



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2022; SP-11(8): 1455-1462  
© 2022 TPI  
[www.thepharmajournal.com](http://www.thepharmajournal.com)  
Received: 02-05-2022  
Accepted: 07-06-2022

**Dr. Mohit Mahajan**

Ph.D. Scholer, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

**Dr. Shiv Prasad**

Professor, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

**Dr. CB Singh**

Professor, Department of Veterinary Animal Genetics and Breeding, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

**Dr. Sunil Kumar**

Associate Professor, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

**Shivanshu Tiwari**

Senior Research Fellow, Department of Veterinary Animal Genetics and Breeding, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

**Dr. Gourav Tevatai**

MVSc Scholer, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

**Corresponding Author**

**Dr. Mohit Mahajan**

Ph.D. Scholer, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand, India

## Effect of supplementing bypass protein, bypass fat, rumen protected niacin and choline on body condition score, body weight and estrus expression in pre-pubertal frieswal heifers

**Dr. Mohit Mahajan, Dr. Shiv Prasad, Dr. CB Singh, Dr. Sunil Kumar, Shivanshu Tiwari and Dr. Gourav Tevatai**

### Abstract

Early estrus expression and early conception is the goal of all farmers rearing pre-pubertal heifers. The current experiment was designed with the objective to reduce the age of the first estrus increasing the overall lifetime productivity. Pre-pubertal heifers were fed the experimental diet composed of bypass protein, bypass fat, rumen-protected choline, rumen-protected niacin and probiotics. The addition of the experimental diet led to a significant improvement in the body condition score for the heifers starting from the first month, there was also an early accomplishment of ideal 3.5 BCS in the experimental heifers. The body weight of heifers during the experiment recorded a significant improvement during the experiment in the treated group. The weight gain was significantly more in the treated group than in the control group at all stages of the experiment. The estrus induction rate was high and related to the increased weight gain and BCS, in comparison to the control group. It was recorded that the average age of estrus induction was reduced by two months in the experimentally fed heifers.

**Keywords:** Bypass fats, bypass proteins, niacin, choline, estrus induction, frieswal

### Introduction

Over the last five decades, animals across India have undergone up-gradation in their genetic potential by the virtue of first-generation biotechnological advancement that is artificial insemination, howbeit the animals fail to achieve their full productivity potential, this is directly related to the quantity and quality of feed consumed for the fulfilment of nutritional demand of production and reproduction. The bulk of livestock in tropical nations such as India, get access to low-quality grasses, crop leftovers, and agro-industrial by-products. Puberty is defined as the period when sexual organs are functionally active and developed, demonstrating emblematic ovulation with plasma progesterone concentrations of more than 1 ng/ml from the developed corpus luteum (Evans *et al.*, 1995) [12]. In heifer, maturity is governed by body weight rather than age. Underfeeding or an imbalanced feed composition causes a reduced growth rate. The addition of low energy and protein diet to animals leads to a decrease in lifetime productive and reproductive performance, especially delayed puberty and sexual maturity (Ranjan *et al.*, 2012) [34]. Thus, the present study has been planned with the objective to reduce the age of puberty and sexual maturity under field conditions with nutritional intervention.

### Materials and Methods

The present experiment was conducted on cattle under field conditions covering animals from the Tarai region of Udham Singh Nagar, Uttarakhand, India and at Instructional Dairy Farm (IDF), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Sixty pre-pubertal heifers (Frieswal cattle) 18 months old, were randomly enrolled in this study and maintained under the AICRP project with the farmers and ten pre-pubertal heifers (Frieswal cattle) 18 months old were randomly selected and enrolled in the study from the IDF, Pantnagar, Uttarakhand, in order to analyse animals under controlled farm conditions. These cows were kept under natural light, housed in a concrete or dry sheds and covered in free stalls. Green fodder ad-labium, dry fodder, concentrate and area-specific mineral supplementation were adjusted to cover the cows' requirements of maintenance and production that were formulated to meet or exceed their requirements.

