Indian J. Anim. Nutr. 2021. 38 (2): 151-159
doi: 10.5958/2231-6744.2021.00022.0
Indian Journal of Animal Nutrition

Effect of Bypass Fat Supplementation on Growth, Onset of Puberty, Biochemical Metabolites and Reproductive Parameters in Jersey Crossbred Prepubertal Heifers

Kinkar Debbarma[1*], Muthupalani Karunakaran[1], Debajyoti Sarkar[1], Mohan Mondal[2], Ajoy Mandal[3], Subrata Koloi[4], Sachin Tripura[5] and Asish Debbarma[6]
[1]Department of Animal Reproduction, ERS, ICAR-National Dairy Research Institute, Kalyani, India;
[2]Department of Animal Physiology, ERS, ICAR-National Dairy Research Institute, Kalyani, India;
[3]Department of Animal Genetics & Breeding, ERS, ICAR-National Dairy Research Institute, Kalyani, India;
[4]Division of Animal Genetics & Breeding, ICAR-National Dairy Research Institute, Karnal, India;
[5]Department of Animal Nutrition, ERS, ICAR-National Dairy Research Institute, Kalyani, India;
[6]Department of Livestock Production & Management, ERS, ICAR-National Dairy Research Institute, Kalyani, India

ABSTRACT

Bypass fat can increase the energy density of ration without having any adverse effects on feed intake and digestion. The experiment was conducted to investigate the effects of supplementation of bypass fat on the onset of puberty in dairy heifers. The trial was conducted with 12 prepubertal divided into control (C) and Treatment (T) groups. The ration for the experimental animals in control group were not supplemented with bypass fat, whereas heifers in treatment group received bypass fat at 50g/day/h for a period of 150 days. Bypass fat supplementation significantly ($P<0.05$) improved the mean live body weight, body condition score (BCS) and body fat thickness (BFT) in supplemented heifers. Bypass fat supplementation had no significant ($P>0.05$) effect on the proportion of heifers attaining puberty (50.00 *vs.* 33.33%) as well as the age at onset of puberty (605.67±22.67 *vs.* 626.50±28.50 days). There was no significant ($P>0.05$) difference observed between the control and treatment groups in the mean size of dominant follicle, CL and ovary. The overall mean uterine horn diameter during the trial period was significantly ($P<0.05$) higher in bypass supplemented group than in control group. The number of small, medium and large follicles did not vary between groups ($P>0.05$) in heifers during the study period. No significant ($P>0.05$) effect on plasma NEFA level was observed, however plasma glucose level was significantly ($P<0.05$) higher in bypass fat supplemented heifers. Hence, it can be concluded that bypass fat supplementation at 50g/day/h improved the body growth of supplemented heifers but did not had any beneficial effect on onset of puberty in heifers.

Key words: Bypass fat, Energy, Heifers, Jersey, Onset of Puberty.

INTRODUCTION

The dairy industry is dependent on dairy cow's reproductive success; since the lactation cycle is reliant on the cow's ability to become pregnant and produce milk after delivery. Nutrition is one of the many significant factors responsible for reproductive activity. Various reproductive problems, such as delayed puberty, anoestrous, repeat breeding, retained foetal membranes, abortion, weak calf syndrome, *etc.,* could eventuate due to poor nutrition in cows, thus reducing reproductive efficiency.

Malnutrition can delay the onset of puberty in dairy cattle during the heifer stage. Early maturing cows will yield more milk during their entire productive time, but delayed puberty is a big concern for dairy cows. The nutritional plan is the most significant aspect affecting the age of maturity (Poy and Panday, 1971). In the tropical zone, puberty occurs in the *Bos indicus* breeds between 16 and 40 months (McDowell *et al.,* 1976) but is associated with the body weight instead of the age of the animal. The body weight of heifers at puberty was observed to be between 55% and 60% of adult body weight (Perry, 2016). Malnourished females lack ovarian activity due to inhibition of pulsatile luteinizing hormone (LH) (Rasby and Funston, 2016). The LH pulse generation system in the hypothalamus and its

*Corresponding author: E-mail: kinkardbrma@gmail.com; Kinkar Debbarma, Department of Animal Reproduction, ERS, ICAR-National Dairy Research Institute, Kalyani, India, Pin: 741 235

pre-pubertal spike is affected by the nutritional plane (Schillo *et al.,* 1992). Nutrition influences the production and release of GnRH (gonadotrophin releasing hormone), FSH (follicle stimulating hormone), LH, and GH (Growth hormone) owing to its function on the hypothalamus and the anterior pituitary. Ovarian follicular development and steroid production are also affected by diet. Thus, greater growth rates will reduce the negative feedback of estradiol on the secretion of LH and thus promote follicular growth.

