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Growth, Reproductive Performance, and Health Attributes of Crossbred Heifers fed High Plane of Energy and Protein

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ABSTRACT: The high feeding of energy and protein enhances the growth of dairy heifers, safety evaluation physiology and health attributes are very essential to established safety. The experiment was planned to evaluate the effect of high energy and protein on growth and reproductive performance as well as the health attributes of crossbred heifers. A total of twenty-one crossbred heifers were selected and blocked in three treatment groups- T1, T2, and T3 based on body weight and age. The control-T1 group was fed a ration with 100% ME and 100% CP of ICAR requirement, the T2 group was fed 125% ME and 125% CP of control, and the T3 group was fed a diet with 100% ME and 125% CP of control diet. The body weight at biweekly intervals was a consideration for the growth performance. The blood was collected from each crossbred heifer at 0, 90, and 140 days of the experiment before feeding and watering. The total body weight gain of heifers was higher (p<0.05) in T2 and T3 groups compared to T1. The daily body weight gain was significantly higher in the T2 group compared to (T1, T3) other. The gain in heart girth was higher (p<0.05) in the T2 group compared to the T1 and T3 groups. All reproductive parameters (AI/conception, age at conception-days, weight at conception-kg) was non-significantly (p>0.05) differing among the treatments. The feeding of high ME and CP TMR resulted in a 40.17 days lower age at conception and 8.64 kg higher body weight at conception compared to T1. The blood (RBC, Hb) and serum biochemical parameters (glucose, total protein, albumin, globulin, A/G ratio, creatinine, AST or SGOT, ALT or SGPT) were within normal physiological range and were not improved by high ME-CP (T2) and high CP (T3) TMR. The WBCs count and BUN were high on feeding high ME-CP (T2) and high CP (T3) TMR. The high ME-CP (T2) TMR has increased and high CP (T3) TMR has reduced total cholesterol and HDL. Only high CP (T3) TMR has reduced LDL levels of crossbred heifers than high ME-CP (T2) and control (T1) TMR.

Keywords: Crossbred heifers, energy, growth, health attributes, protein, reproduction.

INTRODUCTION

The global demand and consumption of livestock products increase as the human population and urbanization increase. To fulfill this necessity, minimizing raising costs can be a way which can be possible by minimizing resource input and nutrient output which lead to improved growth and feed efficiency subsequent to good reproductive efficiency and future lactation performance (Akins, 2016). To decrease age at first calving, heifers can be fed for accelerated growth rates prior to puberty and bred at an earlier age but without fattening. By decreasing age at first calving, the potential exists to increase lifetime profitability (Brown *et al.*, 2005). Adequate body weight and age (22-24 months) at calving improve production life and reduce feeding costs. Maximizing average daily

growth and betterment of health is the main objective of the rearing program for calves and heifers. Among all inputs, feeding covers 60%, so improvement in feed utilization efficiency of ruminant diet is a special concern (Oldham, 1984). Only excessive high dietary protein level without corresponding energy increases in the diet of heifers leads to a more structural growth rate which lowers the rate of increase in BCS, suggesting an increase in lean tissue growth, which may not sufficient for on set reproduction and subsequent milk production in dairy cattle. Too high-level nutrition can cause fat deposition of the udder and impairs the mammary development of heifers (Sejrsen and Purup 1997; Harrison et al., 1983) and are detrimental to future milk production. The experiment was planned to evaluate the effect of high energy and protein on growth and

Vaidh et al.,

Biological Forum – An International Journal 14(4): 579-585(2022)

579

reproductive performance as well as the health attributes of crossbred heifers.