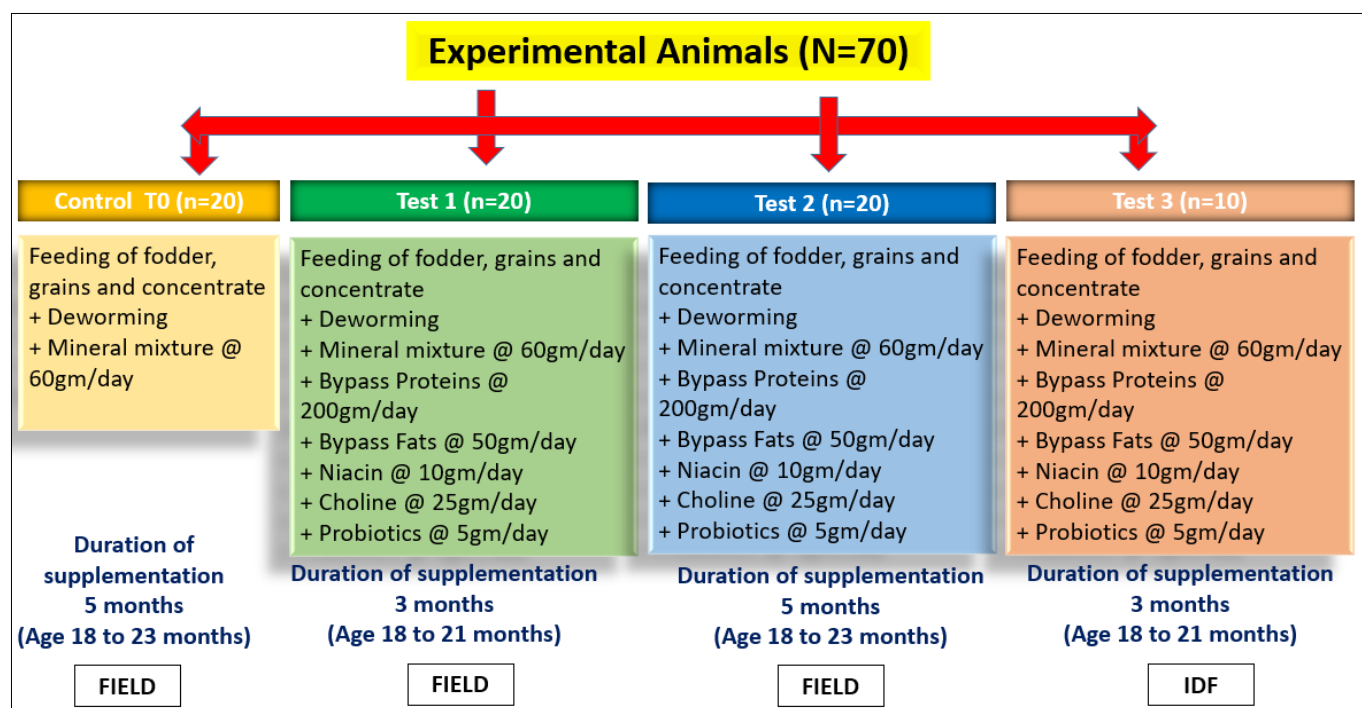
Cows had free access to clean drinking water. These heifers selected were of sound health with no apparent clinical signs of any disease or deficiency. Vaccinated against Haemorrhagic Septicaemia (HS), Black Quarters (BQ) and Foot and Mouth Disease (FMD) according to the state disease prevention policy. Deworming for Ecto and endo parasites was done with NILZAN super (6.0% w/v Oxytoclozanide BP (vet), 3.0 w/v Levamisole Hydrochloride BP (vet), 0.764% Cobalt Sulphate administered @ 1ml/3kg body weight orally) and with HITEK, Virbac Animal Health India Pvt Ltd (Ivermectin IP, 10 mg administered @ 1 ml/50 kg body weight S/C) before the initiation of the experiment and repeated every 3 months

All the animals were subjected to estrous detection twice a day and artificially inseminated on the basis of the AM-PM rule (Dransfield *et al.*, 1998) [9] using Frieswal semen straws. The experimental supplement was formulated by mixing bypass protein, bypass fat, rumen protected choline, rumen protected niacin and probiotics mixed in pre-determined quantities (Table 1).

