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RESEARCH ARTICLE

Age and Weight at Puberty in Nili-Ravi Buffalo Heifers Reared on Three Dietary Energy Restriction Periods followed by Compensatory Growth

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ABSTRACT

Twenty two weaned heifers about 6-8 months old and 98.57±5 kg average BW were divided into two groups; either fed continuously as per NRC recommendations (control) or stair-step nutritional regimen (SSNR). For control group total mixed rations were formulated by adapting the large dairy breeds heifer's nutrient requirements for growth rate of 0.6 kg/day considering body weight of 100, 200 and 300 kg at the beginning of phase I (from 8 to 13 months), phase II (from 14 to 19 months) and phase III (from 20 to 25 months), respectively. The SSNR group, in each phase started on restricted energy diet (80% ME of control) for 4 months and ended with compensatory energy (120% ME of control) for 2 months. There was no difference in daily DMI, weight gain and FCR in heifers raised on SSNR versus control diet. One heifer from each group had retarded growth, and was excluded from reproductive studies. Proportion of heifers attaining puberty by 18-23 months of age did not differ (P>0.05) between SSNR (80%) and control group (70%). Pubertal age (649±21.76 vs 639±21.46 days) and BW (382±14.00 vs 364±12.48 kg) were not affected by SSNR compared to control diet. Similarly, differences in services per conception or conception rate between the two groups were non-significant. However, SSNR reduced feed cost by Rs 16.69/kg BW gain compared to control. It can thus be concluded that SSNR shows economic advantage in rearing of buffalo heifers from post-weaning to conception age without any negative effect on reproductive efficiency compared with heifers fed as per NRC recommendations.

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INTRODUCTION

For efficient dairy farming, a package of heifer rearing is needed to achieve optimal rates of growth that promote early puberty at minimum expense. Recent research has revealed that feed cost can be reduced without major adverse effect on reproduction through altering pattern of body weight gain (Roberts *et al*., 2009) by reducing energy levels for a limited time and then compensating this effect by supplying the energy in excess to exploit the compensatory growth (Kim *et al*., 1998). This feeding scheme is actually 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) and beef (Park *et al*., 1998) heifers. Heifers raised on stair-step feeding compared to NRC standard feeding practices had similar end weights while consuming less feed, resulting in improved growth efficiency, better mammary gland development and enhanced lactation potential (Ford and Park, 2001). It has been observed that feeding heifers on a well-controlled nutritional regimen (energy restriction followed by realimentation) during certain hormone-sensitive growth stages had no adverse effects on first estrus, first conception, gestation period and services per conception compared to National Research Council (NRC) standard feeding practices (Choi *et al*., 1997; Jin *et al*., 2004).

Most of cattle and buffalo heifers in Pakistan are being reared conventionally on some green fodder and

poor quality roughages, resulting in slow growth rates (Jabbar *et al*., 2006) and delayed attainment of puberty (Bhatti *et al*., 2007). Average age at puberty of buffalo is 37 months (Bashir, 2006). However, a few studies have indicated that Pakistani buffaloes have potential to attain puberty at 18 to 24 months of age (Jabbar, 2004). For buffaloes there is no feeding standard available therefore, nutrient requirement of large dairy cattle (National Research Council) is commonly used as a reference for buffalo feed formulation (Jabbar, 2004) because buffalo's body weight is more close to the body weight of Holstein Friesian. Basra *et al*. (2003) and Singh *et al*. (2009) have reported the effect of dietary energy levels (80, 100 and 120% ME of NRC requirements of large cattle breeds) in Nili-Ravi and Bhadwari buffalo heifers.

However, no information on SSNR is available in buffaloes and there is strong need of more research work that can reveal its effects in reducing the age at puberty by providing energy restricted followed by high energy diets in stair-step nutritional regimen. The present study, therefore, was planned to evaluate the effects of low energy followed, by high energy based total mixed diets on pubertal age, body growth and conception rate in buffalo heifers.

