice daily at 6:00 and 16:30. Milk yield of individual buffalo was recorded twice daily for 90 days. Milk yield was measured in kilogram with the help of spring balance.

Milk Sample Collection

Representative milk sample (15 ml) was taken fortnightly using a mixture of morning and evening milk. Collected milk sample was preserved with preservative bronopol ($C_3H_6BrNO_4$) and kept in refrigerator till milk analysis.

Blood Sample Collection

Representative blood sample (10 ml) was taken from the jugular vein of the individual buffalo on 45th and 90th day of the experimental period. Serum was extracted from an individual blood sample and kept under refrigeration until serum biochemical analysis.

Proximate Analysis of Feed

Analysis of the feed sample was done with Weendee Proximate Analysis at Nutrition Laboratory, IAAS, Rampur, Chitwan. Dry matter, crude protein, crude fibre, ether extract, nitrogen-free extract and ash were analyzed by the method of Association of Official Analytical Chemist.

Analysis of Milk Sample

Collected milk sample was analyzed fortnightly in Pokhara model dairy of livestock development farm, Lampatan, Pokhara with the help of ultrasonic milk analyzer (Master Classic LM2). Milk was analyzed for fat, solid not fat, protein, lactose and electrical conductivity.

Serum Biochemical Analysis

All serum samples were analyzed within 3 days of storage using respective commercial kits and Ultraviolet Visible Spectrophotometer (Thermo-spectronic 2000, Genesys™ 10 series/10 UV, New York, USA) at National Avian Laboratory (NAL), Bharatpur, Chitwan.

Data Analysis

The data analysis was performed using Ms Excel and M STAT C version 1.3 Michigan State University (1975). Test of significance among treatment means was performed using the least significant difference (LSD).

RESULTS

Bodyweight

The average body weight of buffaloes was 518.14±38.95 kg, 515.71±42.12 kg and 516.85±20.51 kg initially in the three groups and the corresponding values on the 90th day of the experiment were 523.71±37.89 kg, 526±41.56 kg and 544.57±27.86 kg respectively (Table **2**).

Highest body weight gain (27.71 kg) was observed in high RUP supplied group and the least body weight gain (5.57 kg) was observed in low RUP group (Figure **2**). Average body weight gain of the buffaloes in the high RUP group was significantly higher in comparison with that of buffaloes of the medium and low RUP group ($p<0.05$). However, there was no significant difference in average body weight gain between low RUP and medium RUP group ($p>0.05$).

Lactating Performance

Average daily milk production was significantly higher in medium RUP group in comparison with low and high RUP group ($p<0.01$) (Table **3**) (Figure **3**).

Fortnight milk production was highly significant ($p<0.01$) in medium RUP group of buffaloes during the

Table 2: Mean Body Weight and Mean Weight Gain (kg) of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	On the 1st day of the experiment	On the 90th day of the experiment	Mean weight gain
Low RUP	518.14	523.71	5.57^b
Med RUP	515.86	526	10.29^b
High RUP	516.86	544.57	27.71^a
F value	0.01	0.6	6.21*
Probability	0.99	0.56	0.014
CV%	7.72	7.32	85.24
LSD	ns	ns	14.42