**Table 1:** Composition of experimental diet fed to the cows

S. No.	Attributes	Quantity administered / day
1	Bypass Proteins	200 gm/day
2	Bypass Fats	50 gm/day
3	Rumen Protected Niacin	10 gm/day
4	Rumen Protected Choline	25 gm/day
5	Probiotics	5 gm/day

Heifers were divided randomly into four groups Test 1, Test 2, Test 3 and Control of 20, 20, 10 and 20 heifers respectively. Test groups 1, 2 and 3 were treated with the formulated experimental diet, for a period of three months (18-21 months of age) five months (18-23 months of age) under field conditions and for three months (18-21 months of age) under farm conditions respectively. Both the test groups and the control group were supplemented with mineral mixture @ 60 gm/day along with green fodder, grains and concentrate. (Fig 1)



**Fig 1:** Administration of treatment diet to prepubertal female cows Test 1, Test 2, Test 3 and Control T0 groups

**Parameters studied**

The body condition score of the animal was evaluated as per Paul *et al.*, 2020 [31]. Body condition score was recorded every month for all cows in control and experimental groups before, during and after the experimental period.

The body weight of the cows was calculated using Schaeffer’s formula, the equation used for calculating live weight was  $W = (L \times G^2)/300$ , where W is body weight in lbs, L is the length of the animal from point of shoulder to pin bone in inches, and G is the chest girth of the animal in inches. The final weight was converted into kg (Wangchuk *et al.*, 2018) [45].

Expression of estrus was recorded with behavioural signs of estrus, cows stand to be mounted by other cows, reduction in feed and water consumption; attempt to mount other cows, mucus discharge, sniffing the genitalia of other cows, swelling and reddening of the vulva, chin resting and back rubbing of another cow and increased vocalization. (Röttgen *et al.*, 2018) [37]

**Result and Discussion**

Nutrition is considered to be the major factor controlling the early onset of puberty and sexual maturity (Heinrichs *et al.*, 2005) [22]. It has been proven over the years of scientific experimentation that extremes of body condition, such as <1.5 or >4, will invariably lead to poor reproductive performance (Roche *et al.*, 2009) [35]. A lower body condition score is associated with delayed estrous, higher incidence of anestrums and anovulatory cycles (Ferguson *et al.* 1994) [13], reducing the overall reproductive efficiency (Roche *et al.*, 2009) [35] of cattle. Similarly, sexual maturity in dairy heifers is determined by the body weight rather than the age of the heifer (Gupta *et al.*, 2016; Paul *et al.*, 2020) [20, 31]. Underfeeding and imbalanced feed composition is the leading cause of poor growth rate in field conditions (Gupta *et al.*, 2016) [20] and thus delayed maturity. It is also reported that the Body weight changes do not always indicate the energy status of dairy cows (Schröder and Staufenbiel, 2006) [39], as they

are affected by rumen fill (Berry *et al.*, 2006) [4]. With the aim to reduce the age of estrus in Frieswal heifers, it was reported that Frieswal cattle have an average age at first estrous close to 22 months (Kumar *et al.* 2018) [26].

**Body condition score**

The current study conducted in the field and under controlled farm conditions recorded that the feeding of the experimental diet resulted in significant improvement in the body condition score of the heifers (T1, T2 & T3) starting from the first month of feeding up to the end of the trial, as compared to the T0 control group (Table 2, Figure 8). This states that with the attainment of prerequisite BCS the animals attained early weight leads to sexual maturity which is manifested by fertile estrus, therefore this can be related to early estrous expression recorded in these heifers (Table 4). Similar to the current study, a direct correlation has been reported between the BCS and reproduction (Galina and Arthur, 1998) [15]. Gowda and Devaraj, (2019) [1] also reported that the body condition score (BCS) is an interpretive measure of body tissue reserves that are generally used to monitor energy balance. They can be considered effective indicators for health, reproductive performance and positive metabolic equilibrium of cattle.