MATERIALS AND METHODS

Location and grouping: The present research study was conducted at Livestock Research Station, Anand Agricultural University, Anand for 140 days (ten biweekly periods) from 16/07/2022 to 04/12/2022 with sanction from Committee for the Purpose of Control and Supervision of Experiments on Animals-CPCSEA 364/LRS/2022). New (No. Delhi on the recommendation of Institutional Animal Ethics Committee (IAEC). Experimental heifers were cared for and managed as per directives. Twenty-one healthy crossbred heifers (75% Holstein Friesian × 25% Kankrej) of similar age (T1-383.62±26.68, T2-376.13 ±27.12, T3-373.12 ±27.22 days) and body weight (T1-269.72±10.45, T2- 271.2 ±9.31, T3-272.08±7.90 kg) were selected and grouped into the three (T1, T2, T3) having seven in each group, considering the body weight and age. The crossbred heifers were fed TMR (Table 1): T1 (control)- 100 % each of ME and CP of ICAR (2013), T2-125% each of ME and CP of control, and T3-100% ME and 125% CP of control. The ME of ingredients was taken from the NRC (2001), Anonymous (2012) and of ingredients whose ME was not available as well as of TMR was calculated as per NRC (2001) using the formula ME (Mcal/kg) = TDN (%) \times 0.0361. TMRs were made on a clean cemented floor on daily basis and fed two times a day (10:00, 14:00 hr). The feed offered to crossbred heifers was adjusted at weekly intervals based on changes in body weight. The crossbred heifers were let loose for two hours in the morning under controlled conditions for exercise, during which period they had free access to clean, wholesome, and fresh drinking water. Water was also offered two times (14:00, 17:00 hrs) in a day at a tying place.

Body weight and body measurement: The crossbred heifers were weighed weekly before feeding and watering in the morning (8:00- 8:45 hrs) on the electronic weighing bridge. The data on body weight was presented at biweekly intervals. The body measurements (cm) like withering height, body length and heart girth of all crossbred heifers were taken at the biweekly interval in the morning at 8:00 am before feeding and watering ensuring heifers were on an even surface. Proximate and fibre fraction of TMRs was carried out as per AOAC (1995) and Van Soest *et al.* (1991), respectively.

Reproduction and haemato-biological constituents: The farm policy was to breed crossbred heifers after achieving a body weight of 320kg. The reproductive parameters like the number of heifers conceived, the number of insemination required to conceive, age at conception, and weight at the conception of crossbred heifers were recorded during the experimental period. Blood was collected from each crossbred heifer at 0,90, and 140 days of the experiment. Blood was collected from the jugular vein using a vacuette with all aseptic precautions in the morning before feeding and watering (8:00 to 9:00 hrs). Blood was collected in two separate vacuette: EDTA and clot activator vacuette. EDTA vacuette containing whole blood was used for haematological analysis in the laboratory using Mindray BC-2800Vet Haematology Analyzer after completion of blood collection.

Particular	TMR-T1	TMR-T2	TMR-T3	
Ingredient proportion (kg) on DM basis				
CCM	14.01	19.88	23.29	
Maize Bhardo	15.00	15.00	13.82	
Soybean DOC	0.00	0.00	0.84	
Wheat straw	20.00	19.00	17.73	
Pigeon pea straw	20.00	13.11	18.15	
Green Hybrid Napier	25.00	25.00	23.31	
Premix*	1.00	1.00	1.00	
Salt	1.00	1.00	1.00	
Bypass fat	3.99	6.00	0.86	
Total	100.00	100.00	100.00	
Chemical composition % on DM basis				
DM	51.27 ±0.72	51.45 ±0.89	52.98 ±0.51	
OM	86.19 ±1.31	87.34 ±0.63	86.59 ±1.06	
СР	8.85 ±0.15	10.03 ±0.17	11.43 ±0.24	
EE	6.57 ±0.64	7.87±0.93	3.76±0.24	
CF	33.20 ±1.33	31.80 ±0.94	31.67 ±0.95	
Total Ash	8.53 ±0.31	8.37±0.13	8.70±0.22	
NFE	42.84 ±0.73	41.94 ±0.59	44.43 ±0.79	
NDF	68.38±1.74	65.28±2.44	64.37±1.40	
ADF	38.35±0.56	35.59±0.99	34.90±0.64	
Cal. ME (Mcal/kg)	2.212	2.391	2.159	

Table 1: Ingredient and nutrients composition of total mixed ration (TMR) fed to crossbred heifers.