*Significant at 5%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

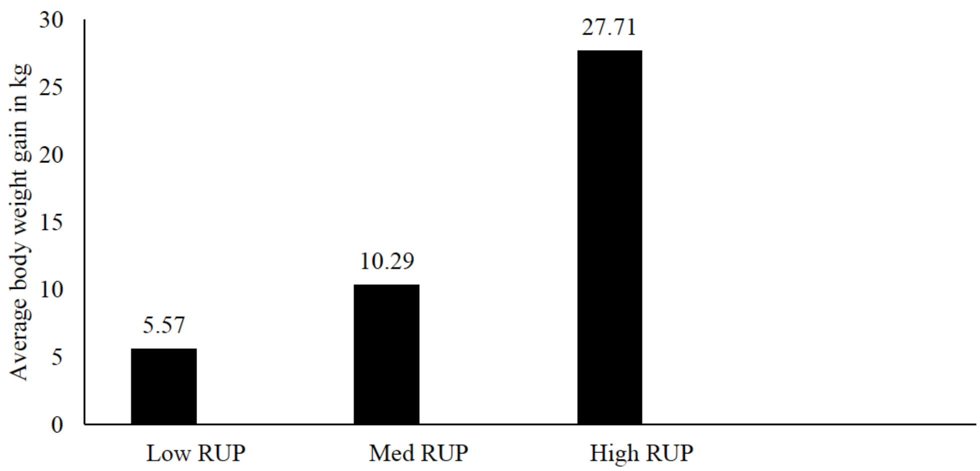

Figure 2: Effect of three different dietary RUP levels on average body weight gain.

Table 3: Fortnightly and Daily Mean Milk Yield (kg) of Buffaloes Fed on Diets Containing Different Level of RUP

Treatment	1st	2nd	3rd	4th	5th	6th	Overall mean	Milk yield/day
Low RUP	110.51	106.53^b	106.39^b	101.87^b	99.66	91.83	102.80^c	6.85^b
Med RUP	119.64	114.44^a	115.29^a	108.21^a	105.07	96.6	109.88^a	7.33^a
High RUP	116.86	113.91^a	110.93^{ab}	101.53^b	97.5	92.46	105.53^b	7.04^b
F value	2.95	8.56**	9.09**	11.43**	3.43	1.82	12.11**	12.11**
Probability	0.091	0.0049	0.004	0.0017	0.06	0.20	0.0013	0.0013
CV%	6.23	3.58	3.52	2.84	5.53	5.44	2.56	2.56
LSD	ns	4.66	4.55	3.43	ns	ns	3.16	0.21

*Significant at 5%, ** Significant at 1%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

entire experimental period. There was significantly higher milk production was seen on 2nd, 3rd and 4th fortnight (p<0.05). However, fortnight milk production in three different RUP groups was statistically similar on 1st, 5th and 6th fortnight (p>0.05) (Table **3**). Average fortnight milk production (109.88 kg) was significantly higher in medium RUP group compared to the low and high RUP group (p<0.01). But, no significant difference on average fortnight milk production was observed between animals fed with high and low RUP diet.

Milk Constituents

Statistical analysis of overall fortnight values of milk composition showed no significant effect on milk fat, SNF, protein, lactose and electrical conductivity (p>0.05) (Tables **4-8**).

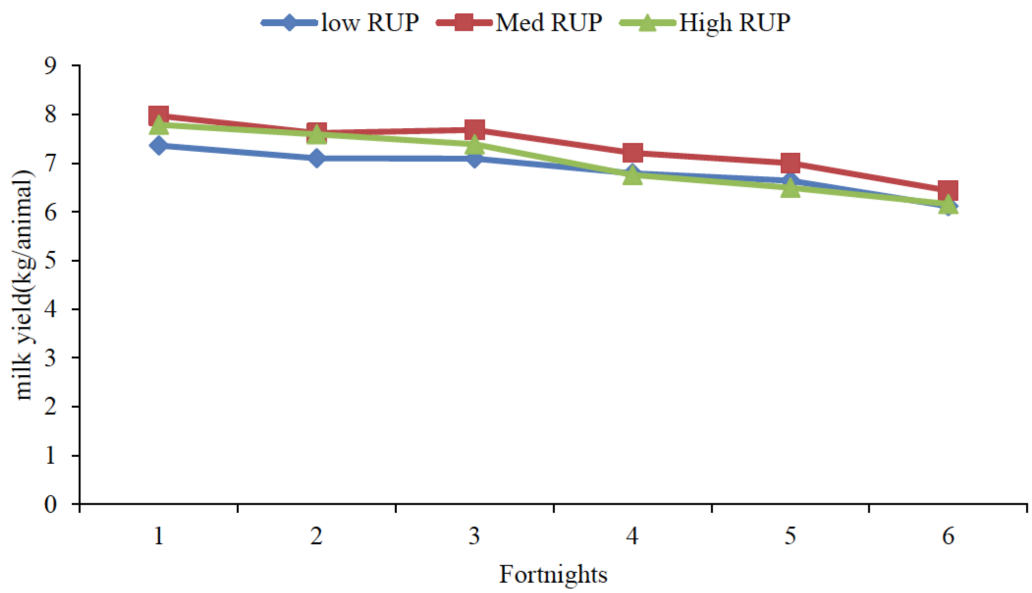

Figure 3: Average daily milk yield of animals in kg fed diets with three different RUP levels.