BCS of 3.5 is considered most optimum for reproduction and production performance in heifers and adult cattle (Cargile and Tracy, 2021) [5]. It was recorded that the experimental heifers attained nearly ideal BCS of 3.5 by the 4<sup>th</sup> month (3.53 ± 0.11) in the T2 group and by the 2<sup>nd</sup> month (3.55 ± 0.10) in heifers of the T1 group under field conditions, marking that with attaining BCS score most of the heifers in the experiment were in estrus and the conception was high (Table 4). The control group failed to achieve this score even by the end of the trial (3.33 ± 0.15) (Table 2, Figure 2). It has been reported by other scientists that a BCS of less than 2 will have poor productive and reproductive potential. Those with BCS of 3 to 4 during their reproductive phase, pregnancy and calving are expected to be good. Elevated BCS greater than 4 up to 5 are obese and are associated with delayed conception, abortion and extending the days for initiation of lactation. (Cargile and Tracy, 2021) [5]

When compared to the initial body condition score at the

beginning of the trial, a significant increase in the body condition score of heifers was recorded by the 3<sup>rd</sup> month in the T1 Group (3.35 ± 0.10) and T2 Group (3.75 ± 0.10) as well as that at the end of the 4<sup>th</sup> month (3.25 ± 0.13) in T2 experimental group. The body condition score for all groups that were fed the experimental diet was greater than as compared to the T0 control group (Table 2, Figure 2). By the end of the trial, it was recorded that the overall body condition score was significantly higher in all groups fed the experimental diet (T1, T2, T3 Groups) as compared to the T0 (control group) at all stages of sampling and recording

With a higher plane of nutrition, feeding of energy-rich feed that by passes ruminal digestion and prevents loss of nutrients during digestion. Additionally, the active availability of niacin and choline in the gut actively contributes to ruminal and hepatic function (Roche, 2006) [35]. This led to the attainment of ideal BCS, early puberty, early ovarian cyclicity and early sexual maturity in these experimentally fed groups. A similar result of preponing puberty by 4 to 6.5 months was reported with the early gain in body weight (Gasser *et al.*, 2006; Sharma, 2011) [18, 41]. Simultaneously, early gain in body weight help in early puberty and an increase in the weight of the ovaries, uterus and teats. It has been reported that a low plane of nutrition leads to low BCS and NEB preventing the development of the corpus luteum via inhibiting the pulsatile release of the luteinizing hormone thereby hindering the overall ovarian function (Grans worthy, 2007; Paul *et al.*, 2020) [31],

All the groups of heifers fed the experimental diet demonstrated a progressive improvement in the body condition score during the trial with Heifers of the T1 and T2 Groups recording significant improvement in the BCS as compared to its precursive examination during the experiment i.e. (3.13 ± 0.09 to 3.75 ± 0.10) and (2.58 ± 0.08 to 3.73 ± 0.09) respectively whereas T3 group reported a non-significant numeric improvement from BCS of 3.56 ± 0.13 to 4.28 ± 0.09 (Table 2, Figure 2). Similarly, it was reported that those cows with low BCS at breeding age and during breeding periods had reduced estrous expression (Heinrichs *et al.*, 2017) [23] and reduced fertility even with protocols of timed artificial insemination (Moreira *et al.*, 2000) [28].

**Table 2:** Body Condition Score of Heifers fed experimental diet in comparison to the Control Heifers in the field and controlled conditions

