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Effect of Low Energy Followed by High Energy Based Total Mixed Diets on Growth Rate, Blood Haematology and Nutrient Digestibility in Growing Buffalo Heifers

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Abstract.- Twenty-two buffalo heifers, 6-8 months old, 98.57±5 kg average body weight were divided into two groups and randomly assigned either control diet for the duration of the experiment which contained 2.55 Mcal/kg of DM (as per NRC, 2001 recommendations of Holstein heifers) or a stair-step diet which contained 2.03 Mcal/kg of DM (80% ME of control-SLE) fed for 120 days followed by 3.01 Mcal/kg of DM (120% ME of control-SHE) for 60 days. Heifers on SLE diet gained ($p<0.05$) less weight (0.51 vs. 0.60 kg/day) but on SHE diet, gained significantly ($p<0.01$) more weight (0.82 vs. 0.58 kg/day) respectively, compared to heifers fed the control diet. Heifers on the SLE diet have poorer FCR (6.73 vs 5.72) but with SHE diet have better ($p<0.01$) FCR (5.23 vs 7.41) compared to control, respectively. Daily DM consumption for the entire feeding period was similar in both groups but heifers reared on stair-step scheme gained 1.67% higher weights than control. Blood was collected on 0, 120 and 180 days of the experiment and showed that all haematological values increased with the advancement of age, with the exception of lymphocyte percentage; however, these values were not affected by dietary treatment. Apparent digestibility of DM, OM and CP remained unchanged on both the SLE and SHE diets, respectively, compared to the control. However, NDF and ADF digestibility increased ($p<0.05$) with the SLE diet but decreased with the SHE diet compared to control diet, respectively. Nitrogen retention was not affected in heifers fed either the SLE or SHE diets compared to the control ($p>0.05$). Feed costs per kg gain of heifers fed stair-step diets were 16.57% less than those fed the control diet.

Key words: Buffalo heifers, growth, digestibility, haematology, stair-step feeding

INTRODUCTION

Profitable dairy farming depends upon sound rearing strategy of heifers to replace the older and non-productive cows through voluntary culling. However, heifer production is the most expensive part of dairy farm operation because it needs more inputs for a longer period with no immediate returns (Heinrichs, 1993). For efficient dairy farming, a complete package of heifer rearing is needed to exploit the performance potential at minimum expense. Feed cost represented 63% (Moore *et al*., 2009) or 84% (Razzaque *et al*., 2010) of the total input costs for heifer's production. Managing heifers to attain puberty with decreased feed inputs and then taking advantage of compensatory gains may have economic advantages. Stair-step feeding schemes are a practical and cost effective approach for dairy heifer production (Park *et al*., 1987). The stair-step feeding scheme is to reduce energy levels for a limited time and then compensate this effect by supplying the energy in excess to exploit the effect of compensatory growth (Kim *et al*., 1998). This feeding scheme is based on a combination of both restriction and realimentation of energy that is designed to induce compensatory growth for developing dairy (Ford and Park, 2001; Park *et al*., 1989) and beef (Park *et al*., 1998), heifers. Ashworth and Millward (1986) defined compensatory growth as the acceleration in growth that occurs when the period of growth restriction ends and favorable conditions are restored. Heifers raised on stair-step feeding compared to NRC standard feeding practices had similar end live weights while consuming less feed, resulting in improved growth efficiency, better mammary gland development and enhanced lactation potential (Ford and Park, 2001). Park *et al*. (1989) observed better growth efficiency and increase milk yield from first calf heifers raised on stair-step feeding than with

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conventionally fed animals.

Researchers have attempted to develop heifer feeding and rearing programs with a particular emphasis on the effects of high feeding intensity on pubertal heifers (Ford and Park, 2001). However, numerous studies have observed that overfattening of the prepubertal heifer by feeding high energy diets resulted in impaired mammary parenchymal development.