Table 4: Fortnightly Mean Milk Fat % of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	1st	2nd	3rd	4th	5th	6th	Overall mean
Low RUP	6.64	6.75	6.59	6.70	7.35	8.34	7.06
Med RUP	6.53	7.26	6.81	6.86	6.57	7.08	6.85
High RUP	6.04	6.93	6.71	7.6	7.27	8.82	7.23
F value	1.78	0.26	0.27	2.72	1.97	2.56	1.76
Probability	0.21	0.77	0.76	0.10	0.18	0.11	0.21
CV%	9.8	19.07	8.42	10.85	11.53	18.44	5.39
LSD	ns	ns	ns	ns	ns	ns	ns

ns (non-significant) at p>0.05.

Table 5: Fortnightly Mean Milk SNF % of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	1st	2nd	3rd	4th	5th	6th	Overall mean
Low RUP	9.38	9.42	10.11	10.30	9.23	9.12[ab]	9.59
Med RUP	9.30	9.86	9.98	10.24	9.05	9.26[a]	9.61
High RUP	9.23	9.42	10.01	10.53	9.37	8.70[b]	9.54
F value	0.35	0.62	0.16	0.73	2.16	4.05*	0.17
Probability	0.71	0.55	0.85	0.50	0.15	0.045	0.84
CV%	3.59	8.83	4.61	4.54	3.17	4.23	2.38
LSD	ns	ns	ns	ns	ns	0.45	ns

*Significant at 5%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

Table 6: Fortnightly Mean Milk Protein % of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	1st	2nd	3rd	4th	5th	6th	Overall mean
Low RUP	3.48	3.50	3.62	3.76	3.43	3.40	3.53
Med RUP	3.45	3.66	3.68	3.63	3.37	3.44	3.54
High RUP	3.39	3.5	3.66	3.85	3.48	3.25	3.52
F value	0.60	0.6	0.23	1.45	1.9	3.56	0.09
Probability	0.56	0.56	0.80	0.27	0.19	0.06	0.91
CV%	4.7	8.93	4.39	6.41	3.12	4.16	2.29
LSD	ns	ns	ns	ns	ns	ns	ns

ns (non-significant) at p>0.05.

Table 7: Fortnightly Mean Milk Lactose % of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	1st	2nd	3rd	4th	5th	6th	Overall mean
Low RUP	4.84	5.00	5.55	5.62	4.89	4.82[ab]	5.12
Med RUP	4.72	5.23	5.52	5.62	4.80	4.91[a]	5.13
High RUP	4.83	5.01	5.49	5.78	4.97	4.58[b]	5.11
F value	0.16	0.58	0.1	0.84	2.02	4.48*	0.04
Probability	0.85	0.57	0.90	0.45	0.17	0.035	0.96
CV%	8.84	8.78	4.04	4.62	3.24	4.38	2.86
LSD	ns	ns	ns	ns	ns	0.24	ns

*Significant at 5%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

Table 8: Fortnightly Mean Electrical Conductivity (mS/cm) of Milk of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	1st	2nd	3rd	4th	5th	6th	Overall mean
Low RUP	5.50	5.38	5.56	5.40	5.43	5.42	5.45
Med RUP	5.51	5.51	5.54	5.49	5.50	5.52	5.51
High RUP	5.50	5.52	5.56	5.48	5.48	5.52	5.51
F value	0.29	1.19	1.15	1.32	1.16	2.29	2.31
Probability	0.75	0.33	0.34	0.30	0.34	0.14	0.14
CV%	0.55	3.45	0.44	2.11	1.72	1.92	1.17
LSD	ns	ns	ns	ns	ns	ns	ns

ns (non-significant) at p>0.05The SNF % and lactose % were significantly higher in medium RUP group than that in low and high RUP group (p<0.05) (Tables **5** and **7**).