MATERIALS AND METHODS

This study was conducted at the National Agricultural Research Centre, Islamabad, Pakistan. The study lasted for 18 months during the period 2008-2009.

Animals and their management: Twenty two Nili-Ravi buffalo heifers with 6-8 months age and average body weight of 98.57±5 kg in the beginning of experiment were used. All heifers were provided the diet as per NRC recommendations for one-month as adjustment period preceding the trial. Heifers were fed twice daily in cement mangers. Heifers were divided into two equal groups and randomly assigned to two dietary treatments i.e., fed as per NRC recommendations (served as control) and stair-step nutritional regimen (served as treated). Fresh water was provided 4-5 times per day. The heifers were housed in individual tie stalls in well ventilated shed. In the morning all heifers were let loose in an open paddock for three hours throughout the experimental period except during severe weather. 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® (Ivermectin) long-acting injection @ 2 ml/animal to control internal and external parasites.

Dietary treatments: Total mixed rations were formulated by adapting the large dairy breed heifers nutrient requirements (NRC, 2001) for growth rate of 0.6 kg/day considering body weight of 100, 200 and 300 kg at the beginning of phase I (from 8 to 13 months), phase II (from 14 to 19 months) and phase III (from 20 to 25 months), respectively for control group. The SSNR group was designed in three phases of 6 months each, started on restricted energy diet (80% ME of control) for 4 months and ended with compensatory energy (120% ME of control) for 2 months. At the beginning of each phase, previous daily gains and intakes were used to formulate experimental diets. Total mixed rations were analyzed for crude protein (CP) (AOAC, 1990), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Van Soest *et al*., 1991). Ingredient and chemical composition of experimental diets are given in Tables 1 and 2. Heifers of both groups were offered 1-2 kg of seasonal green fodder per animal/day.

Total digestible nutrients (TDN) were calculated by Wardeh (1981) equation based on proximate composition (TDN = 40.32+0.5398 CP+0.448 NFE+1.422 EE - 0.7007 CF). Digestible energy (DE) and metabolizable energy (ME) were calculated by using NRC (2001) equations.

Growth rate and feed intake: Feed intake for each animal was recorded daily. Growth was monitored by weighing heifers fortnightly with an electronic scale (Avery Berkel L122) after restriction of feed and water intake for 16 hours. Feed conversion ratio (FCR) was calculated.

Attainment of puberty: Heifers of both groups were monitored for estrus signs twice daily from 15-16 months of age (morning and evening) using a buffalo teaser bull. A heifer that showed heat signs and stood to be mounted by the bull first time in her life was assumed to have attained puberty. The age and live body weight at puberty for each animal were recorded.

Breeding and conception rate: The heifers detected in estrus were bred by natural mating. Heifers not returning to estrus were examined for pregnancy through rectal palpation at 70-90 days post breeding. Numbers of services per conception were also recorded.

Statistical analysis: Data collected were analyzed with a general linear model using student's paired t-test, as described by Steel *et al*. (1997).

RESULTS AND DISCUSSION

Growth rate and feed intake: Over the entire developmental period, there was no difference (P>0.05) in daily dry matter intake and weight gain of heifers raised on SSNR growth pattern versus control diet (Table 3). These results coincide with the findings of Grings *et al*. (1999), who reported that beef heifers raised on stair-step growth pattern did not differ in dry matter intake and average daily gain compared to heifers fed on *ad libitum* intake over the entire feeding period. There is also indication that buffalo heifers have the capacity to adjust the metabolism in response to a subnormal energy diet by increasing dry matter conversion (Campanile *et al*., 2010). In our study, SSNR heifers when moved to high energy diet after restricted energy period compensated their body weights comparable to the heifers fed as per NRC requirements. Peri *et al*. (1993) also reported lower weight gain (0.66 vs 1.10 kg/day) in Holstein heifers fed 85% of NRC requirements for 4 months but these heifers when moved on high energy-high protein diet for 2 months, showed higher weight gain (1.16 vs 0.80 kg/day) but overall weight gain was comparable to the control heifers fed high energy-high protein diet continuously throughout the study period.