Presently the Pakistani population of buffalo is 30.8 million head, contributing approximately 67% of total milk production in the country (Anonymous, 2009-10). It is estimated that 6.45 million buffalo heifers and 4.88 million cow heifers are produced annually. These heifers are being reared conventionally on some green fodder and poor quality roughages resulting in slow growth rates (Jabbar *et al*., 2006) and delayed puberty (Bhatti *et al*., 2007). Average age at puberty of cattle and buffalo is 34 and 37 months, respectively (Bashir, 2006; Rehman, 2006). Furthermore, few studies indicated that Pakistani buffaloes have the potential to attain puberty at 18 to 24 months of age (Jabbar, 2004). Therefore, the present study was conducted to evaluate the effects of low energy followed by high energy based total mixed diets on growth performance, blood haematology and digestibility and nitrogen balance in growing buffalo heifers.

MATERIALS AND METHODS

This study was conducted at the National Agricultural Research Centre, Islamabad, Pakistan (Lat. 33.7°N; Long. 73.1°E; Alt. 508 m). The study lasted for six months during the years 2008-09. Feedstuffs used in experimental diets were analyzed for nutritional profile prior to feed formulation. Feedstuffs including vegetable oil cakes and meals, cereal by-products, cereal grains, sugarcane molasses and wheat straw were analyzed for proximate composition (AOAC, 1990) and cell wall constituents (Van Soest *et al.,* 1991). Total digestible nutrients (TDN) were calculated by regression equation based on proximate composition as described by Wardeh (1981). Digestible energy (DE) and metabolizable energy (ME) was calculated by using NRC (2001) equations.

TDN (%)	=	40.32 + 0.5398 CP + 0.448 NFE + 1.422 EE - 0.7007 CF
DE (Mcal/kg)	=	0.04409 × TDN (%)
ME (Mcal/kg)	=	1.01 × DE (Mcal/kg) – 0.45

Growth trial

Animals, diets and management

Growth performance trial was conducted on twenty-two Nili-Ravi buffalo heifers with 6-8 months age and average body weight 98.57±5 kg at the beginning of experiment. All heifers were provided the control diet for one-month as adjustment period preceding the trial. Heifers were fed at twice daily (morning and evening) in cement mangers. Heifers were divided into two equal groups and randomly assigned two dietary treatments *i.e.*, control and step-wise feeding scheme. The control group was given ration as per NRC energy requirements of Holstein heifers (2.55 Mcal of ME/kg of dry matter) for 180 days. Stair-step diets contained 80% ME of control (2.03 Mcal/kg of DM-SLE) fed for 120 days followed by 120% ME of control diet (3.01 Mcal/kg of DM-SHE) for 60 days (Table I). The stair-step heifers feed level was adjusted with similar dry matter consumption as that of control heifers.

Fresh water was provided 4-5 times per day. The heifers were housed in individual tie stalls in well ventilated sheds with concrete floors and an asbestos sheet roof. In the morning all heifers were let loose in an open paddock for three hours throughout the experimental period except during severe weather and during collection of urine and faeces during the digestibility and nitrogen balance trial. Daily sweeping and cleaning of floors and bathing the heifers with fresh ground water was practiced to provide good hygienic environment. Before starting the experiment heifers were given subcutaneous promectine® injection (Vetaria Pharmaceuticals, Lahore, Pakistan) 2 ml/animal to control internal and external parasites. Individual feed intake was recorded daily. This was done by subtracting the amount of orts/refusal from the feed offered before the morning feeding. Heifers were weighed fortnightly to monitor the growth rate after restriction of feed and water intake for 16 hours throughout the experimental period by using an electronic scale (Avery Berkel L122). Feed conversion ratio (FCR) was calculated as kilograms of feed intake per kilogram of live weight gain.