Body Condition Score (BCS)	Field conditions			Controlled conditions
	T <sub>0</sub> Group	T <sub>1</sub> Group	T <sub>2</sub> Group	T <sub>3</sub> Group
	Heifers Control (n=20)	Heifers fed 3 months (n=20)	Heifers fed 5 months (n=20)	IDF Heifers fed 3 months (n=10)
Pre-Treatment	2.68 ± 0.15 <sup>a,x</sup> (2.00-4.00)	3.13 ± 0.09 <sup>n,x</sup> (2.50-3.50)	2.58 ± 0.08 <sup>m,x</sup> (2.00-3.00)	3.56 ± 0.13 <sup>b,n</sup> (3.00-4.00)
1 month Post-treatment	2.88 ± 0.14 <sup>a</sup> (2.00-4.00)	3.32 ± 0.10 (2.40-4.00)	2.88 ± 0.10 <sup>m,q</sup> (2.00-3.50)	3.78 ± 0.12 <sup>b,n</sup> (3.50-4.50)
2 months Post-treatment	3.03 ± 0.12 <sup>a</sup> (2.00-4.00)	3.55 ± 0.10 (3.00-4.00)	3.05 ± 0.09 <sup>g,m</sup> (2.50-4.00)	4.11 ± 0.11 <sup>b,n</sup> (3.50-4.50)
3 months Post-treatment	3.18 ± 0.14 <sup>a</sup> (2.50-4.00)	3.75 ± 0.10 <sup>b,y</sup> (3.00-4.50)	3.35 ± 0.10 <sup>m,y</sup> (2.50-4.00)	4.28 ± 0.09 <sup>b,n</sup> (4.00-4.50)
4 months Post-treatment	3.25 ± 0.13 <sup>y</sup> (2.50-4.00)	*	3.53 ± 0.11 <sup>y,r</sup> (2.50-4.50)	*
5 months Post-treatment	3.33 ± 0.15 <sup>y</sup> (2.50-4.50)	*	3.73 ± 0.09 <sup>h,r,y</sup> (3.00-4.50)	*

Figures with different superscripts (x, y) within a column for a particular parameter differ significantly (P<0.01)

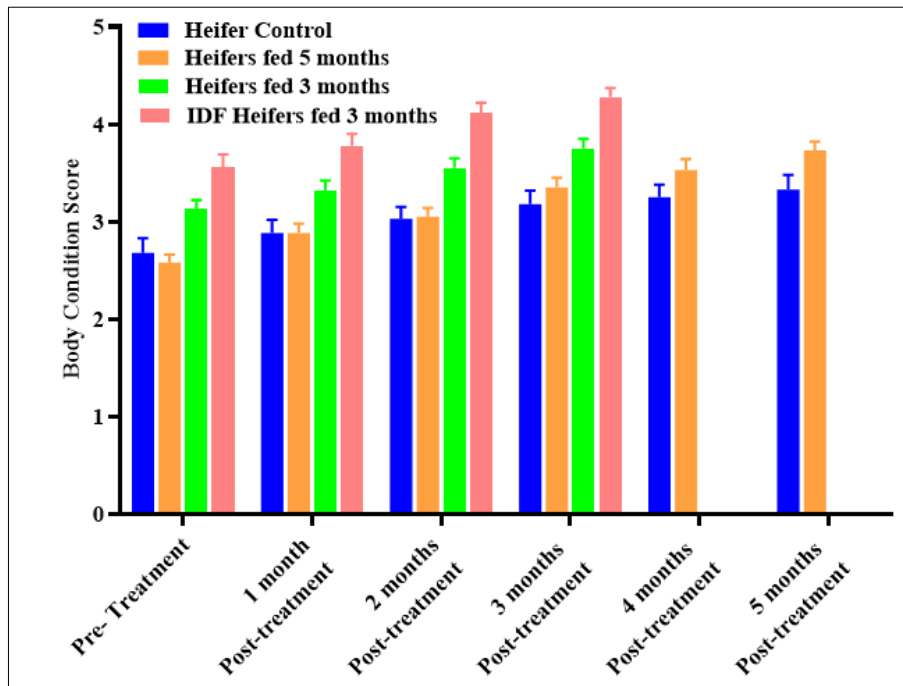
Figures with different superscripts (q, r) within a column for a particular parameter differ significantly (P<0.01)

Figures with different superscripts (g, h) within a column for a particular parameter differ significantly (P<0.01)

Figures with different superscripts (a, b) within a row for a particular parameter differ significantly (P<0.01)