Balanced nutrition and effective management will help to promote growth and early sexual maturity (Heinrichs *et al.,* 2005). Supplementation of high energy concentrates or fats have been used as a technique to increase the energy density of the ration. However, various negative impacts of such supplementation *viz.* decrease in intake and digestion of fibre, acidosis, and chelation of minerals may be observed in such animals. To reduce the detrimental effects of high energy concentrate and fat supplementation, a specialised rumen-inert lipid, popularly known as bypass fat was developed. Bypass fat can resist lipolysis and biohydrogenation in rumen but is digested in abomasum and can help overcome negative energy balance (NEB) without negatively affecting the ruminal digestion and fermentation process. Initially bypass fat was only considered as a source of energy during transition period in dairy cows to reduce NEB and maintain milk production, however later on, it was demonstrated that the fatty acids present in bypass fat may have a positive effect on reproduction (Staples *et al.,* 1998; Rahbar *et al.,* 2014). Thus, bypass fat supplementation may provide the energy required for achieving optimum growth in growing heifers to attain puberty without any adverse effects. Hence, the main objective of this study was to determine the effects of bypass fat supplementation in crossbred Jersey heifers on the onset of puberty and reproductive parameters.

MATERIALS AND METHODS

This study was conducted in the Dairy farm complex of ICAR-National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India. Kalyani is located at the lower Gangetic basin in the Nadia district of West Bengal at an altitude of 11m above sea level. The coordinates of Kalyani are: 22° 58'30" N latitude and 88° 26'4" E longitude. The climatic condition is tropical hot and humid. The study was conducted between the months of October, 2020 to March, 2021. The average climatic conditions during the trial period were *viz.* temperature: 28.3° C (31.8-23° C), rainfall: 16.6 mm (0.00-94.1 mm), and humidity: 46 % (34-73 %).

Twelve (*n*=12) Jersey x Zebu crossbred heifers above 16 months of age with no history of observed sign of estrus and ovaries without any functional structures (corpus luteum or matured follicles), were selected and assigned to one of the two dietary treatment groups for the study. Heifers were similar in age (16.84± 0.36 months), body weight (184.54± 7.46 kg), and BCS (2.75± 0.08), and distributed equally to one of the two groups with six cows in each group: Control (non-supplemented) or treatment (supplemented) group. The trial was conducted for a period of 150 days (5 months). The BW and BCS were measured at fortnightly interval for both the groups. Body weight was recorded using a digital weight recording platform (Spider – 300, Future weighing & engineering). BCS were determined by the same individual for the entire experiment according to a 1 (thin) to 5 (obese) scale (Edmonson *et al.,* 1989). The experimental cows were dewormed and vaccinated regularly according to farm schedule. Subcutaneous back fat thickness was measured at the end of trial period by real time ultrasound (Linear ultrasonograph DIGI 1100 CD-E VET, SSMED Ltd. probe- 6.5 MHz).

All the experimental animals were housed in the experimental sheds, which was well-ventilated having cemented floor with individual feeding and watering arrangement throughout the experimental period. Proper cleanliness and healthy surroundings were ensured throughout the experimental period. All cows were fed with concentrate mixture, 20 to 30 kg of seasonally available green fodders and paddy straw according to the farm protocol. The treatment group was supplemented with bypass fat at the rate of 50 g/

animal/day for a period 05 150 days. Bypass fat was procured from ICAR- Central Coastal Agricultural Research Institute, Old Goa, Goa.

Blood samples were collected at early morning at fortnightly interval from both the groups by means of jugular vein puncture using sterile needles. The blood samples were collected into centrifuge tubes containing EDTA (10%), put in an ice bucket and carried to the laboratory within 20 minutes of collection. Blood samples were centrifuged at 3000 rpm for 30 min and plasma was separated and kept in the labelled storage vials of 5 ml capacity and stored at -20°C till analysis. Samples were later analysed for blood glucose and plasma non-esterified fatty acids (NEFA) levels. Blood glucose was analysed by spectrophotometry using AUTOSPAN® Glucose (ARKRAY, Inc.). Plasma NEFA was analysed by the copper soap extraction method modified by Shipe *et al.* (1980).

The onset of puberty in heifers was monitored by observing the behavioural signs of estrum, presence of cervico-vaginal mucus discharge, rectal palpation of genital organs and ultra-sonographic examination of ovaries. Heifers were observed for the visual signs of estrus twice in a day and the ultra-sonographic evaluation of reproductive tract was carried out at 7 days interval. Reproductive tract score (RTS) values are gauged through rectal palpation and ultrasound examination of the uterine horns and ovaries as per the method of Andersen *et al.* (1991). Puberty and sexual maturity of heifers was gauged based on ovarian development and palpable size of the reproductive tract.

Intensity of estrus in all the experimental animals was scored, based on the score card (maximum score: 100) designed by Layek *et al.* (2011). Each observation of sign of the estrus was assigned a score as per the scale and the sum of the scores was recorded. Duration of behavioural estrus in all the animals was estimated in hours from the time of first appearance of estrous to the time of detection of last estrous sign.