Table I.- Ingredients and nutrient composition of experimental diets

Ingredients (%)	Control	Stair-step⁺	
		SLE	SHE
Maize oil cake	17.00	-	20.00
Cotton seed meal	12.00	-	15.00
Cottonseed cake	-	32.00	-
Sunflower meal	1.00	13.00	-
Canola meal	6.00	2.00	3.00
Rice polish	5.00	-	7.00
Wheat bran	7.00	1.00	5.00
Corn gluten feed	5.00	12.75	2.00
Corn grains	12.00	-	15.00
Vegetable oil	1.00	0.25	6.00
Wheat straw	25.00	30.00	18.00
Constant ingredients⁻	9.00	9.00	9.00
Chemical composition (% DM)			
CP§	16.16	15.84	16.11
CF	15.41	24.00	12.52
NDF	29.34	43.81	24.00
ADF	19.19	30.47	15.61
Ca	0.69	0.75	0.65
P	0.57	0.64	0.55
ME (Mcal/kg)	2.55	2.03	3.01

[+]Stair-step low energy diet (SLE) consisting of 2.03 Mcal/kg of metabolizable energy (ME)/kg of DM for 120 days then switched to high energy diet (SHE) consisting of 3.01 Mcal of ME/kg of DM for 60 days and control heifers fed as per NRC requirement consisting of 2.55 Mcal of ME/kg of DM for 180 days.

[−]Constant ingredients consisted of molasses 6%; dicalcium phosphate 1%; limestone 0.5%; sodium chloride 0.5%; urea 0.5% and premix 0.5%.

[§]Where CP= Crude protein, CF= Crude fibre, NDF= Neutral detergent fibre, ADF= Acid detergent fibre, Ca= Calcium, P= phosphorous, ME= Metabolizable energy and DM=Dry matter

Blood haematology

Blood samples were drawn at 0, 120 and 180 days of the experiment from the jugular vein of heifers and immediately put into 5 ml heparinised venoject® EDTA (K_3) tubes and mixed thoroughly for the measurement of haematological parameters i.e., red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb), haematocrit, lymphocyte, monocyte and eosinophils were determined by method of Benjamin (1985) by using Beckman Coulter® AcT Diff™ Haematology Analyzer at National Veterinary Laboratories, Islamabad.

Digestibility and N balance trial

A total tract digestibility and nitrogen (N) balance trial lasting 15 days (10 days for the adjustment period and 5 days for sample collection) was carried out on the 105th and 165th days of experiment. Four heifers of similar body weight were used for each trial, and were placed in individual digestibility pens (measuring 4 ft × 9 ft) equipped with the facility to collect faeces and urine separately. Total faeces were collected (with plastic bags placed behind the heifers) for 5 days after heifers were given 10 days of acclimatization to become accustomed to the environment. Feed and orts samples were collected daily. Samples were pooled over a period, dried at 60°C for 48 hours, ground through a 2 mm screen Wiley mill (standard model 4) and analyzed for dry matter (DM), organic matter (OM), crude protein (CP) by the methods described by AOAC, (1990) and neutral detergent fibre (NDF) and acid detergent fibre (ADF) by the method described by Van Soest *et al.* (1991). For five days, faeces were weighed and mixed daily, and a representative samples (2%) were taken, stored at -20˚C, and subsequently thawed, dried at 60˚C for 48 hours, ground through a 1mm screen for chemical analysis. Apparent digestibility of DM, OM, CP, NDF and ADF were determined.

Total urine passed was collected for 5 days into plastic bucket and urine was acidified by daily addition of 30 ml 6 N HCl to urine collection vessels to avoid N losses due to ammonia volatilization and to prevent bacterial growth. A representative sample (2%) was taken from each animal and analyzed for nitrogen estimation (AOAC, 1990) and N retention: N intake – (Urine N losses + Faecal N losses)

Statistical analysis

Data collected were analyzed with a linear model using student's paired t-test described by Steel *et al*. (1997). Data are given as means plus or minus the standard error of the mean.