CCM= Compounded Concentrate Mixture, DOC= deoiled cake, DM=Dry matter, OM=Organic matter, CP=Crude protein, EE=Ether extract, CF=crude fibre, NFE=Nitrogen free extract, NDF=Neutral detergent fibre, ADF=Acid detergent fibre, Cal. ME= Calculated metabolizable energy, Mcal= Maga calory

* Premix Each kg contained 202g calcium, 11.8g phosphorus, 10g magnesium, 5.4g sodium, 9g sulphur, 12.76g zinc, 1g copper, 0.125g cobalt, 4g manganese, 0.03g selenium, 0.4g iodine, 6g iron, 0.02g chromium, 1100000 IU vitamin A, 220000 IU vitamin D3, 200mg vitamin E, 8.8 mg vitamin B12, 2759 mg niacin, 5000mg methionine activity, and 10000mg lysin.

The serum was separated by centrifugation at 2000 rpm for 8 minutes in the REMI research centrifuge machine. Serum was collected in a clean sterilized Eppendorf tube and stored at -20°C for further analysis of serum parameters with the help of CCK 300 Clinical Chemistry Analyzer (Bangalore, India) at Veterinary Clinical Complex, Anand. Glucose was detected by the GOD-POD method, SGPT and SGOT by the Modified IFCC method, BUN by the GLDH-UREASE method, creatinine by Modified Jaffe's method, total protein by the Biuret method, albumin by BCG Dye method, total cholesterol by CHOD-PAP method, HDL and LDL cholesterol by Direct Enzymatic method. Globulin level was calculated by subtracting albumin from total protein content. The albumin to globulin ratio was calculated by dividing albumin content (g/dl) by globulin content (g/dl). For comparing the mean on haemato-biological parameters average of 90- and 140day was taken heifer-wise.

Statistics: The data generated were presented as the mean of treatment with standard error. The data were analyzed as per Snedecor and Cochran (2014) as one-way ANOVA using Web-based Agriculture Statistic software Package-WASP 2.0 (Jangamand Wadekar 2004). The difference between the mean was taken as significant at probability <0.05.

RESULTS AND DISCUSSION

Ration composition: The nutrient composition of total mixed ration (TMRs) is given in Table 1. The crude protein and ether extract were higher in T2 TMR and only crude protein was higher in T3 TMR compared to control T1 TMR.

 Table 2: Body weight and body measurements of crossbred heifers fed different TMR.

Parameter	T1	T2	Т3	P value	
Body weight (kg)					
Initial	267.4±11.79	267.43±9.95	267.97±8.39	-	
Final	350.31±13.16	369±10.13	365.2±7.47	-	
Total gain	82.91 ^b ±5.24	101.57 ^a ±4.24	97.23 ^a ±3.63	0.031	
Daily gain	0.592 ^b ±0.04	0.730 ^a ±0.03	0.694 ^b ±0.03	0.008	
Heart Girth (cm)					
Initial	142.71±2.52	139.86±1.53	143.43±1.26	-	
Final	168.64±2.51	170.79±2.01	167.79±1.61	-	
Gain	25.93 ^b ±1.31	30.93 ^a ±1.06	24.36 ^b ±1.87	0.020	
Body Length (cm)					
Initial	131.29±1.12	130.29±2.14	132.93±1.10	-	
Final	145.79±2.07	147.43±3.07	150.79±1.29	-	
Gain	14.50±1.45	17.39±1.82	17.86±1.35	0.324	
Wither Height (cm)					
Initial	117.14±1.46	120.00±0.62	120.29±0.92	-	
Final	128.93±1.52	133.26±1.74	133.07±1.14	-	
Gain	11.79±1.70	13.26±1.22	12.79±0.97	0.760	

Growth performance:

The data on growth parameters were presented in Table 2. The mean body weight of crossbred heifers was nonsignificantly (p>0.05) different from each other. The total body weight gain of heifers was significantly higher (p<0.05) in T2 and T3 groups compared to T1. The daily body weight gain was significantly higher in the T2 group compared to (T1, T3) other.

The higher level of both metabolizable energy + crude protein and crude protein improved the total and daily gain in body weight of crossbred heifers. Similar to the present study results, a significantly higher total gain in BW (kg) was reported in Holstein heifers (Williams et al., 2022), in crossbred heifers (Lunagariya et al., 2019), and in growing Murrah buffalo calves (Prusty et al., 2016) on the feeding of densified diet in term of energy or protein or both. The feeding of densified diet in terms of energy and protein level also resulted in a significantly higher daily gain in Murrah buffalo calves (Prusty et al., 2016), in Angus crossbred steers (Fluharty et al., 1994), in Italian Friesian heifers (Pirlo et al., 1997) and in Holstein heifers (Bethard et al., 1997). In contrast to the present study, non-significant (p>0.05) gain in daily body weight were observed in Holstein heifers (Gabler and Heinrichs 2003a; Hoffman et al., 2001), in growing Angus × Chinese Xiangxi yellow cattle (Li et al., 2014), in Chinese Holstein heifers (Dong *et al.*, 2015), in male buffalo calves (Tauqir *et al.*, 2011), in Nili-Ravi buffalo calves (Shahzad *et al.*, 2011) and in HF bull calves (Brosh *et al.*, 1995) on feeding diet densified with energy and protein.