Blood Constituents

Average blood glucose, blood urea nitrogen (BUN) and total protein (TP) were significantly different among treatment groups (p<0.05) (Tables **9-11**).

Average blood glucose concentration was significantly higher in the high and medium RUP group in comparison to low RUP group (p<0.05) (Table **9**). However, there was no significant difference in blood glucose concentration among the three groups on 45th and 90th day of the experimental period (p>0.05). Average blood urea nitrogen concentration (26.86 mg/dL) was significantly (p<0.05) higher in low RUP group in comparison to the medium and high RUP group (Table **10**). There was no significant (p>0.05) difference on average blood urea nitrogen

Table 9: Mean Serum Glucose (mg/dL) of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	On 45th day	On 90th day	Overall mean
Low RUP	59.96	54.01	56.99[b]
Med RUP	61.08	56.38	58.73[b]
High RUP	66.59	71.87	69.23[a]
F value	0.59	2.38	4.79*
Probability	0.56	0.13	0.02
CV%	19.53	27.4	12.99
LSD	ns	ns	9.33

*Significant at 5%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

Table 10: Mean Blood Urea Nitrogen (mg/dL) of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	On 45th day	On 90th day	Overall mean
Low RUP	28.46[a]	25.25	26.86[a]
Med RUP	22.73[b]	23.42	23.08[b]
High RUP	23.64[b]	19.15	21.39[b]
F value	4.76*	3.79	5.58*
Probability	0.03	0.052	0.019
CV%	14.98	18.83	13.18
LSD	4.35	ns	3.65

*Significant at 5%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

Table 11: Mean Serum Total Protein (g/dL) of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	On 45th day	On 90th day	Overall mean
Low RUP	7.08[a]	7.25	7.17[a]
Med RUP	6.14[b]	7.03	6.59[b]
High RUP	6.09[b]	6.60	6.35[b]
F value	6.33	2.72	8.13
Probability	0.013*	0.106	0.0059**
CV%	9.13	7.64	5.86
LSD	0.69	ns	0.46

*Significant at 5%, ** Significant at 1%, ns (non-significant) at p>0.05 and means in a column with the same superscript are not significantly different.

concentration between medium and high RUP group. Average total protein (TP) concentration (7.17 g/dL) was significantly higher (p<0.01) in low RUP group in comparison with medium and high RUP group (Table **11**). However, there was no significant difference in total protein concentration between medium and high RUP group (p>0.05). Average serum aspartate trans-aminase concentration (101.03 IU/L) was higher in low RUP group than that in medium (93.71 IU/L) and high (89.72 IU/L) RUP group, but was not significantly different among the three dietary RUP groups (p>0.05) (Table **12**).

DISCUSSION

Bodyweight

Dietary supplementation of rumen un-degradable protein had a significant (p<0.05) effect on body weight gain of buffaloes of the three groups (Table **2**). Highest

Table 12: Mean Serum Aspartate Trans-Aminase (IU/L) of Buffaloes Fed on Diets Containing Three Different Levels of RUP

Treatment	On 45th day	On 90th day	Overall mean
Low RUP	97.27	104.79	101.03
Med RUP	87.34	100.07	93.71
High RUP	86.75	92.69	89.72
F value	1.54	1.58	2.17
Probability	0.25	0.24	0.15
CV%	13.94	12.94	10.85
LSD	ns	ns	ns

ns (non-significant) at p>0.05.

body weight gain (27.71 kg) was observed in high RUP supplied group and the least body weight gain (5.57 kg) was observed in low RUP group which was statistically significant ($p<0.05$) (Figure **4**). The higher body weight gain in animals fed with high RUP was probably due to the extra supply of dietary RUP plus metabolizable proteins for body tissue synthesis compared to others. Higher RUP in the diet is likely to bypass relatively higher ruminal-digestibility and increased flow to the intestine which is later on used for body tissue synthesis thus resulting in higher body weight gain.