RESULTS

Growth trial

The results on DM intake, daily weight gain, FCR and economic efficiency of growing buffalo heifers fed total mixed ration based control versus stair-step diets are given in Table II. Heifers fed the SLE diet gained significantly lower ($p<0.05$) daily weights than heifers fed control diet. However, these heifers when fed the SHE diet gained significantly more ($P<0.01$) live weight compared to heifers fed the control diet. Average daily feed consumption (DM) of heifers fed the SLE or SHE diets was similar to those fed the control diet, respectively. Feed conversion ratio of heifers fed the SLE diet was poorer compared to heifers fed the control diet. But FCR was significantly ($p<0.01$) better in animals offered the SHE diet after having been maintained previously on the SLE diet than those fed control diet continuously. Over the entire feeding period, daily gain was 1.67% higher but daily dry matter consumption was similar resulting improved FCR ($p<0.01$) in stair-step fed heifers relative to the control group. In the present study, the cost per kg of the control diet was PKRs. 16.14, SLE diet PKRs. 12.60 and SHE diet PKRs. 20.06. Buffalo heifers raised on the stair-step diets incurred a lower feed cost (91.96 vs 107.20 PKRs) for one kilogram live weight gain than control heifers fed continuously as per the NRC energy requirement over the entire developmental period (Table II).

Table II.- Growth performance (Mean±SEM) of buffalo heifers fed experimental diets.

Parameters	Feeding Scheme		P-Value
	Control	Stair-step	
BW§ (kg)			
Day 0	98.62±5.15	98.53±5.00	0.491
Day 120	170.82±7.94	160.07±6.21	0.354
Day 180	205.82±8.96	209.15±7.45	0.376
ADG (kg/day)			
Day 0-120	0.60±0.03	0.51±0.03	0.041
Day 120-180	0.58±0.03	0.82±0.04	0.000
Day 0-180	0.60±0.03	0.61±0.03	0.323
DMI (kg/day)			
Day 0-120	3.37±0.10	3.39±0.10	0.488
Day 120-180	4.22±0.11	4.18±0.09	0.304
Day 0-180	3.65±0.10	3.65±0.09	0.490
FCR (kg/kg)			
Day 0-120	5.72±0.23	6.73±0.31	0.004
Day 120-180	7.41±0.34	5.23±0.24	0.000
Day 0-180	6.25±0.23	6.03±0.23	0.204
Feed cost per kg gain, Pak Rs[+].	107.20	91.96	-

[+]Cost per kg of control diet was Rs. 16.14, SLE diet Rs. 12.60 and SHE diet Rs. 20.06.
[§]Where BW= Body weight, ADG= Average daily gain, DMI= Dry matter intake, FCR= Feed conversion ratio and Pak Rs= Pakistani rupees

Blood haematology

The WBCs (x10 ^{3}/ml), RBCs (x10 ^{6}/µl) count, Hb (g/dl) and the hematocrit and percentage lymphocytes, monocytes and eosinophils in blood of heifers fed control versus stair-step diets were almost similar (Table III). All haematological values obtained increased with the advancement of age of heifers, with the exception of lymphocytes which were significantly decreased from 0 to 180 days of the experimental period in heifers of both groups.

Digestibility and N balance trial

The results of nutrient digestibility are presented in Table IV. Apparent digestibility of DM, OM and CP tended to decrease in heifers fed the SLE diet but increased when heifers were fed the SHE diet thereafter, as compared to heifers fed control diet but the difference was non-significant ($p>0.05$). Digestibility of NDF and ADF increased significantly ($p<0.05$) in heifers fed the SLE diet compared to the controls. But digestibility of both NDF and ADF decreased significantly ($p>0.05$), when heifers changed from the SLE to SHE diet compared to the control diet. Nitrogen retention was not affected (Table V) in heifers fed either SLE or SHE diets compared to the control ($P>0.05$).

DISCUSSION

In ruminant nutrition DMI has great importance because it determines the availability of required nutrients for health, growth and reproduction (NRC, 2001). In the current study, DMI did not differ ($p>0.05$) between heifers fed stair-step versus control diets throughout the study period. Although overall less dietary energy was used by stair-step fed heifers to attain parallel body weights to the heifers fed control diet: this may be due to compensatory growth. These results are in

Table III.- Blood profile (Mean±SEM) of buffalo heifers fed experimental diets.