The gain in heart girth of crossbred heifers (Table 2) was significantly higher (p<0.05) in the T2 group compared to the T1 and T3 groups. The gain in body length and wither height of crossbred heifers differed non-significantly (p>0.05). The combination of both ME and CP in TMR was found effective in improving the heart girth of crossbred heifers. The significantly higher heart girth was reported in Angus × Chinese Xiangxi yellow growing cattle, (Li et al., 2014), in crossbred heifers (Lunagariya et al., 2019), in Holstein heifers (Williams et al., 2022) and in crossbred bull calves (Akhter et al., 2017) fed energy protein densified diet. A non-significant (p>0.05) improvement in body length was observed in crossbred bull calves (Akhter et al., 2017) and in crossbred heifers (Lunagariya et al., 2019) on feeding energy and protein densified diet. A non-significantly (p>0.05) wither height and body length in Holstein heifers (Gabler and Heinrichs 2003a) in crossbred heifers (Lunagariya et al., 2019) on feeding a 6% densified diet and that of Holstein heifers (Rincker et al., 2011) on feeding high protein + fat diet at a higher rate (2.1% of body weight) was reported. Williams *et al.* (2022) also reported nonsignificant hip height on the feeding of a high-energy diet to Holstein dairy heifers. These findings support the outcome of the present study. The contradicting and significant (p<0.05) improvement in wither height was reported in Holstein heifers (Gabler and Heinrichs 2003a; Bethard *et al.*, 1997; Daccarett *et al*, 1993), in Italian Friesian heifers (Pirlo *et al.*, 1997), in Holstein heifer calves (Brown *et al.*, 2005) on the feeding of energy and protein densified diet. A higher significant (p<0.05) gain in body length and wither height was reported in Holstein heifers (Hoffman *et al.*, 2001) on the feeding of protein densified diet. experimental period. All reproductive parameters (AI/conception, age at conception-days, weight at conception-kg) was non-significantly (p>0.05) differing among the treatments. The feeding of high ME and CP TMR resulted in a 40.17 days lower age at conception and 8.64 kg higher body weight at conception compared to T1. The feeding of high CP TMR lowered both age (52.67days; 10.23%) and body weight (4.00 kg; 1.18%) at conception in crossbred heifers than in the T1. Similarly, a non-significant (p>0.05) effect on the number of services required to conceive, age at conception, and body weight at conceptionwas reported body weight at conception fed energy and protein densified diet (Rincker *et al.*, 2011; Pirlo *et al.*, 1997).

Reproduction performance: In each treatment, six out of seven heifers were conceived during the

Parameter	T1	T2	T3	P value
No. of heifer conceived	6	6	6	-
No. of AI/ conception	1.33±0.28	1.67±0.21	1.33±0.21	0.454
Change over control	-	+0.34	0.00	
Percent change over control	-	+25.56	0.00	
Age at conception (days)	514.67±16.24	474.50±22.92	462.00±37.43	0.379
Change over control	-	-40.17	-52.67	
Percent change over control	-	-7.80	-10.23	
Weight at conception (kg)	337.68±6.89	346.32±8.53	333.58±8.42	0.530
Change over control	-	+8.64	-4.00	
Percent change over control	-	+2.56	-1.18	

Table 3: Reproductive performance of crossbred heifers fed different TMR.