Ultrasound examination (Transrectal ultrasonograph DIGI 1100 CD-E VET, SSMED Ltd. probe- 9 MHz) was carried out at weekly intervals in experimental animals. Monitoring of follicular growth (small<4 mm; medium 4-8 mm and large>8 mm) was carried out. On each examination and for each animal in control and treatment groups, the diameter of uterine horn (cm) was assessed. The diameter and area of ovaries, presence of corpora lutea and dominant follicles and their sizes were also measured.

Statistical analysis

The data were captured in excel and analysed using suitable statistical package. The model contained the diet and interval as fixed parameters, two-way ANOVA was used to compare the effect of bypass fat supplementation on the mean differences between the control and treatment group. Data were presented as least square means ± standard error of the mean (SEM). The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The live body weight increased along with increasing age in both the groups throughout the trial period. The overall mean body weight during the 150 days trial period was significantly higher ($P<0.05$) in treatment (220.47±5.85 kg) group than in control (209.34±4.95 kg) group. There was no significant ($P>0.05$) difference in final body weight at the end of trial period between control (231.83±17.18 kg) and treatment (251.5±11.94 kg) group. The average daily weight gain during the 150 day trial period was non-significantly ($P>0.05$) higher in treatment group (417.78±18.65g/day) than in control group

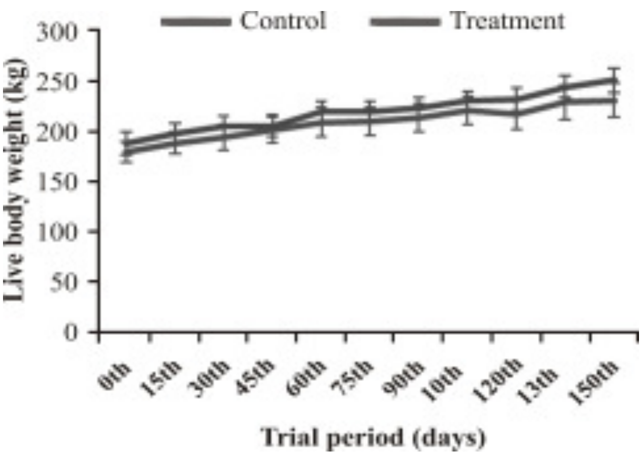

Fig 1. Live body weight (kg) of heifers during trial period (Mean ±SEM)

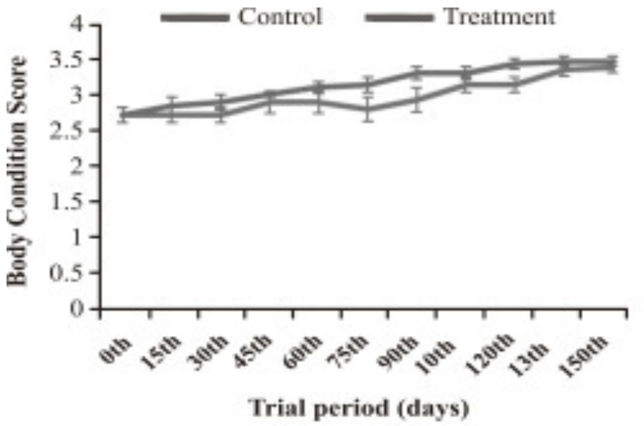

Fig 2. Body Condition Score (BCS) during trial period in experimental heifers (Mean ±SEM)

(343.89±47.94g/day). Vahora *et al.* (2012) observed significantly higher average daily gain in bypass fat supplemented growing buffalo heifers. Gajera (2013) observed significant improvement in total weight gain and average daily body weight gain in bypass fat supplemented Jaffarabadi heifers. Rosadiuk *et al.* (2021) observed that the overall, average daily gain and body weight were greater for heifers offered increased plane of nutrition during both the pre- and post-weaning phases. The ADG can be improved by increasing levels of nutrition, and in general around 750 g/day ADG is optimal for dairy heifers (Wathes *et al.,* 2014) with lower rates delaying puberty and age at first calving (AFC). In the present study, we observed the ADG to be lower than 750 g/day in both control (343.89±47.94g/day) and treatment (417.78±18.65g/day) group, which may be the reason of delayed puberty in both the groups during our study.

The overall mean BCS for 150 days period was significantly ($P<0.01$) higher in treatment group (3.18±0.08) than the control group (3.00±0.07). The average gain in BCS during the trial period was non-significantly higher in treatment group (0.75±0.09) than in control group (0.67±0.11). Campanile *et al.* (2010) found significant improvement in BCS of Murrah buffalo heifers fed high energy diet in contrast to those fed a low energy diet. Carvalho *et al.* (2013) found higher energy intake to increase the BCS of zebu heifers significantly without altering the BW at puberty.