The findings we reported here were by the result of [11] who reported increased daily weight gain of growing ruminants fed diets high in dietary RUP. Similarly, [12-14] found a similar result with equal or greater body weight gain with depressed dry-matter take when fed at RUP at 40% of the diet. However, the current study results were not by, [15-18] who recorded no significant difference between the two groups of cows fed with varying level of dietary CP as RUP regarding the body weight. That might be due to protein protection from ruminal fermentation and balanced amino-acids (AA) supplied by high RUP diet. Variation in the results by various authors could be due to variation in climatic conditions, species, breed of animals and also partly due to the considerable variation among the feeds on ruminal degradation and intestinal digestion of proteins as mentioned by [19].

Lactating Performance

The dietary RUP level significantly influenced the milk yield of buffaloes during the entire experimental period ($p<0.05$) (Table **3**). Higher milk yield in medium RUP group might be due to more amino-acids available at the intestinal level that provided more essential amino-acids for milk protein synthesis as mentioned by [20]. There was significantly higher milk production in medium RUP group on 2nd, 3rd and 4th fortnight ($p<0.05$). However, fortnightly milk production in three different RUP groups was statistically similar on 1st, 5th and 6th fortnight ($p>0.05$). Low milk production in low RUP diet was probably due to less CP content as mentioned by other researchers [21-23]. Similar results on milk yield were observed by [23-26] by changing the protein levels in the diet. This could be because an increase in RUP in the diet will increase glucose, protein and other nutrients available to mammary gland for milk synthesis. Average daily milk production was highly significant in the medium RUP group in comparison with the low and high RUP group ($p<0.01$). Significantly higher milk production in medium RUP might be due to a proper balance of RUP and RDP that is necessary for efficient nitrogen utilization and subsequently low level of wastage of nitrogenous products to the environment. The higher milk yield could also be due to the appropriate amount of metabolites necessary for protein synthesis especially glucose precursors and amino-acids [27, 28]. But, there was a decrease in milk yield with high RUP indicating that the law of diminishing return applies to milk production as un-degraded proteins reaching small intestine is increased [29]. This result agreed with the result of [24, 26] who observed the positive response of increased dietary RUP level in milk production in medium to high producers, fed mainly on crop residue-based diet. Similarly, [25] verified the effects of changing protein levels in diet on milk composition and milk quality. This is since an increase in RUP in the diets will increase glucose and other nutrients available to mammary gland due to increased gluconeogenesis, sparing of glucose from oxidation and increased glucose from glycerol released from adipose tissue breakdown; for milk secretion [2, 30-32]. This result was in contrast with the result of [18] who noticed average daily milk yield of animals fed with low RUP diet higher than those fed with high RUP diet. The failure of un-degradable protein to increase milk production in a study by [18] may be due to the low productivity of cows or low availability of proteins post-ruminally. In other studies too, increasing rumen un-degradable protein (RUP), or replacing rumen degradable protein (RDP) sources with RUP sources, in concentrates had no consistent effect on milk production or composition [33-35]. This could partly be because the effect of RUP can be observed only when there is an adequate rumen digestible protein (RDP) in the diet of an animal. For a high yielding cow, especially in early lactation, a smaller proportion of the protein is generally supplied by the rumen microbes and more need to escape rumen degradation than the case with lower genetic merit cows [34, 36, 37]. During the early stage of lactation, dairy cows are in a negative energy balance which could be exacerbated by feeding excess rumen un-degradable proteins as it requires energy to metabolize excess proteins [30]. Significantly ($p<0.01$) higher milk production observed in buffaloes fed with medium RUP might be due to proper balance of RDP and RUP for different metabolites needed for milk synthesis and the low level of wastage of nitrogen products to the environment as mentioned by [27, 28].

