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FEEDING OF TMR AT DIFFERENT DIETARY ENERGY LEVELS: INTAKE, GROWTH PERFORMANCE, FEED EFFICIENCY, STRUCTURAL DEVELOPMENT, AND ECONOMICS IN PRE-PUBERTAL NILI-RAVI BUFFALO HEIFERS

E. U. Khan[1]; T. N. Pasha[1], M. A. Rashid[1*] and M. Aleem[2]

[1]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
[*]Corresponding author's e-mail: drafzal@uvas.edu.pk

ABSTRACT

The diet of growing buffalo heifers altering in energy levels can potentially impact growth performance and its relative parameters. The present study was conducted with objectives to evaluate the influence of varying dietary energy levels on dry matter intake (DMI), live weight gain, average daily gain (ADG), feed efficiency (FE), structural measurements and body condition score (BCS) in growing pre-pubertal Nili-Ravi buffalo heifers. Eighteen heifers at an age of 220 ± 8 days, weighing 140.5 ± 2.8 Kg and BCS of 2.9 ± 0.1 were randomly assigned to one of the three dietary treatments (n=6 heifers/treatment). Experimental duration was 16 weeks including 14 weeks of data collection and a 2 week pre-experimental adaptability where TMR of NRC 100% recommendations was offered. Three treatments were; 1-ME100%, 2- ME 112% and 3- ME 125% TMR diets containing dietary ME levels of 100%, 112% and 125% of NRC 2001 recommendations for pre-pubertal growing heifers, respectively. These higher levels of energy were achieved by increasing the concentrate level as well as by adding by-pass fat in respective diets. Formulated TMR's were iso-nitrogenous, made for 150 to 250 Kg live BW heifers, and aimed to achieve a target ADG of 0.8 kg/d. Offering was individual, at 2.8% live BW in two equal feedings and adjusted every fortnight while orts were collected daily. Body weight, BCS and structural measurements were taken at start and biweekly thereafter. Total DMI and DMI as ~~a~~ % of BW were lower ($P \leq 0.05$) for the ME 125% as compared to the ME 112% and ME 100%. The heifers fed ME112% gained more weight (total and ADG) ($P \leq 0.05$) than those fed ME100%. Whereas, FE improved with increasing level of energy in the diets above NRC (2001) recommendations. Structural measurements and BCS of heifers remained unaffected ($P > 0.05$) for all the treatments. The cost per unit gain in ME100% and ME112% treatments was comparable; whereas, it was higher ($P \leq 0.05$) in ME125% treatment. The results of present study implicate that a target ADG of 800 g can be achieved by feeding TMR with 12% higher energy ~~level~~ than the NRC recommendations. Feeding TMR diet with ME 112% of NRC also improved FE in pre-pubertal Nili-Ravi buffalo heifers at a comparative cost.

Key words: Buffalo heifers, target growth, pre-pubertal, dietary energy levels.

INTRODUCTION

The future of any dairy operation depends upon a successful program for raising heifers and converting them into good milk producers, which ideally should exceed the current level of milk production in herd. The average age at first calving in buffaloes is 55 months in Pakistan which is much higher when compared to 29 months in Holstein (Rehman, 2006). Heifer rearing is one of the neglected segments of livestock production in most of developing countries. The main reason for this negligence might be due to lower growth rates and longer pre-pubertal periods without any income generation. One of the positive approaches to decrease this cost is implementation of efficient feeding management (Le Cozler *et al*. 2008) to lessen the age of sexual maturity, which cuts down the non-producing period (Ettema and Santos, 2004).

Dietary energy is an important and limiting nutrient for optimal growth performance of growing ruminants (Tauqir *et al*. 2011). However, in prevalent circumstances of overall feed and nutrients shortage for animals in Pakistan (Pasha, 2013), buffalo heifers frequently fail to achieve recommended targets of live weight and ADG at specified age (Bhatti *et al*., 2007). Inclusion of appropriate ME in the diet can help to optimize utilization of nutrients and achieve the growth targets in pre-pubertal heifers (Lammers and Heinrichs, 2000).