The BFT (mm) was significantly higher ($P<0.05$) in treatment group (22.94±0.85 mm) than in control group (21.17±0.61 mm) at the end of 150th day trial period. Similar to our findings, Ortega *et al.* (2020) observed significant improvement in fat thickness in the rump region in heifers fed with high energetic-protein supplement in post-weaning period. Chelikani *et al.* (2003) observed back fat thickness to be lower in dairy heifers fed with low protein and energy containing diet in comparison to that of high protein and energy containing diet.

The overall mean RTS during the trial period of 150 days was similar ($P>0.05$) in both treatment (3.42±0.08) and control (3.32±0.11) group. RTS was developed as an indirect technique of predicting pubertal age and an indication of heifer nutritional requirements before the breeding season (Anderson *et al.* 1991). Heifers were assigned a score ranging from 1 to 5 for their reproductive tract. A RTS of one suggested an immature, non-cycling reproductive system, whereas an RTS of five suggested a cycling heifer with a functional corpus luteum. On the final trail day (150th day) of the experiment, two heifers in the control group and three heifers in the treatment group had an RTS of 5, suggesting cyclicity with a functional corpus luteum. Parallel to our findings, Speer *et al.* (2020) observed that increased energy diet supplementation had no effect on heifer RTS and that all heifers showed similar reproductive maturity near

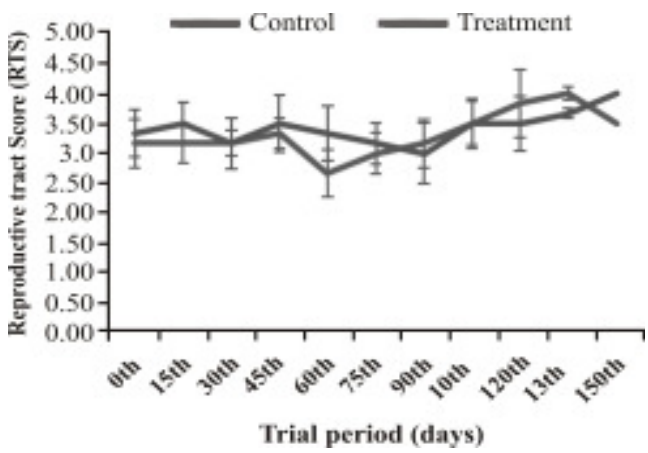

Fig 3. Reproductive tract score (RTS) in experimental heifer groups (Mean ±SEM)

the breeding season. Rosasco *et al.* (2017) found that increasing nutritional supplementation had no effect on RTS in beef heifers.

The average numbers of specific type of follicles based on size (small<4 mm; medium 4-8 mm and large>8 mm) at a given interval throughout the trial period was observed using ultrasound examination. The overall mean number of small follicles was 6.05±0.46 and 5.67±0.36 in control and treatment groups, respectively. The overall mean number of medium follicles was 4.83±0.24 and 4.98±0.37 in control and treatment groups, respectively. The average number of large follicles was 0.91±0.07 and 0.92±0.08 in control and treatment groups, respectively. There was no significant (P>0.05) difference in number of small, medium as well as large follicles between the groups. Similar to our findings, Rosasco *et al.* (2017) found no effect of increased nutritional supplementation on antral follicle count (AFC) in beef heifers, which is consistent with our findings. Stokes *et al.* (2018) observed that trace mineral supplementation had little influence on the AFC of Angus breed heifers. Speer *et al.* (2020) found that supplementing a higher calorie diet had no effect on total follicle count in heifers across treatment groups. The similarity in follicular growth between groups can be attributed to the absence of an energy shortage in the research animals (Mattos *et al.* 2000).

The mean maximum diameter of the follicles (mm) during the 150 day trial was almost similar (P>0.05)

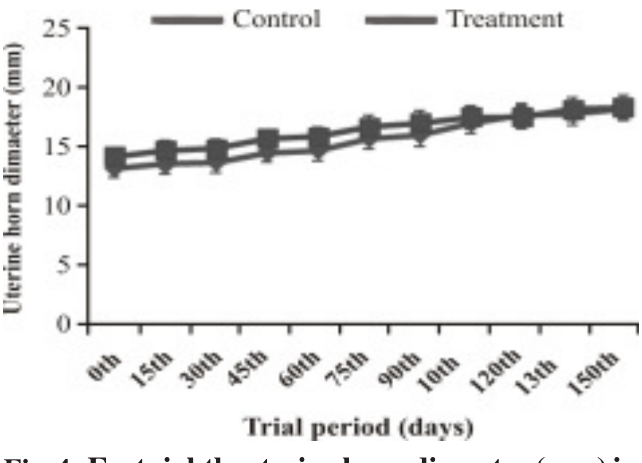

Fig 4. Fortnightly uterine horn diameter (mm) in experimental heifers (Mean ±SEM)

between treatment (12.67±0.99 mm) group and control group (12.00±0.97 mm). The overall mean area of the largest follicle (mm^2) during the 150 days trial was slightly higher in treatment (434.89±77.06) group than in control group (391.98±58.28), however the difference was non-significant (P>0.05). In a study on postpartum buffaloes, Ganie (2011) observed no significant effect of bypass fat supplementation on the largest follicle and preovulatory follicle diameter; nevertheless, it was improved in contrast to the control. Similarly, Petit *et al.* (2002) and Katiyar *et al.* (2017) observed that supplementing with bypass fat had no effect on the size of dominant follicles in postpartum buffalo cows.