Table 4: Haemato-biochemical	parameters in	different TMR	groups of	crossbred heifers

Parameter	T1	T2	Т3	P value		
Haematology						
WBC (10 ³ /µL)	9.79 ^b ±0.59	11.96 ^a ±0.39	11.64 ^a ±0.38	0.004		
RBC (10 ⁶ /µL)	10.01±1.13	9.68±0.47	9.21±0.44	0.752		
Hb (g/dl)	10.30±0.54	12.17±0.78	11.76±0.59	0.371		
	Energy and protein nutrition					
Glucose (mg/dl)	60.71±2.55	60.07±2.17	59.79±2.28	0.960		
Total protein (g/dl)	6.31±0.09	6.29±0.13	6.35±0.11	0.928		
Albumin (g/dl)	3.19±0.04	3.11±0.07	3.21±0.07	0.413		
Globulin (g/dl)	3.12±0.08	3.18±0.13	3.13±0.13	0.082		
A/G Ratio	1.03 ± 0.03	1.00 ± 0.05	1.06 ± 0.06	0.744		
Liver function test						
AST-SGOT (U/L)	73.79±2.06	80.52±3.75	73.29±3.36	0.205		
ALT-SGPT (U/L)	46.67±1.64	44.98 ± 1.84	45.75±2.21	0.822		
Kidney function test						
Creatinine (mg/dl)	1.53±0.05	1.41±0.04	1.53±0.08	0.213		
BUN (mg/dl)	18.44 ^b ±0.41	19.73 ^a ±0.42	$20.80^{a} \pm 0.47$	0.002		
Cholesterol profile						
Total Cholesterol (mg/dl)	220.86 ^b ±8.54	276.86 ^a ±15.38	139.57°±6.88	0.000		
HDL-c (mg/dl)	118.00 ^b ±4.81	135.64 ^a ±6.64	72.29 ^c ±3.70	0.000		
LDL-c (mg/dl)	112.14 ^a ±18.37	108.93 ^a ±8.03	54.36 ^b ±2.75	0.001		

Haematological and serum biochemical parameter: Haematology: The WBCs count was significantly higher (p<0.01) in the T2 and T3 groups than in the T1. The RBCs count ($10^6/\mu$ L blood) and haemoglobin-Hb concentration (g/dl) of crossbred heifers in different groups did not differ significantly (p>0.05). The WBCs (9.79-11.96 × $10^3/\mu$ L blood) and RBC (8-15 × $10^6/\mu$ L blood)count of crossbred heifers were within the normal physiological range (Aiello and Moses 2016) indicated the high energy + protein and high protein in the diet of crossbred heifers has no detrimental effect on the health. Similarly, a significantly higher WBCs count has been observed in Sahiwal calves fed a high protein and high fat + protein diet (Sharma *et al.*, 2020). A significant (p<0.05) improved RBC and a nonsignificant effect on WBCs count was reported in Holstein multiparous cowsfed a high protein diet (Holter *et al.*, 1987), contrasting the present finding. The Hb value reported in this experiment was within the normal range (8-15 g/dl) as reported by Kaneko (2008). Similarly, Medhi *et al.* (2018); Sachurman and Kesler (1974) reported non-significant results (p>0.05) in growing yak and in Holstein & Brown Swiss calves on the feeding of the densified diet with different levels/ratios of protein and energy. Contrary, Holter *et al.*(1987) reported significantly (p<0.05) improved haemoglobin (g/dl) levels in Holstein multiparous cowsfed protein densified diet.

Energy and protein nutrition: The blood glucose (mg/dl), serum total protein (g/dl), albumin (g/dl),

Vaidh et al., Biological Forum – An International Journal 14(4): 579-585(2022)

globulin (g/dl), and A/G ratio differed non-significantly among the different groups, indicating optimum energy and protein nutrition of crossbred heifers. The serum glucose (45-75 mg/dl), serum total protein (6.74-7.46 g/dl), serum albumin (3.03-3.55 g/dl), serum globulin level (3.0-3.48) in cow was reported by Kaneko (2008) and the value reported in the present study was within the normal range. The metabolizable energy protein and protein level in TMR not influenced the serum concentration of glucose, total protein, albumin, globulin and albumin to globulin ratio in crossbred heifers. Similarly, non-significant (p>0.05) results on serum glucose, serum total protein, serum albumin, serum globulin level, and serum albumin to globulin ratio were observed in growing animals on feeding different levels of protein (DCP) and energy (TDN) in diet (Medhi et al., 2018; Sharma et al., 2020; Elihasridas et al., 2019; Schurman and Kesler 1974). In contrast, significantly higher (p<0.05) serum total protein content was observed in Holsteinheifers fed a high CP diet (Hoffman et al., 2001), and in crossbred heifers fed high energy protein diet (Hadiya et al., 2019). Hoffman et al. (2001) in Holsteinheifers and Holter et al. (1987) in multiparous Holstein cows reported significantly (p<0.05) higher albumin levels with higher CP content in the diet.