Figures with different superscripts (m, n) within a row for a particular parameter differ significantly (P<0.01)

\*Could not be sampled



**Fig 2:** Body Condition Score of Heifers fed experimental diet under field conditions and controlled farm conditions compared to the Control Heifers

Heifers (T3 Group) fed experimental diet under controlled conditions recorded the most significant improvement in the BCS ( $4.28 \pm 0.09$ ) but also had a significantly higher BCS from the beginning of the trial ( $3.56 \pm 0.13$ ) compared to the T0 control group (Table 2, Figure 2). This is correlated to the balanced and superior quality of ration fed along with regular deworming and mineral supplementation provided to the heifers of the T3 Group at the IDF farm under controlled management. A good BCS help in attaining superior fertility and prevent metabolic failure (Paul *et al.*, 2020) [31] and vice versa (Roche *et al.*, 2009) [35].

It has been proven that the addition of niacin and choline prevents over-fattening of the heifers, BCS > 4 (Cargile and Tracy, 2021) [5], as over-conditioned cows have a higher incidence of infertility. Niacin stimulates the adipose–circulatory–hepatic axis and prevents various metabolic diseases in cattle (Yuan *et al.*, 2012) [47]. Choline is a key nutrient for maintaining a normal concentration of fat in the liver as it is chiefly involved in fatty acid transport (McGuffey, 2017) [27]. It also promotes the formation of low-density lipoproteins (VLDL) to remove the excess NEFA (Garg *et al.*, 2012) [16]. Choline is also involved in the metabolism of fatty acids in the liver (Shahsavari *et al.*, 2016) [40], thereby keeping a check on fat and fat metabolism in the body. The supplementation of this experimental diet improves the cattle body condition score, helps in attaining early fertility in terms of puberty, sexual maturity, pregnancy and overall higher lifetime productivity

### Body weight

A significant improvement in the body weight of the heifers was also recorded in heifers fed experimental diet i.e., T1, T2 & T3 Groups during the trial, the significance of the change in body weight of the heifers in the T0 (control group) was recorded much later

With the feeding of the experimental diet, it was recorded that the body weight of the heifers fed experimental diet had a significant change by the end of 2<sup>nd</sup> month in heifers of T2

Group ( $238.01 \pm 7.54$  kg), T1 Group ( $256.31 \pm 9.18$  kg) and T3 Group ( $256.85 \pm 10.98$  kg) under controlled conditions as compared to that of the T0 (control group) that reached a significant difference by the end of 3<sup>rd</sup> month ( $221.38 \pm 12.68$  kg) when compared to their preliminary body weight ( $171.11 \pm 6.54$  kg;  $160.50 \pm 9.91$  kg;  $178.32 \pm 8.32$  kg &  $168.32 \pm 6.32$  kg for T1, T2, T3 & T0 respectively) (Table 3, Figure 3). The recorded weight gain had close proximity to the reports of Kumar *et al.*, (2018) [26], who studied the weight gain in Frieswal cattle, but they reported the average body weight of Frieswal heifers to be  $257 \pm 1.68$  kg at 18 months of age and  $323 \pm 1.63$  kg at 24 months of age. The weight recorded at first calving was  $412.12 \pm 2.83$  kg, which was higher than the body weight of Frieswal cattle recorded under field conditions. Heifers fed the experimental diet (T2 Group & T0 Group) were able to attain a similar body weight as reported by Kumar *et al.*, (2018) [26] at 24 months of age. Thus, a significant improvement in body weight is recorded in our findings.

Similarly, the change in the body weight was found significantly increased in heifers fed experimental diet (T1, T2, T3 Groups) by the end of 3<sup>rd</sup> month as compared to the control group (T0) which recorded a significant change by the end of 4<sup>th</sup> month of feeding as compared to the body weight of heifers at the end of 1<sup>st</sup> month in their respective groups (Table 3, Figure 3).