NRC nutrient recommendations are applied worldwide for diet formulation of ruminants, being more applicable to cattle than that of buffalo. Nutrient needs of buffalos in tropical regions differ from the dairy cattle because of differences in breed size, feeds, climate and digestive physiology (Singh *et al*. 2015). A number of nutritional studies involving varying energy levels to that of NRC (2001) recommendations have been carried out in both buffalos and cattle (Mandal and Yadav 2000; Basra *et al*. 2003; Paul and Patil 2007; Fiaz *et al*. 2012) with variable outcomes. In Pakistan, efforts have been made to study the influence of increasing energy levels in Sahiwal heifers (Fiaz *et al*. 2012) and stair step feeding regimens (certain period on a lower nutrition plane and then taking compensatory growth at higher nutrition plane) in buffalo heifers (Anjum *et al*. 2012) with significant reduction in age at puberty when adequate nutrition is provided. There appears to be no published work available on pre-pubertal growth rates with varying energy levels. Therefore, an experiment was conducted on pre-pubertal Nili Ravi buffalo heifers (7 to 11 mo of age) to evaluate the impact of dietary energy levels beyond the NRC (2001) recommendations on growth performance, DMI, FE and structural development for achieving target ADG with the aim to reduce age at puberty.

MATERIALS AND METHODS

Experimental animals and management: The research was conducted at the BRI, Pattoki, Pakistan. All the procedures were conducted in compliance with protocols approved by ACUC-UVAS. The study involved 18 Nili-Ravi buffalo heifers aged 220 ± 8 d and with similar initial BW of 140.5 ± 2.8 kg, and mean BCS of 2.9 ± 0.1. All heifers were ear tagged for their identification and moved to a naturally ventilated barn. Separate and individual feeding space of 0.8 m was provided to each animal with unrestricted access to fresh water. The heifers were also allowed to move and rest in an open area from 3 h post the morning feeding until feeding in the afternoon. The open area had two fresh water mangers of 50 cubic feet capacity for free access to water. Injectable doramectin 10 mg/mL (Dectomax®; Zoetis, USA) was administered to all heifers at the beginning of the experiment to protect against internal and external parasites. Treatment of any sick animals was carried out as per veterinary practices and protocols of the farm.

Experimental design and feeding management: Eighteen growing buffalo heifers were randomly assigned to three treatments (n = 6 / treatment) under completely randomized design. Three treatments were; 1- ME100%, 2- ME 112% and 3- ME 125% TMR diets containing dietary ME 100%, 112% and 125% of NRC 2001 recommendations for growing heifers, respectively. Formulated TMRs were iso-nitrogenous, made for 150 to 250 kg live BW heifers, and aimed to achieve a target of 0.8 kg/d ADG. Higher levels of ME in the ME112% and ME125% treatments were achieved by increasing the concentrate level as well as increasing the by-pass fats content in the respective diets (Table 1).

Experimental duration was 16 weeks including 14 weeks of data collection and a 2 week pre-experimental adaptation period. During the adaptation period, animals were fed on a common TMR diet and transitioned on to their respective dietary treatments. Animals were fed individually at 2.8% of their live BW twice daily at 08:00 and 16:00. Daily DM allowance was adjusted for live BW every fortnight, as well as to minimize refusals ≤ 5% of daily offering.

Data collection and parameters studied: Individual DMI was measured daily; whereas, body weights and body measurements including heart girth (HG), withers height (WH), hip width (HW) were measured approximately 4 h post-feeding (in the morning) at the beginning and fortnightly for the rest of trial period. Body condition score was assessed at the beginning of the experiment l and thereafter every 2 weeks using 1 to 5 scale with 0.5 increment (1 being emaciated and 5 being obese) (Anitha *et al*. 2011). During entire experiment, BCS was performed by the same two people each time and averaged for each observation.