Parameters studied	Feeding Scheme		P-Value
	Control	Stair-step	
Day 0			
White blood cells ($x10^3$/ml)	8.75±0.81	8.98±0.77	0.438
Red blood cells ($x10^6$/µl)	7.33±0.24	7.34±0.14	0.482
Hemoglobin (g/dl)	9.12±0.23	9.05±0.15	0.409
Hematocrit (%)	32.51±0.60	31.45±0.49	0.116
Lymphocytes (%)	53.91±1.03	55.73±1.28	0.187
Monocytes (%)	4.00±0.26	3.64±0.29	0.220
Eosinophils (%)	6.91±0.71	6.36±0.65	0.284
Day 120			
White blood cells ($x10^3$/ml)	12.95±0.68	12.93±0.33	0.481
Red blood cells ($x10^6$/µl)	9.02±0.51	9.22±0.37	0.362
Hemoglobin (g/dl)	12.48±1.49	11.17±1.05	0.244
Hematocrit (%)	43.23±2.86	41.75±1.05	0.313
Lymphocytes (%)	52.91±2.05	51.55±1.12	0.296
Monocytes (%)	3.18±0.25	2.64±0.27	0.096
Eosinophils (%)	4.45±0.81	5.64±1.05	0.184
Day 180			
White blood cells ($x10^3$/ml)	13.86±0.64	13.51±0.83	0.380
Red blood cells ($x10^6$/µl)	8.43±0.18	8.33± 0.30	0.392
Hemoglobin (g/dl)	11.46±0.27	11.32±0.452	0.404
Hematocrit (%)	36.03±0.83	35.47±1.33	0.373
Lymphocytes (%)	42.30±2.30	42.09±2.59	0.479
Monocytes (%)	4.40±0.47	4.82±0.36	0.258
Eosinophils (%)	6.50±0.75	8.18±0.65	0.066

(Mean±SEM) of buffalo heifers fed experimental diets

Table IV.- Percent nutrient digestibility (Mean±SEM) by buffalo heifers fed experimental diets.

Digestibility (%)	Feeding scheme		P-Value
	Control	Stair-step	
Day 120			
DM§	61.59±1.25	60.40±1.09	0.290
OM	63.88±1.38	63.14±0.59	0.348
CP	56.69±2.56	51.49±3.12	0.169
ADF	49.38±0.77	57.99±0.61	0.004
Day 180			
DM	57.45±2.55	60.56±0.47	0.194
OM	61.80±2.12	65.64±0.24	0.134
CP	54.83±0.68	58.21±1.70	0.017
ADF	45.13±3.25	42.07±1.89	0.337
NDF	50.11±1.30	45.63±0.37	0.079

§Where DM= Dry matter, OM= Organic matter, CP= Crude protein, ADF= Acid detergent fibre, NDF= Neutral detergent fibre

n=4 heifers per treatment

Table V.- Nitrogen balance (Mean±SEM) in buffalo heifers fed experimental diets.

Digestibility (%)	Feeding scheme		P-Value
	Control	Stair-step	
Day 120			
N intake (g/day)	79.79±1.68	80.44±4.52	0.409
Faecal N (g/day)	34.40±2.36	38.32±2.86	0.026
Urine N (g/day)	22.88±0.57	25.29±1.21	0.041
N retention (g/day)	22.50±1.80	16.83±2.66	0.262
% of intake N	28.28±2.37	20.62±2.58	0.181
Day 180			
N intake (g/day)	106.83±4.86	99.73±4.91	0.003
Faecal N (g/day)	48.32±2.68	41.44±0.46	0.012
Urine N (g/day)	45.57±2.26	42.25±1.85	0.270
N retention (g/day)	13.98±3.35	16.41±3.64	0.243
% of intake N	13.98±3.35	16.41±3.64	0.313

n=4 heifers per treatment

line with the findings of Clanton *et al*. (1983) who reported that beef heifers raised on a stair-step feeding scheme (compensatory growth) needed less feed to reach an equal body weight than heifers fed for constant growth rate. Contrary to this Grings *et al*. (1999) found no change in dry matter intake in beef heifers raised on stair-step versus control diets.