The overall mean ovarian diameter (mm) during the 150 day trial was insignificantly higher (P>0.05) in treatment (20.70±0.39) group than in control group (20.12±0.39). Similarly, the mean area of the ovary (mm^2) during the 150 day trial was higher in treatment (974.54±33.94) group than in control group (939.72±36.17), however the difference was non-significant (P>0.05). In support of the present findings, Freetly *et al.* (2014) found that diet had no influence on ovarian weight and size in peripubertal heifers. Speer *et al.* (2020) observed that increased energy diet supplementation had no effect on ovarian length and height in heifers across treatment groups.

The overall mean uterine horn diameter during the 150 day trial period was significantly (P<0.05) higher in treatment group (16.39±0.40 mm) than in control group (15.62±0.56 mm). The uterine horns of the prepubertal heifer are often flaccid and undeveloped. However, as puberty approaches and in response to increasing stimulation by ovarian estrogens, the uterus and cervix become bigger and display more smooth muscle tone when palpated manually (De Carvalho, 2014). Akhtar (2002) observed a substantial improvement in uterine horn diameter in Nili-Ravi buffalo heifers fed a greater amount of dietary energy. Summers *et al.* (2012) recorded increased uterine horn diameter in heifers administered a corn gluten feed-based supplement throughout development period.

The heifers were monitored for onset of puberty by observing the estrus signs and development of corpus luteum (CL) in ovaries during 150 days trial period. Out of 6 heifers, 2 in control group and 3 in treatment group attained puberty during the trial period. The difference in percentage of heifers that attained puberty was insignificant (P>0.05) between treatment (50.00%) and control group (33.33%). The age at onset of puberty (days) was insignificantly (P>0.05) lower in treatment group heifers (605.67±22.67 days) than in control heifers (626.50±28.50 days).

Using the concept of "targeted body weight," nutritional management involving continuous, high rates of growth is one approach for promoting the timely onset of sexual maturity. As thumb rule, this strategy sets a target of 60 to 65 percent of mature body weight (BW) for individual heifers to have achieved puberty. Feeding heifers a high-concentrate diet during crucial developmental windows causes alterations in metabolic endocrine state, as seen by increased circulatory concentrations of leptin, insulin, and IGF-1, which can trigger the onset of puberty months later (De Carvalho *et al.,* 2014; Speer *et al.,* 2020). The results of our current investigation show that bypass fat supplementation has no influence on the age at pubertal onset in prepubertal heifers. Similar to the current observation, Lammoglia *et al.* (2000) fed dietary fat to beef heifers and found no effect on pubertal age, but did found an effect on the proportion of heifers reaching puberty. Chelikani *et al.* (2003) provided prepubertal Holstein heifers with diets differing in dietary

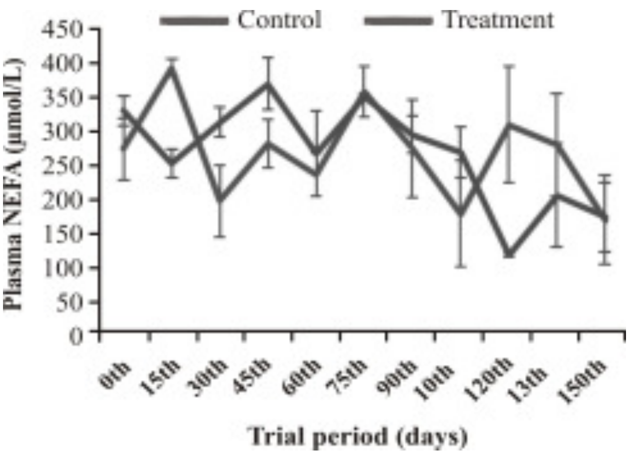

Fig 5. Plasma NEFA (µmol/l) during trial period in experimental heifers (Mean ±SEM)