Laboratory analysis: Orts were collected daily before the morning feeding and then weighed, subsampled, composited for each week and stored at -20ºC for further analysis. Preserved samples of rations offered and orts were thawed and composited on a bi-weekly basis for proximate analysis. A forced air oven was used to dry the samples at 60 ºC for 48 h and then samples were ground through a 2 mm screen using a Wiley mill (model 2; Arthur H. Thomas Philadelphia, USA). Following the procedures of AOAC (2002), dried ground samples proceeded for proximate analysis; laboratory DM content was determined by drying in a forced air oven for 4 h at 105 ºC, Kjeldahl apparatus was used to determine CP of the samples as N x 6.25 (Gerhardt, Kjeldatherm, Germany), ether extract was measured through Soxhlet apparatus (Behr Labor-Technik, GmbH, Germany) and ash content was determined after incinerating the samples in a muffle furnace at 500ºC for 4 h. Fibre contents (NDF and ADF) were determined using the procedures of Van Soest *et al*. (1991). All of the laboratory analyses were performed at the Department of Animal Nutrition, UVAS.

Economics: The cost of three TMRs made for ME100%, ME112% and ME125% treatments was Pak Rs. 15.75, 18.59 and 23.56, respectively. The daily feeding cost was the TMR consumed daily by each calf and it was summed for the whole trail to get total for individual heifer. To calculate the cost per unit gain, the total cost was divided by total weight gain of each calf during the whole study period.

Statistical analysis: All data were analyzed through one way ANOVA technique under CRD using SPSS (Version 21). Standard error of means and least square means were calculated on the basis of treatments. Statistical differences at the value of $P \leq 0.05$ were considered significant and were separated through Tukey's Post Hoc multiple comparison test.

RESULTS

DMI, BW changes and feed efficiency: Results of DMI, BW, and FE are presented in Table 2. The total DMI and DMI as ~~a~~ % of BW were lower (P≤0.05) for the ME 125% compared to the ME 112% and ME 100%. Table 2 also shows that daily CP intake was lowest (P 0.05) in the ME125% treatment; however, daily ME intake was lowest (P≤0.05) in the ME100%. The heifers fed ME112% gained more weight (total and ADG) (P≤0.05) than those fed ME100% but did not differ (P>0.05) to those fed the ME125% diet. Furthermore, FE improved with increasing level of energy in the diets above NRC (2001) recommendations.

Structural measurements and BCS: The structural measurements and BCS are presented in Table 3. Increasing energy levels in the diet had no effect (P> 0.05) on WH, HG, HW or BCS. Similarly, increasing energy levels in the diet had no effect (P≤0.05) on the gain in all of body measurement parameters over the experiment.

Economics: Total feed cost and daily feeding cost of the calves in the ME100% treatment was lower (P≤0.05) as compared to the ME112% and ME125%. The cost per unit gain in the ME100% and ME112% treatments was comparable; whereas, it was higher (P≤0.05) in the ME125% treatment.

Table-1. Composition and nutritional analysis of the experimental diets

	Diet		
	ME 100%[1]	**ME 112%[2]**	**ME 125%[3]**
Ingredients	**%**	**%**	**%**
Corn (ground)	14	18	22
Wheat bran	6.3	6.5	5.4
Rice polish	6.0	6.5	6.5
Molasses (sugar cane)	6.0	6.0	6.0
Megalac ®[5]	0	3.7	8.7
Soybean meal	6.5	6.5	6.5
Canola meal	5.0	5.0	5.0
Sunflower meal	3.5	3.0	3.0
Urea	1.0	1.1	1.2
Salt (NaCl)	0.5	0.5	0.5
Dicalcium phosphate	1.0	1.0	1.0
Mineral mix[6]	1.2	1.2	1.2
Wheat straw	15	15	15
Corn silage[4]	34	26	18
Nutrient composition			
Dry matter (%)	57.2	63.2	68.0
Crude protein (%)	15.0	15.0	15.0
Metabolizable energy (M.cal/kg)[7]	2.33	2.62	2.91
Neutral detergent fiber (%)	36.1	32.8	29.2
Acid detergent fiber (%)	19.1	17.3	15.5
Fat (%)	3.3	6.4	10.5
Ash (%)	5.7	5.9	6.2
Calcium (%)	0.9	1.0	1.2
Phosphorus (%)	0.5	0.5	0.5
F : C[8]	49:51	41:59	33:67