Liver function test: The value of liver function tests like serum aspartate amino transferase (AST-SGOT; U/L), and serum aminotransferase (ALT-SGPT; U/L) differed non-significantly among the different groups indicating a higher level of energy + protein and protein safe to feed to crossbred heifers.AST values reported in the present study were within the physiological range (60-125 U/L) for cow species as per Aiello and Moses (2016). Similarly, a non-significant AST and ALT value was observed in Holstein heifers fed a 6% higher energy protein diet (Hadiya *et al.*, 2019) and in growing yak fed high protein (DCP) and energy (TDN) diet (Maeko, 2008) may be due to biological variation as it was similar across the treatment group.

Kidney function test: The value of creatinine (mg/dl) concentration indicating kidney function has differed non-significantly among the different groups. The BUN (mg/dl) levels in groupsT2 and T3 were significantly (p<0.05) higher than in T1. The serum creatinine value in the present study was within the physiologically normal range (1.0-2.0 mg/dl) as reported by Kaneko (2008) indicating normal kidney function of crossbred heifers. Similarly, feeding a high-protein diet resulted in a non-significant (p>0.05) difference in creatinine content in multiparous Holstein cows(Holter et al., 1987). The feeding of high protein and energy in the diet results in higher blood urea nitrogen (BUN), putting a greater load on the kidney to clear from blood but the value reported in the present study (20.80 mg/dl) indicates optimum protein nutrition (Hammond, 1998). Similarly, significantly higher (p<0.05) serum urea nitrogen was reported in Chinese Holstein heifers fed the high ME diet (Dong et al., 2015), and in Holstein heifers fed a high CP: ME ratio diet (Gabler and Heinrichs 2003a; Gabler and Heinrichs 2003b; Hoffman et al., 2001). Whereas Medhi et al. (2018) observed non-significantly (p>0.05) blood urea nitrogen content in growing yaks fed a high energy and protein ratio diet.

Cholesterol profile: The total cholesterol and HDL-c concentration (mg/dl)were significantly (p<0.05) higher in the T2 group than in the T1 as in this group the crossbred heifers were fed energy and protein-dense diet which showed higher body weight at the time of conception (Table 3). A high level of HDL was important for antioxidant and anticoagulant activity in the animal body to prevent various diseases (Andersen et al., 2013). The feeding of high CP TMR (T3) significantly (p<0.05) reduced cholesterol concentration in crossbred heifers compared to the control. The serum cholesterol value reported in the present study was within the normal range (65-220 mg/dl) as reported by (Jackson and Cockcroft 2007). Similarly, significantly higher (p<0.05) cholesterol levels were observed in Holstein heifers (Park et al., 1987; Hadiya et al., 2019) and in male Simmental cattle (Elihasridas et al., 2019) on the feeding of high energy and high protein diet. Whereas Holter et al. (1987)reported decreased (p<0.05) total cholesterol level (mg/dl) in multiparous Holstein cows fed a high protein diet.Similarly, Park et al. (1987) reported higher HDL levels in Holstein heifers fed densified diet during the compensatory phase. Contrasting to the present finding, Elihasridas et al. (2019) reported lower HDL levels in Simmental cattle fed a high-energy diet.

CONCLUSION

The feeding of the high plane of nutrition in terms of ME + CP (T2) and high CP (T3) TMR improved growth, and body weight at conception at a lower age, without adversely affecting health attributes like haematology (WBC, RBC, Hb), energy and protein nutrition (serum glucose, total protein, albumin, globulin, A/G ratio) liver function (AST, ALT), and kidney function (creatinine, BUN).

FUTURE SCOPE

This research is useful to the dairy farmer to improve the growth rate of crossbred heifers by feeding high energy, protein total mixed ration without compromising physiology and health.

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Vaidh et al.,

Biological Forum – An International Journal 14(4): 579-585(2022)

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