Table 1: Ingredients composition (%) of total mixed rations

Ingredients (%)	Phase I (8-13 months)				Phase II (14-19 months)				Phase III (20-25 months)			
	Restricted for 4 months		Compensatory for 2 months		Restricted for 4 months		Compensatory for 2 months		Restricted for 4 months		Compensatory for 2 months	
	SSNR	Control	SSNR	Control	SSNR	Control	SSNR	Control	SSNR	Control	SSNR	Control
Maize oil cake	-	17.0	20.0	17.0	-	7.0	16.0	7.0	-	7.0	19.0	7.0
Cottonseed meal	-	12.0	15.0	12.0	-	10.0	15.0	10.0	-	3.0	10.0	3.0
Cottonseed cake	32.0	-	-	-	28.0	5.0	-	5.0	26.0	16.0	1.0	16.0
Sunflower meal	13.0	1.0	-	1.0	19.0	4.5	1.0	4.5	19.0	5.0	1.0	5.0
Canola meal	2.0	6.0	3.0	6.0	4.0	4.0	1.0	4.0	1.5	3.0	1.0	3.0
Rice polish	-	5.0	7.0	5.0	-	3.0	8.0	3.0	-	2.0	9.0	2.0
Wheat bran	1.0	7.0	5.0	7.0	-	9.0	1.0	9.0	1.0	8.0	3.0	8.0
Corn gluten feed	12.75	5.0	2.0	5.0	0.5	5.0	1.0	5.0	1.0	4.0	3.5	4.0
Corn grains	-	12.0	15.0	12.0	-	10.0	22.0	10.0	-	11.0	17.0	11.0
Vegetable oil	0.25	1.0	6.0	1.0	-	1.0	3.5	1.0	-	0.5	3.0	0.5
Wheat straw	30.0	25.0	18.0	25.0	42.0	35.0	25.0	35.0	44.0	33.0	25.0	33.0
Cane molasses	6.0	6.0	6.0	6.0	4.0	4.0	4.0	4.0	5.0	5.0	5.0	5.0
Urea	0.5	0.5	0.5	0.5	-	-	-	-	-	-	-	-
Constant ingredients[§]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total	100	100	100	100	100	100	100	100	100	100	100	100

[§]Constant ingredients: Dicalcium phosphate 1.00%, limestone powder 0.50%, sodium chloride 0.50% and mineral premix 0.50%; [+]Buffalo heifers fed Stair-step Nutritional Regimen (SSNR) with low energy diet for 4 months followed by high energy for 2 months during each phase.

Table 2: Chemical composition and cost of total mixed rations

Variables	Restricted phase		Compensatory phase	
	SSNR[§]	Control	SSNR	Control
Phase I (8 to 13 months)	4 months		2 months	
CP (%)	15.84	16.16	16.11	16.16
NDF (%)	43.81	29.34	24.00	29.34
ADF (%)	30.47	19.19	15.61	19.19
ME (Mcal/kg)	2.03	2.55	3.01	2.55
Cost per kg (Rs)	12.60	16.14	20.06	16.14
Phase II (14 to 19 months)	4 months		2 months	
CP (%)	13.70	13.41	13.65	13.41
NDF (%)	50.92	39.14	27.36	39.14
ADF (%)	35.91	25.96	18.50	25.96
ME (Mcal/kg)	1.89	2.35	2.80	2.35
Cost per kg (Rs)	12.15	14.95	18.08	14.95
Phase III (20 to 25 months)	4 months		2 months	
CP (%)	12.76	12.31	12.89	12.36
NDF (%)	51.15	38.56	28.03	38.56
ADF (%)	36.23	25.70	18.67	25.70
ME (Mcal/kg)	1.84	2.30	2.76	2.30
Cost per kg (Rs)	11.51	14.35	17.50	14.35

[§]Where SSNR=Stair-step Nutritional Regimen, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber and ME=Metabolizable energy.