[1] ME 100% = Ration of 100% ME recommendations of NRC (2001); [2] ME 112% = Ration of 112% ME recommendations of NRC (2001); [3] ME 125% = Ration of 125% ME recommendations of NRC (2001); [4] Corn silage contained 33% DM, 42.4% NDF, 23% ADF and 7.2% CP on DM basis; [5] Megalac ® is rumen protected fat, made by reaction of calcium with fatty acids; [6] 100 kg Mineral mixture included DCP 70.81 kg, NaCl 18.91 kg, $MgSO_4$ 8.64 kg, $FeSO_4$ 0.89 kg, $MnSO_4$ 0.49 kg, $ZnSO_4$ 0.22 kg, $CuSO_4$ 0.03 kg, KI 8.77 g, $CoCl_2$ 0.89 g and $NaSiO_3$ 1.50 g; [7] Metabolizable energy calculated using the equations of NRC (2001); [8] Forage to concentrate ratio in TMR offered (DM basis)

Table-2. Effect of varying energy levels (100, 112 and 125% of NRC recommendations) in TMR diet on intake, growth performance, feed efficiency and production cost of pre-pubertal Nili-Ravi buffalo heifers.

	Diet				
	ME 100%	**ME 112%**	**ME 125%**	**SEM**	***P-value***
Total DM intake (kg)	439.5^{a}	458.8^{a}	392.0^{b}	10.7	0.002
DMI (% of BW)	2.52^{a}	2.57^{a}	2.31^{b}	0.07	0.000
ME intake (M.cal/d)	10.45^{b}	12.26^{a}	11.73^{a}	0.28	0.002
Crude protein intake (g/d)	672.75^{a}	702.00^{a}	604.75^{b}	16.32	0.004
Body weight gain (kg)	71.21^{b}	81.18^{a}	74.21^{ab}	2.32	0.032
Average daily gain (kg/d)	0.726^{b}	0.829^{a}	0.759^{ab}	0.02	0.031
Feed efficiency	0.162^{c}	0.177^{b}	0.188^{a}	0.14	0.000
Total cost (Rs)	11,935^{b}	14,215^{a}	14,207^{a}	342.9	0.000
Daily feeding cost (Rs)	121.8^{b}	145.1^{a}	144.9^{a}	3.50	0.000
Cost per unit gain (Rs)	167.9^{b}	175.3^{b}	191.5^{a}	7.05	0.001

Table-3. Effect of varying dietary energy levels (100, 112 and 125% of NRC recommendations) on structural measurements and body condition score of pre-pubertal Nili-Ravi buffalo heifers

	Diet				
	ME 100%	**ME 112%**	**ME 125%**	**SEM**	***P value***
Hip height (cm)					
Initial	94.92	95.17	94.83	0.80	0.961
Final	105.83	105.58	105.33	0.86	0.929
Gain	10.92	10.42	10.50	0.41	0.682
Hip width (cm)					
Initial	36.33	35.33	35.25	0.54	0.313
Final	43.50	43.33	42.25	0.54	0.267
Gain	7.17	8.00	7.00	0.26	0.038
Heart girth (cm)					
Initial	116.17	116.92	114.75	1.76	0.705
Final	132.92	133.83	131.08	1.99	0.628
Gain	16.75	16.92	16.33	0.83	0.879
BCS					
Initial	2.92	3.04	2.88	0.08	0.352
Final	3.83	3.92	3.83	0.09	0.761
Gain	0.92	0.88	0.96	0.06	0.651