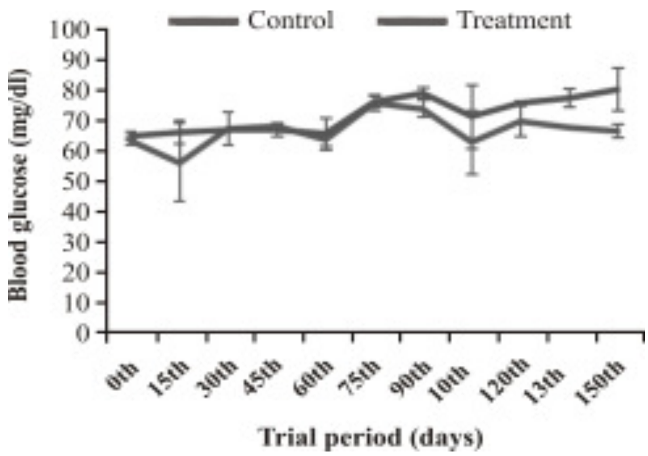

Fig 6. Blood glucose (mg/dl) during trial period in experimental heifers (Mean ±SEM)

energy and protein density, but observed that dairy heifers achieved puberty with consistent body weight and body composition independent of dietary modification. Shike *et al.* (2013) investigated the effect of supplementary fat on beef heifers but found no change in the proportion of pubertal heifers. Manthey *et al.* (2017) fed dairy heifers with distillers' dry grain of high fat content but found no effect of diet on heifer age and body weight at puberty. Oliveira *et al.* (2008) opined that the influence of a greater protein and energy diet on the age at puberty was unclear; however exposing heifers to a male significantly lowered the age at puberty in heifers.

The estrus intensity score (P>0.05) in heifers attaining puberty was similar in both treatment (56.00±1.15) and control group (55.50±1.50). Layek *et al.* (2011) reported that the estrus intensity score in *Bos indicus* breeds is generally around 50%, which was similar to the findings of the present study. Mucus discharge was observed as the most commonly expressed estrus behaviour in heifers attaining puberty. The duration of estrus (hours) in treatment group (21.33±4.81) and control group (18±6.00), did not differ statistically (P>0.05).

The mean blood NEFA level during 150 day trial period was similar (P>0.05) in both treatment group (267.92±23.26 µmol/l) and control group (268.76±21.23 µmol/l) and did not differ significantly. There have been conflicting studies of plasma NEFA levels in dairy cows fed with bypass fat. Manriquez *et al.* (2019) found that bypass fat supplementation had no effect on NEFA levels in supplemented cows. Postpartum cows given bypass fat had significantly lower plasma NEFA levels, according to Singh *et al.* (2016) and Nirwan *et al.* (2019). However, Dhami *et al.* (2017) and Ranaweera *et al.* (2019) observed an elevated level of plasma NEFA in bypass fat supplemented postpartum cows. Jorritsma *et al.* (2003) observed the plasma NEFA levels to be increased in heifers subjected to acute fasting indicating NEFA level rises in case of negative energy balance.

The mean blood glucose level during 150 day trial period was significantly ($P<0.01$) higher in treatment group (72.04±1.81 mg/dl) than in control group (66.98±1.65 mg/dl). Similar to our findings, Yelich *et al.* (1996) observed plasma glucose levels to be higher in heifers fed to high-gain in comparison to those fed to low-gain diet. Campanile *et al.* (2010) found significantly greater concentration of glucose in Murrah buffalo heifers fed high energy diet in contrast to those fed a low energy diet. Cappellozza *et al.* (2014) observed that beef heifers supplemented with cracked corn, soybean meal and urea had higher glucose levels in contrast to heifers without any supplementation. Ortega *et al.* (2020) observed significant increase in the mean glucose level in heifers supplemented with high levels of energy and protein supplement in the post-weaning period. Rosadiuk *et al.* (2021) observed overall glucose level to be higher in heifers fed with high plane of nutrition in both preweaning as well as postweaning period. The increase in glucose level in dietary fat supplemented animals might be due to glucose sparing effect of dietary fatty acids (Voigt *et al.,* 2005). Additionally, fat feeding is known to induce insulin resistance therefore leading to increase in glucose level of animals fed with dietary fats (Palmquist and Moser, 1981).

CONCLUSIONS

Based on the present study it was concluded that, bypass fat supplementation at 50 g/day over a period of 150 days improved the body growth and condition in supplemented heifers. However, supplementation of bypass fat at the above mentioned level did not have any significant effect on reproductive tract development and onset of puberty in prepubertal heifers. Further studies on bypass fat supplementation on prepubertal heifers at higher inclusion level, larger sample size, different age group and trial interval can be done to further validate the effect of bypass supplementation on onset of puberty in prepubertal heifers.

ACKNOWLEDGEMENTS

The author would like to thank The Director, ICAR-National Dairy Research Institute, Karnal, Haryana and Head, ERS, ICAR-National Dairy Research Institute, Kalyani, West Bengal for facilitating the research.

REFERENCES

Akhtar, M.S. 2002. Determination of pubertal age in Nili-Ravi buffalo heifers under two different feeding regimens. University of Veterinary and Animal Science, Lahore, Pakistan.