Buffalo heifers previously fed the SLE diet exhibited greater ($p<0.05$) compensatory growth when moved onto the SHE diet compared to the heifers continuously fed on control diet. Higher daily weight gain of heifers on SHE diet after the restricted energy period could be due to compensatory growth. The findings of the present study are in agreement with the results of Peri *et al*. (1993) who reported lower weight gains (0.66 vs 1.10 kg/day) in Holstein heifers fed 85% of NRC (1988) requirements for 4 months but these heifers when moved onto a high energy-high protein diet for 2 months, showed higher weight gain (1.16 vs

0.80 kg/day) compared to the heifers (control) fed high energy-high protein diet continuously. Other workers (Fox *et al.,* 1972; Park *et al*., 1998; Poland *et al*., 2005) reported that animals on restricted growth, when placed on full-fed displayed higher daily gains and feed efficiencies. In the current study, stair-step heifers attained body weight equal to their counterparts with in two months. These results are in line with those of Park *et al*. (1987) and Yambayamba and Price (1997) who reported that heifers reared on stair-step diets gained faster and fully recovered their live weights parallel to the weights of heifers fed on *ad libitum* feeding during the first 6 to 8 weeks of compensatory high energy feeding. Similarly, Barash *et al.* (1998) found that bull calves recovered their weights within 5 to 6 weeks following a similar change in dietary energy on offer; however, the degree of compensation was influenced by many factors like nutrient restriction, length and severity of feed restriction and the length and feeding level during compensatory period (Freetly *et al*., 2001).

Feed conversion ratio was worst in buffalo heifers fed the SLE diet compared to heifers fed the control diets over the initial phase of the experiment. But FCR was significantly improved when these heifers moved onto the SHE diet compared to the heifers fed the control diet. We speculate that during the energy restricted period most of the energy was used for maintenance requirements and probably very little dietary energy was spared for live weight gain, thereby resulting in poorer FCR in heifers on SLE diet. Decrease in N retention in heifers fed SLE diet may be another contributing factor for worst FCR. Fox *et al*. (1974 and Park *et al*. (1987) reported that during the realimentation period a greater proportion of consumed energy is available for compensatory growth. Intake restriction in beef cattle by offering feed 8.5% below than *ad libitum* feeding, reduced daily gain 5.2% and improved feed efficiency 3.2% reported by Hicks *et al*. (1987). Like this study, other studies (Choi *et al*., 1997; Yambayamba and Price, 1997; Ford and Park, 2001; Jin *et al*., 2004) have demonstrated that the heifers raised on step-wise feeding scheme had improved efficiency of gain by consuming less feed compared to their counterparts fed for continuous growth rate. Response of heifers on step-wise diets to weight gain and FCR was directly proportional to the dietary energy levels.

Over the entire developmental period, 1.67% greater weight gain was found in heifers raised on the stair-step growth pattern than heifers fed control diet. These results are in agreement with that of Ford and Park (2001) who reported that the heifers fed energy restricted diet exhibited higher subsequent growth rate when more dietary energy was offered than did heifers fed the control regimen. Similarly, Park *et al*. (1987) found higher weight gain and less dry matter consumption in stair-step heifers fed diets alternatively with 15% below or 40% above nutrient density of NRC requirements compared to control heifers fed diet to met the NRC requirement for growth rate of 0.45 kg/day. Our results also coincided with the findings of those of Choi *et al*. (1997) and Jin *et al*. (2004), who reported that dairy heifers that were raised on stair-step feeding scheme according to 3, 2, 4, 2, 5 and 2 months alternatively with 20% below or 25% above ME of NRC requirements had higher weight gain than those heifers (control) fed as per NRC (2001) requirement throughout the study period.