Table 3: Growth performance (Mean±SE) of Nili-Ravi buffalo heifers fed SSNR versus control diets (8 to 25 months of age)

Parameters	Feeding Regimen		P-Value
	SSNR	Control	
Initial BW (kg)	98.5±5.0	98.6±5.2	0.49
Final BW (kg)	440.0±15.3	419.9±22.0	0.15
Daily weight gain (kg)	0.63±0.02	0.59±0.03	0.11
Average daily DM intake (kg/head)	5.53	5.52	-
Feed conversion ratio	8.78	9.36	-
Feed cost per kg weight gain (Rs)*	125.11	141.80	-

±=standard error of means; SSNR=Stair-step Nutritional Regimen; BW=Body weight; *Average cost per kg of control diet was Rs. 15.15 and stair-step diet Rs. 14.24.

Contrary to the findings of the present study, Ford and Park (2001) reported that heifers raised on stair-step growth pattern gained significantly more weight (0.95 vs 0.80 kg/day) compared to conventionally fed control heifers. Freetly *et al*. (2001) demonstrated that there are many factors which may influence the response of compensatory growth for heifers undergoing compensatory gain e.g., nutrient restriction, length and severity of feed restriction and the length and feeding level of the refeeding phase.

In our study feed costs incurred per kg weight gain of heifers fed SSNR was 13.34% less (Rs 125.11 vs. 141.80) than those fed control diet (Table 3). In the present study, one buffalo heifer from each group (9.09%) did not attain required body weight at the same age in spite of best health cover and feeding probably due to poor genetic potential, so these animals were excluded from reproductive studies.

Attainment of puberty: Proportion of heifers attaining puberty by 18-23 months of age did not differ (80 vs 70%) between SSNR and control group (Table 4). These findings are consistent with previous reports (Choi *et al*., 1997; Jin *et al*., 2004) in which Holstein heifers raised on stair-step feeding scheme did not differ in onset of puberty from the heifers fed as per NRC requirements.

Behavioral signs of estrus in buffalo heifers were not influenced by SSNR compared to those fed as per NRC requirement. In both groups, overall behavioral signs of estrus were swollen vulva (73%), mucous discharge (60%), frequent micturition (33%), bellowing (7%) and reduced feed intake (27%).

Age at puberty: Age at puberty of buffalo heifers was not influenced by SSNR (Table 5) as the heifers in this group attained puberty only 10 days later than the control heifers (649 vs 639 days). Choi *et al*. (1997) also reared Holstein heifers on stair-step feeding and reported that stair-step feeding had no negative effect on first estrus, first conception and services per conception compared to the heifers fed as per NRC requirements. Borghese *et al*. (1996) fed two diets (low or high energy levels) to Italian buffalo heifers of nine months age. Heifers on high energy diet attained puberty one month earlier (24.5 months of age) than heifers reared on low energy diet (25.5 months of age).

Pubertal age of buffalo heifers of 540-590 days (18-23 months) in the present study coincides with the findings of Jabbar (2004), who reported that Nili-Ravi buffaloes fed rations containing 80, 100 and 120% metabolizable energy of NRC attained puberty at the age of 698, 679 and 659 days, respectively. Another study concerning puberty in buffaloes (Rafiq *et al.,* 2008) showed that the onset of first estrus in Nili-Ravi buffalo heifers occurred at the age of 774, 728 and 993 days fed on 2, 4 and 0 kg/day per animal concentrate plus seasonal

available green fodder, respectively, throughout the study period. Bashir (2006) reported pubertal age of 1110 days (37 months) in buffaloes on conventional feeding system. The most probable reason of delayed puberty in buffaloes raised under conventional management may be imbalanced nutrition as buffalo heifers reared on SSNR or as per NRC requirements attained puberty about 12 months earlier than the heifers reared on conventional feeding system. Therefore, nutritional management was considered to be very important for efficient reproductive performance.