Andersen, K.J., LeFeverF, D.G., Brinks, J.S. and Odde, K.G. 1991. The use of reproductive tract scoring in beef heifers. *Agric. Practice*. 12: 19-26.

Campanile, G., Baruselli, P.S., Vecchio, D., Prandi, A., Neglia, G., Carvalho, N.A.T., Sales, J.N.S., Gasparrini, B. and D'Occhio, M.J. 2010. Growth, metabolic status and ovarian function in buffalo (Bubalus bubalis) heifers fed a low energy or high energy diet. *Anim. Reprod. Sci.* 122: 74-81.

Cappellozza, B.I., Cooke, R.F., Reis, M.M., Moriel, P., Keisler, D.H. and Bohnert, D.W. 2014. Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: II. Performance, reproductive, and metabolic responses of replacement heifers. *J. Anim. Sci.* 92: 2725-2734.

Carvalho, M.V., Diniz-Magalhães, J., Pereira, A.S.C., Santos, M.V. and Silva, L.F.P. 2013. Effect of chronic infusion of leptin and nutrition on sexual maturation of zebu heifers. *J. Anim. Sci.*, 91: 1207-1215.

Chelikani, P.K., Ambrose, J.D. and Kennelly, J.J. 2003. Effect of dietary energy and protein density on body composition, attainment of puberty, and ovarian follicular dynamics in dairy heifers. *Theriogenology.* 60: 707-725.

De Carvalho Cardoso, R. 2014. *Nutritional Programming of Puberty in Beef Heifers* Doctoral dissertation, Texas A

&M Universityu, Texas, USA.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G. 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72: 68-78.

Freetly, H.C., Vonnahme, K.A., McNeel, A.K., Camacho, L.E., Amundson, O.L., Forbes, E.D., Lents, C.A. and Cushman, R.A. 2014. The consequence of level of nutrition on heifer ovarian and mammary development. *J. Anim. Sci.* 92: 5437-5443.

Gajera, A. 2013. *Effect of Supplementing Rumen Bypass Nutrients on Growth of Jaffarabadi Heifers* Doctoral Dissertation, JAU, Junagadh.

Ganie, M.A. 2011. *Enhancing Post-Partum Fertility with Supplementary Feeding of Glucogenic Precursor (Calcium Propionate) and Rumen Innert Fat Supplement in Dairy Buffaloes* Doctoral Dissertation, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Heinrichs, A.J., Heinrichs, B.S., Harel, O., Rogers, G.W. and Place, N.T. 2005. A prospective study of calf factors affecting age, body size, and body condition score at first calving of Holstein dairy heifers. *J. Dairy Sci.* 88: 2828-2835.

Jorritsma, R., De Groot, M.W., Vos, P.L.A.M., Kruip, T.A.M., Wensing, T. and Noordhuizen, J.P.T.M. 2003. Acute fasting in heifers as a model for assessing the relationship between plasma and follicular fluid NEFA concentrations. *Theriogenology.* 60: 151-161.

Katiyar, G.S., Sharma, R.K., Mudgal, V., Jerome, A., Phulia, S.K. and Singh, I. 2017. Fertility response in post-partum buffaloes following bypass nutrient supplementation. *Indian J. Anim. Sci.* 87: 960-964.

Lammoglia, M.A., Bellows, R.A., Grings, E.E. and Bergman, J.W. 1999. Effects of prepartum supplementary fat and muscle hypertrophy genotype on cold tolerance in newborn calves. *J. Anim. Sci.* 77: 2227-2233.

Layek, S.S., Mohanty, T.K., Kumaresan, A., Behera, K. and Chand, S. 2011.Behavioural signs of estrus and their relationship to time of ovulation in Zebu (Sahiwal) cattle. *Anim. Reprod. Sci.*, 129: 140-145.

Manriquez, D., Chen, L., Melendez, P. and Pinedo, P. 2019. The effect of an organic rumen-protected fat supplement on performance, metabolic status, and health of dairy cows. *BMC Vet. Res.* 15: 1-14.

Manthey, A.K., Anderson, J.L., Perry, G.A. and Keisler, D.H. 2017. Feeding distillers dried grains in replacement of forage in limit-fed dairy heifer rations: Effects on metabolic profile and onset of puberty. *J. Dairy Sci.* 100: 2591-2602.

Mattos, R., Staples, C.R. and Thatcher, W.W. 2000. Effects of dietary fatty acids on reproduction in ruminants. *Rev. Reprod.* 5: 38-45.

McDowell, R.E., Hollon, B.F., Camoens, J.K. and Van Vleck, L.D. 1976. Reproductive efficiency of Jerseys, Red Sindhis, and crossbreds. *J. Dairy Sci.* 59: 127-136.

Nirwan, S.S., Mehta, J.S., Kumar, A., Kumar, P., Kumar, A. and Singh, V. 2019.Effects of bypass fat on postpartum reproductive performance in dairy cattle. *Indian J. Dairy Sci.* 72: 194-200.