Haematological values of buffalo heifers were not influenced by dietary treatments. All haematological values were with in the normal physiological range as reported by Fraser (1991) in "The Merck Veterinary Manual" and are similar as reported by Majeed *et al*. (1985) in Nili-Ravi buffalo heifers. Eppard *et al*. (1997) reported that various haematological parameters changed during developmental stages of animals. Patil *et al*. (1992) observed average erythrocytic count of 6.77 million/µl in Murrah buffaloes and Khadjeh and Papahn (2002) reported 7.37 million/µl in Iranian buffaloes. However, lower erythrocytic count (5.90 million/µl) was reported by Chandra *et al*. (2008) in 2-3 years old Murrah buffaloes. The variation in values of erythrocytic count may be due to breed, season and feeding practices. Average leukocyte count was 13.20 thousand/µl in Nili-Ravi buffaloes (Majeed *et al*., 1985) and 12.59 thousand/µl in Nili-Ravi buffalo heifers (Jabbar, 2004) however; Patil *et al*. (1992) observed lower leukocyte count (7.96 thousand/µl) in Murrah buffaloes. Similarly, decreased leukocyte count with advancement of age was reported by Chandra *et al.* (2008) who noticed 4.54 thousand/µl in 2-3 years old buffaloes to 2.51 thousand/µl in over 10 years' old buffaloes. Patil *et al*. (1992) reported haemoglobin concentration as 11.81 g/dl in Murrah buffaloes. Majeed *et al*. (1985) reported such values as 11.30 g/dl in Nili-Ravi buffaloes. Kumar *et al*. (1990) found highest haemoglobin concentration in buffaloes with overall mean value of 12.76 g/dl. Khaliq and Rehman (2010) reported packed cell volume as 33.36% in lactating Nili-Ravi buffaloes. Likewise, Majeed *et al*. (1985) observed packed cell volume 35.0% in Nili-Ravi buffaloes and Khadjeh and Papahn (2002) reported as 36.65% in Iranian buffaloes. Chandra *et al*. (2008) and Patil *et al*. (1992) reported packed cell volume 27 and 30.58% in Murrah buffaloes, respectively. The probable reason for the decreased values of packed cell volume may be due to the different breed, weather, and feeding practices.

Ciaramella *et al.* (2005) found significantly reduced lymphocyte numbers in buffaloes that were above eight years of age. Khaliq and Rehman (2010) reported mean lymphocytes, monocytes and esinophils values as 58.23%, 5.92% and 2.61% respectively in lactating Nili-Ravi buffaloes of 7-10 years old fed on mixed ration. Buffaloes over ten years of age showed higher eosinophil levels (Ciaramella *et al*., 2005) compared to those in immature females (Canfield *et al*., 1984).

Apparent DM, OM and CP digestibility decreased by 1.97, 1.17 and 10.1% in buffalo heifers fed SLE diet than heifers fed control diet but the difference was non-significant ($p>0.05$) as reported by Singh *et al.* (2009), who observed non-significant ($p>0.05$) differences in apparent DM, OM and CP digestibility among Bhadawari buffalo heifers fed different energy levels (ME of 100, 80 and 120% of NRC, 2001 requirements). In the current study, buffalo heifers previously on SLE diet when shifted on to SHE diet, exhibited 5.41, 6.21 and 6.16% greater DM, OM and CP digestibility than heifers fed control diet that may have contributory effect in enhancement of feed efficiency and ultimately compensatory growth. Fox *et al*. (1972; 1974) reported that previously intake restricted cattle when allowed at *ad libitum* intake, their efficiency to utilize nutrients was improved or may have improved the digestibility of nutrient coefficients (Yambayamba *et al*., 1996). The rapid utilization of nutrients by the heifers on SHE diet after SLE diet might have resulted in more nutrient demand for the initiation of anabolic process (Blum *et al*., 1985) which ultimately enhanced fat deposition.

In the present study, apparent digestibility of fibre (NDF and ADF) is inversely proportional to dietary energy levels. Digestibility of ADF and NDF increased ($p<0.05$) in heifers fed SLE diet than those fed control diet. These results are supported by Hart and Glimp (1991) who reported that ADF digestibility increased in intake restricted lambs compared to those fed *ad libitum* intake. Improved feed efficiency with restricted intake is possibly, due to reduction in the size of liver and intestine that reduces the maintenance requirement of animal as reported by Baldwin *et al*. (1980).