Body weight at puberty: It is generally recognized that when heifers attain 55 to 65% of mature body weight, there is no risk of complexity in breeding and calving percentage (Freetly *et al*., 2001). In the current study, buffalo heifers raised on SSNR attained puberty at 382 kg body weight (69.45% of mature body weight), while the control heifers attained puberty at 364 kg body weight (66.21% of mature body weight), where mature weight of Nili-Ravi buffaloes was considered to be 550 kg (Khan 2009). Rafique *et al*. (2008) reported that the onset of first estrus in Nili-Ravi buffalo heifers occurred at body weight of 388 to 431 kg. In a study made on Murrah buffaloes (a breed comparable with Nili-Ravi in weight and physiology), Haldar and Prakash (2005) reported that heifers fed individually a roughage-concentrate based diet to provide weight gain of 0.4 kg/day, attained puberty at an average age of 946 days (31.53 months) with a body weight of 380.67 kg.

In the present study, 2 out of 10 heifers (20%) of both groups that attained required proportion of mature body weight before breeding season did not come in standing heat. Although investigation was not made in depth to identify the cause but there may be some pathological factors (hypoplasia of reproductive organs, etc.) involved in non occurrence of puberty even after attainment of proper body weight in a small proportion of population. Maximum proportion of heifers came in estrus during breeding season (October by 21-23 months of age). These results indicate that attainment of puberty in buffaloes not only relies on body weight but also has relation with breeding season as majority of buffaloes are bred during winter months (Khan, 2009).

Breeding and conception rate: The present study showed that heifers fed on SSNR required less number of services per conception than those fed on control diet but the difference was non-significant (P>0.05). Similarly, dietary treatments did not affect pregnancy rate in both groups (Table 5).

It is concluded that buffalo heifers raised on energy restriction followed by realimentation (SSNR) had comparable reproductive efficiency to buffalo heifers fed as per NRC recommendations. Eighty percent of the animals of SSNR group attained puberty by 18-23 months age as compared to 70% animals from NRC group. As energy restriction followed by realimentation was cost effective and also did not disturb reproductive parameters, it may be recommended for raising buffalo heifers. The impact of such a regimen on calving and postpartum productive performance remains to be investigated.

Table 4: Attainment of puberty and estrus symptoms in buffalo heifers fed SSNR and control diets

Parameters	Feeding Regimen		
	SSNR (n=10)	Control (n=10)	Overall (n=20)
Heifers attaining puberty by 25 months of age (%)	80	70	75
Pubertal by (%)			
April	12.50	14.28	13.33
(18-19 months age)	(1/8)	(1/7)	(2/15)
September	12.50	-	6.66
(21.4 months age)	(1/8)		(1/15)
October	75.00	85.71	80.00
(22-23 months age)	(6/8)	(6/7)	(12/15)
Estrus symptoms			
Swollen vulva (%)	75	71	73
Mucous discharge (%)	62	57	60
Frequent micturition (%)	25	42	33
Bellowing (%)	12	-	7
Reduced feed intake (%)	25	28	27

SSNR= Stair-step Nutritional Regimen.

Table 5: Age and body weight at puberty and conception rate in buffalo heifers fed SSNR and control diets (Mean±SE)

Parameters	Feeding Regimen		
	SSNR	Control	P value
Age at first estrus (days)	649±21.7 (530-675)	639±21.5 (525-677)	0.405
Live BW at first estrus (kg)	382±14.0 (335-435)	364±12.5 (338-430)	0.126
Percent mature BW at first oestrus[2]	69.45±3.2	66.21±2.2	0.076
Services per conception	1.33±0.2	1.83±0.3	0.182
Conception rate (%)	50	57	-

SSNR= Stair-step Nutritional Regimen; BW= Body weight; [2]Mature weight has been considered to be 550 kg for Nili-Ravi buffaloes (Khan, 2009).

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