Oliveira, C.M.G., Oliveira Filho, B.D., Gambarini, M.L., Viu, M.A.O., Lopes, D.T. and Sousa, A.P.F. 2009. Effects of biostimulation and nutritional supplementation on pubertal age and pregnancy rates of Nelore heifers (*Bos indicus*) in a tropical environment. *Anim. Reprod. Sci. 113*: 38-43.

Ortega, R.M., Paulino, M.F., Detmann, E., Rennó, L.N., Moreno, D.S., Márquez, D.C., de Almeida, D.M., de Melo, L.P. and Moura, F.H. 2020. Nutritional strategies for heifers under grazing system: productive and nutritional performance, metabolic profile and ovarian activity. *Trop. Anim. Health Prod.* 52: 1013-1022.

Palmquist, D.L. and Moser, E.A. 1981. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. *J. Dairy Sci.* 64: 1664-1670.

Perry, G.A. 2016. Factors affecting puberty in replacement beef heifers. *Theriogenology.* 86: 373-378.

Petit, H.V., Dewhurst, R.J., Scollan, N.D., Proulx, J.G., Khalid, M., Haresign, W., Twagiramungu, H. and Mann, G.E. 2002. Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats. *J. Dairy Sci.* 85: 889-899.

Poy, K. and Panday, R.D. 1971. Effect of higher levels of protein on production of milk and reproductive performance in lactating buffaloes. *Indian J. Anim. Nutr.* 5: 52-57.

Rahbar, B., Safdar, A.H.A. and Kor, N.M. 2014. Mechanisms through which fat supplementation could enhance reproduction in farm animal. *Eur. J. Exp. Biol.* 4: 340-348.

Rasby, R.J. and Funston, R.N. 2016. Invited review: Nutrition and management of cows: Supplementation and feed additives. *Prof. Anim. Sci*. 32: 135-144.

Rosadiuk, J.P., Bruinjé, T.C., Moslemipur, F., Fischer-Tlustos, A.J., Renaud, D.L., Ambrose, D.J. and Steele, M.A. 2021. Differing planes of pre-and postweaning phase nutrition in Holstein heifers: I. Effects on feed intake, growth efficiency, and metabolic and development indicators. *J. Dairy Sci.* 104: 1136-1152.

Rosasco, S.L., Scholljegerdes, E.J., Cox, S.H., Dunlap, R.C., Hallford, D.M. and Summers, A.F. 2017. Influence of increased nutrient intake before and after breeding on performance and reproductive efficiency of beef heifers. ASAS Western Section, 162.

Schillo, K.K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. *J. Anim. Sci.* 70: 1271-1282.

Shike, D.W., Ireland, F.A. and Faulkner, D.B. 2013. Influences of supplemental fat, differing in fatty-acid composition, on performance, plasma fatty-acid content, and reproduction of developing beef heifers. *Prof. Anim. Sci.* 29: 580-586.

Shipe, W.F., Senyk, G.F. and Fountain, K.B. 1980. Modified copper soap solvent extraction method for measuring free fatty acids in milk. *J. Dairy Sci.* 63: 193-198.

Singh, M., Yadav, G., Roy, A.K. and Thakur, S. 2016. Productive performance and metabolic hormonal profile in cows supplemented with prilled fat. *Indian J. Tradit. Knowl.* 15: 292-296.

Speer, H., Riley, H., Cushman, R., Freetly, H. and Drewnoski, M. 2020. November. Alternative heifer development systems utilizing corn residue and cover crops. *J. Anim. Sci.* 98: 128-128.

Staples, C.R., Burke, J.M. and Thatcher, W.W. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *J. Dairy Sci.* 81: 856-871.

Vahora, S., Parnerkar, S. and Kore, K.B. 2012. Effect of feeding bypass nutrients to growing buffalo heifers under field conditions. *Livest. Res. Rural Dev.* 24: 39.

Voigt, J., Gaafar, K., Kanitz, W., Precht, D., Becker, F., Schneider, F., Spitschak, M., Schönhusen, U., Junghans, P., Aschenbach, J.R. and Gäbel, G. 2005. Utilization of glucose and long-chain fatty acids in lactating dairy cows fed a fat-enriched diet. DTW. *Deutsche Tierarztliche Wochenschrift*. 112: 423-425.

Wathes, D.C., Pollott, G.E., Johnson, K.F., Richardson, H. and Cooke, J.S. 2014. Heifer fertility and carry over consequences for life time production in dairy and beef cattle. *Animal*. 8:91-104.

Yelich, J.V., Wettemann, R.P., Marston, T.T. and Spicer, L.J. 1996. Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates. *Domest. Anim. Endocrinol.* 13: 325-338.

Received on 02.08.2021 and accepted on 11.09.2021