BCS: body condition score

DISCUSSION

Dry matter and nutrient intake: Feeding buffalo heifers a diet containing 125% of NRC recommendations for ME resulted in a significant decrease in DMI (compared to 112% and 100% of NRC recommendations), with a difference of 47.5 kg and 66.8 kg compared to the ME100% and ME112% treatments, respectively. Similarly, DMI% of BW remained 9.09% and 11.3% lower than that of the ME100% and ME112% treatments, respectively. The DMI of animals is typically decreased as the energy concentration in diet is increased (Mertens 1994). Similar results have been reported for studies conducted on growing male buffalo calves (Nair *et al*. 2004; Mahmoudzadeh *et al*. 2007; Shahzad *et al*. 2011). In this experiment, energy density of the ration was enhanced by increasing the ratio of concentrate in diet which likely shifted fermentation to more ruminal propionate production and consequently a hypophagic response in high energy fed animals, as supported by previous studies (Frobish and Davis 1977; Oba and Allen 2003).

As a consequence of the decrease in DMI of animals fed ME125%, CP intake was 11.2% and 16.1% higher in ME100% and ME112% treatments, respectively. However, due to the interaction between DMI and energy density, ME intake was higher for the ME112% compared to the ME100% and ME 125%. The similar daily ME intakes for ME112% and ME125% further supports that there is a relationship between DMI and energy density. This phenomenon is well elaborated by Illius and Jessop (1996) in their model of metabolic constraints on voluntary intake.

Changes in body weight: Body weight gain and ADG was higher in the ME112% compared with those fed on the ME100% diet (Table 5.2). Heifers fed on the ME112% gained 12.3% more weight than the ME100% fed heifers. In this experiment, heifers were targeted to achieve a daily gain of 0.80 kg/d; an ADG of 0.829 kg/d in the ME112% treatment compared to 0.726 kg/day in the ME100% suggested that buffalo heifers required 12% more energy than NRC recommendations to maintain a growth rate of 0.80 kg/d. The dietary energy is at first utilized for basal metabolism and maintenance requirements and then remaining part is utilized for growth (Ensminger and Ensminger 1993). Comparatively, higher energy availability can be attributed to higher ADG in the ME112% treatment.

Studies in growing animals have emphasized that synchronization of protein and carbohydrates is crucial for growing heifers due to limitation in DMI and fermentation capacity (Casper *et al*. 1994). Other research reported a strong relationship between protein and energy for nutrient utilization and better ADG (Gabler and Heinrichs 2003). The TMR used in the study were formulated to be iso-nitrogenous and to vary in ME, obtained by altering the forage to concentrate ratio; characteristics of better energy availability from concentrate at a favorable protein level in ME112% TMR may account for the higher ADG. Similar to our findings work by Dennis (2016) reported that Holstein heifers fed on iso-nitrogenous TMRs with high and low non fibrous carbohydrates NFC levels resulted in better ADG for high NFC treatment.

Our results for ADG are in line with findings of Bortone *et al*. (1994), authors reported that pre-pubertal Holstein heifers should be fed diets containing ME at 115% of NRC recommendation to maintain ADG. Similarly, Baruah *et al*. (1988) and Singh *et al*. (2009) when investigated the effect of diets with higher energy level to that of NRC, in growing male and female buffalo calves respectively, found a significant increase in ADG. Feeding 25% extra energy to the ME125% heifers and achieving an ADG of 0.759 kg/d suggested that there is no benefit of increasing dietary energy levels over 112% of NRC recommendation. One possible explanation for lower ADG could be the iso-nitrogenous contents of diets, and consequent lowering of CP to ME ration for not properly utilizing the increasing dietary energy contents. Feeding diets over 115% might not benefit farmer and could increase cost per unit gain.