Milk Constituents

Almost similar values of milk fat, SNF, protein, lactose and electrical conductivity in our study could be due to level of dietary protein or degradability as mentioned by [38]; Rajagopalan and Murty 1983 cited in [23]. Higher SNF in 6th fortnight could be because as the days on lactation progress, the average milk yield decreases with increase in SNF content. The higher SNF percentage and lactose value in medium RUP might be due to the proper amount of post ruminal digestible proteins necessary for lactose synthesis. Similar results were observed [18, 39, 40] by increasing dietary protein protection. In contrast to our findings, [17] found no significant difference among three treatment groups in terms of milk fat, lactose and SNF % by increasing dietary RUP level in early lactating dairy goats. This might be due to the difference in animal species used for the experiment. The variations on results observed by various researchers may be due to difference in type and quality of roughage, frequency of feeding, climatic conditions and animal species used for experiment as reported in crossbred cattle by [18, 40] who reported significant difference in percentage of milk fat, SNF and total solid in low RUP group than that in high RUP group. The higher efficacy of low RUP diet may be due to high RDP content which is easily metabolized and converted to microbial amino-acids and also due to balanced amino-acids delivered by RUP supplement. It was found by [17] that the contrasting result with no significant differences among three treatment groups in terms of milk fat, lactose and solid not fat percentage by increasing dietary RUP level on early lactating dairy goats. This might be due to the difference in animal species used for the experiment.

Blood Constituents

The dietary RUP level significantly influenced blood glucose, BUN and TP ($p<0.05$) (Table **9**) with average blood glucose concentration being significantly higher in high and medium RUP group in comparison to low RUP group ($p<0.05$). This finding was by the finding of [18, 41, 42] who reported higher blood glucose level in cows fed with high RUP diet than that in cows fed with low RUP diet. Lower rumen degradability of high RUP diet might have increased nutrient flow into the small intestine which might have increased the absorption of glucose. Feeding high rumen-protected protein probably helped to save energy wastage in urea synthesis thus resulting in high glucose concentration in blood.

Significantly high blood urea concentration in animals fed with low RUP might be due to less production of ammonia in the rumen due to low RDP supply [23]. Besides, highly efficient utilization of dietary nitrogen for microbial protein synthesis might have explained this result. On the contrary, [17, 43, 44] found raised serum BUN level in dairy cows and dairy goat respectively by feeding diets with high crude protein level. This might be due to insufficient energy substrate available for rumen microbes for efficient utilization of nitrogen (N).

The increased concentration of blood urea in animals fed with low RUP agree with the data from [45, 46] who observed increased dietary RUP levels decrease ruminal pH and increase ruminal NH_3-N. As a result of excess nitrogen available in the rumen, ruminal NH_3 is absorbed and appears in blood as BUN. BUN and MUN in lactating goat, cattle and lactating buffalo, respectively. Thus, significantly ($p<0.05$) higher blood urea nitrogen concentration seen in case of low RUP group might be the fact that too much RDP increases ruminal ammonia (NH_3) resulting in high blood urea nitrogen.

Average total protein concentration (7.17 g/dL) was highly significant ($p<0.01$) in low RUP group in comparison with medium and high RUP group (Table **11**). Similar findings have been reported by [17, 47] who found significantly ($p<0.05$) higher TP concentration in low RUP group than in the medium and high RUP group of dairy goat. The higher plasma protein level in low RUP compared to other group was probably due to more efficient utilization of absorbed amino-acids, indicating the efficiency of milk production decrease with increase in RUP.

Average serum aspartate trans-aminase concentration (101.03 IU/L) was higher in low RUP group than that in medium (93.71 IU/L) and high (89.72 IU/L) RUP group. This result was in agreement with the findings of [17] who found higher AST concentration in low RUP group than in the med and high RUP group of dairy goat. High blood urea nitrogen found in case of low RUP group might have increased liver load and increased AST concentration.

CONCLUSION

The results of the present study showed that medium dietary RUP (43.54% of dietary CP) decreases the urea nitrogen concentration in the blood which in turn increases milk yield in early lactating buffaloes. It was found from the study that medium dietary RUP (43.54% of dietary CP) level increases the milk yield in early lactating buffaloes. Diet in high RUP (50.04% of dietary CP) increases the bodyweight whereas medium dietary RUP decreases the urea nitrogen concentration in blood. Therefore the liver load is decreased to metabolize ammonia into urea. Hence, diet containing medium RUP level can improve lactation performance and health in lactating buffaloes.

CONFLICT OF INTEREST

I/We hereby declare no conflict of interest among the authors.

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