In the current study, after energy restriction when heifers were fed SHE diet, NDF and ADF digestibility declined significantly compared to heifers fed control diet. Mould *et al*. (1983) and Hoover (1986) reported that dietary non-structural carbohydrates had negative effect on fiber digestion because of readily fermentable nature and eventually decline rumen pH which may have restricted fiber digestion. This lack of response of heifers fed SHE diet (20% high energy) than control diet may suggest that high energy diets contain more starch may limit microbial growth by not supply of available energy and N simultaneously as ruminant diets containing more degradable starch has a tendency to decrease rumen pH and rumen ammonia N concentration (Cabrita *et al*., 2006). Cecava *et al*. (1991) reported that NDF digestibility (67.5 vs 71.7% of intake) and ADF (74.40 vs 73.10% of intake) digestibility was not affected by steers fed low-forage: high concentrate (ME of 2.90 Mcal/kg of DM) and high forage: low concentrate diet (ME of 2.17 Mcal ME/kg of DM). The difference was might be due to the different feed ingredients as they used green roughages which may contribute to fiber digestibility while in the present study, wheat straw was used as dry roughage which has negligible nutritional values compared to green roughages.

Nitrogen balance was positive for all heifers; however, N retention whether expressed as grams per day or as a percentage of N intake decreased significantly ($p<0.05$) which may be associated with higher faecal and urinary N losses for heifers fed SLE diet than heifers fed control diet. Results of the present study are in line with the findings of Blum *et al.* (1985) who observed lower growth rate and N balances in food restricted steers than those in control animals. However, N retention was numerically higher ($p>0.05$) in heifers fed SHE diet leading to compensate the growth rate than the heifers fed control diet. Hornick *et al*. (1998) fed Belgian blue bulls raised on limited diet low in energy to support growth rate of 0.50 kg/day followed by control diet rich in energy on *ad libitum* intake until the animals slaughtered. They found lowered N balance on limited intake but N balance was significantly ($p<0.05$) higher in compensatory period as compared with the restricted period may be ascribed due to higher N intake. Similar findings were reported by Fox *et al*. (1972) who reported that steers on restricted growth period when placed on compensatory diet, showed increased growth rate and nitrogen retention. Cecava *et al*. (1991) observed lower ($p<0.05$) apparent N digestibility (68.40 vs 75.00%) and higher faecal N excretion (67.40 vs 50.70 g/day) with low energy diet (ME of 2.17 Mcal/kg of DM; high-forage: low concentrate) compared to high energy diet (ME of 2.90 Mcal/kg of DM; low forage: high concentrate diet) in steers. Similar findings were observed by Huntington and Prior (1983) who conducted research to evaluate the effects of metabolizable energy intake by beef heifers on apparent digestibility of various nutrients. They reported linear ($p<0.05$) increased intake of ME (5.98-15.83 Mcal/day) and N retention (-6.3 to 25.6 g/day) in heifers as they fed increased energy level.

Nitrogen excretion through faeces and urine was significantly higher on SLE diet than control diet. On SHE diet faeces N losses were significant lower than control however, urine N losses remained unchanged between the groups in the present study. These results are comparable with those observed by Singh *et al*. (2009), who reported that feacal N excretion (18.50, 16.90 and 17.80 g/day) and urinary N excretion (8.40, 10.90 and 6.00 g/day) were not affected by diets containing three energy levels fed to Bhadawari buffalo heifers. However, higher ($p<0.05$) excretion of N in urine (Sultan and Loerch, 1992) and faeces (Merchen *et al*., 1987; Sultan and Loerch, 1992) have been observed in lambs fed low energy diet than those fed high energy diet. The differences with the present results may be attributed either to the difference in the feed composition or the difference in species. Buffalo heifers raised on stair-step growth pattern incurred 16.57% lesser feed cost for one kilogram live weight gain than control heifers fed continuously as per NRC energy requirement over the entire developmental period. This study demonstrated that rearing of buffalo heifers on stair-step feeding has great potential of economic advantage.

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