Some inconsistent results have been reported for the dietary energy manipulation in growing male buffalo calves and heifers. In their study with pre-pubertal Sahiwal heifers by Fiaz *et al*. (2012) evaluated 88, 100 112 and 124% dietary energy levels to that of NRC (2001) recommendations. Contrary to our findings authors recommended that diets with124% ME level during prepubertal phase for optimal growth rates suggesting that buffalo heifers are efficient energy utilizers compared to Sahiwal heifers. Other researchers documented higher ADG in yearling buffalo heifers fed on diet containing energy 100% of NRC recommendations (Jabbar *et al*. 2009; Shahzad *et al*. 2011). Difference in ADG reported by Shahzad *et al*. (2011) can be due to fact that diets used in the study were not iso-nitrogenous. Other factors including genetic variation, age of animals, previous nutritional management, and difference in duration of the studies might have affected the growth rates (Bhatti *et al*. 2007).

Feed efficiency: Provision of dietary energy higher to that of recommended by NRC (2001) improved the feed efficiency (FE) by 9.19% and 16.71% in the ME112% and ME125%, respectively compared to ME100%. Singh *et al*. (2009) also found a higher FE in buffalo heifers fed a high energy diet with ME 120% of NRC (2001) recommendations. Previous studies involving evaluation of diets with increasing dietary energy levels reported similar results of enhancement in FE in buffalo male calves, buffalo heifers and Sahiwal heifers (Mahmoudzadeh and Fazaeli 2009; Shahzad *et al*. 2011; Anjum *et al*. 2012; Fiaz *et al*. 2012). Investigators attributed the improved FE to the availability of excess energy being utilized for faster growth. The higher FE for ME125 can be attributed to lower DMI compared to ME100%.

The lower FE is likely due to the relatively higher forage to concentrate ratio of ME100%. Zanton and Heinrichs (2007) documented that FE and digestibility were lower in high forage diets. The increase in FE in both ME 112% and 125% diets is likely due to improvement in digestibility of organic matter and greater nitrogen retention (Moody *et al*. 2007; Zanton and Heinrichs 2009).

Structural development and BCS: Measurements of structural development (WH, HG and HW) and BCS remained unaffected by increasing the dietary energy, although these parameters increased over the duration of the experiment. These findings are in agreement with Zanton and Heinrichs (2007) who reported that structural measurements were not affected by feeding high forage or high concentrate diets which were varying for their energy values. Similar findings of structural measurements as well as for BCS were reported by Anderson *et al*. (2015) and Manthey (2016) when they fed high energy diets by increasing fat percentage by adding high fat distiller grains with different forage to concentrate ratios.

Work by Anitha *et al*. (2011) proposed that for buffaloes a BCS 3.5-3.99 was associated with increased ovarian activity. By the end of the experiment all of the buffalo heifers had a BCS within this range. These findings indicate that diets neither caused fat deposition in excess nor made the buffalo heifers over conditioned during study (Anderson *et al*. 2015). Iqbal *et al*. (2014) also reported similar results of structural measurements and BCS when evaluated the feeding of different concentrate levels in growing Nili-Ravi buffalo heifer calves.

Economics: Lower total and daily feeding cost in ME100% treatment can be can be attributed to a lower price of ME100% TMR as compared to the ME112% and ME125% treatments. On the other hand, cost per unit gain was comparable among ME110% and ME112% and remained 12.3% and 8.5% lower to the ME125% treatment respectively. Under current scenario, the feeding of 12% higher energy than that of NRC standards, to pre-pubertal heifers, can be economical and beneficial.

Conclusion: In pre-pubertal Nili-Ravi buffalo heifers, a target ADG of 800 g can be achieved by feeding TMR with 12% higher energy level than NRC recommendations. Feeding TMR diet with ME 112% of NRC also improved feed efficiency at a competitive cost. Further studies are required to evaluate ideal CP to ME ratio when energy contents are higher than NRC recommendations.

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