It was recorded that by the end of the 2<sup>nd</sup> month heifers of the T1 group had a significant improvement in body weight ( $256.31 \pm 9.18$  kg) as compared to the T0 (control group) ( $208.85 \pm 9.98$  kg) at the end of the 2<sup>nd</sup> month. Similarly, heifers of group T2 ( $287.58 \pm 11.43$  kg) and T3 heifers ( $312.38 \pm 14.68$  kg) recorded a significant improvement in their body weight by the end of 3<sup>rd</sup> month compared to the control group ( $221.38 \pm 12.68$  kg) (Table 3, Figure 3). It was recorded that heifers attained an average of 60 to 65 percent of mature body weight may be considered a benchmark for breeding (Endecott *et al.*, 2013) [11]. Thus, correlating to a maximum number of heifers that demonstrated estrus. The



mature body weight at first calving was 412.12±2.83 kg (Kumar *et al.* 2018) [26], which is achievable among the experimental heifers by the end of their gestation, considering their final body weight at the end of the experiment.

The experimental diet was rich in energy and bioavailability in terms of bypass fats and bypass protein. Thus, heifers had a better growth rate and attained higher body weight in a short time, similar results were recorded with the feeding of only rumen undegradable protein in Frieswal heifers (Project report, CIRC, Meerut, 2013). Protein is considered to be the key limiting nutrient in the diet of tropical cattle including Indian zebu due to its significant ruminal breakdown, leading to poor availability and conversion of dietary protein into growth (Nisa *et al.*, 2008) [29]. Protein fermentation in the rumen prior to enzymatic digestion in the lower tract leads to reduced protein utilization efficiency (Singh *et al.*, 2019) [42].

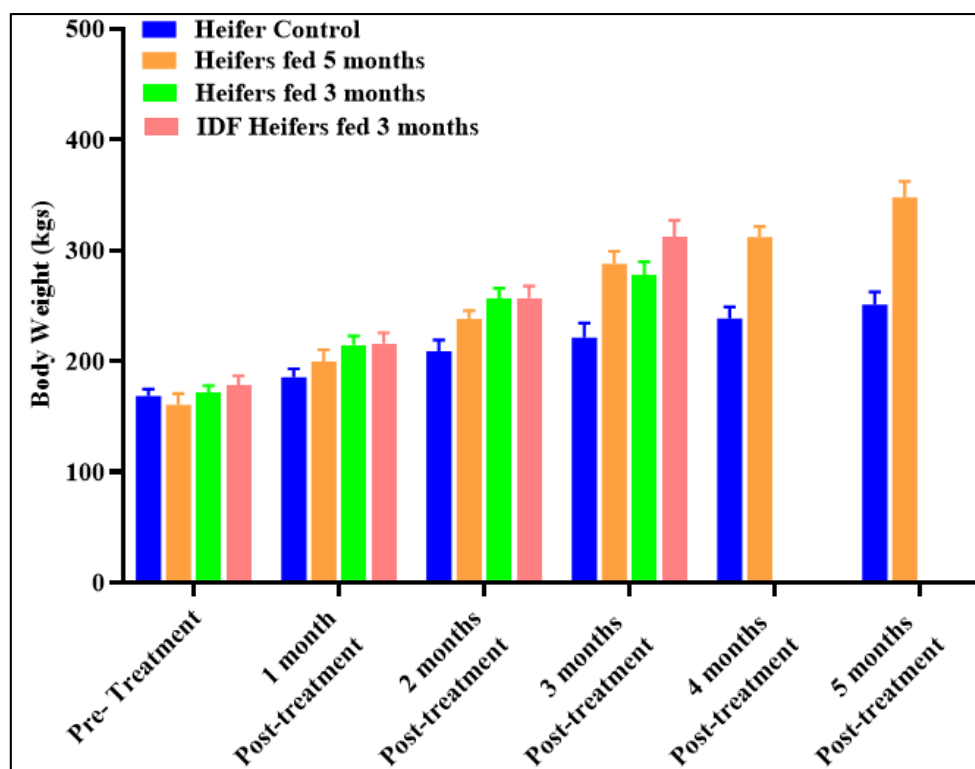
With bypass proteins, the protein availability increased and led to better growth and development.

The addition of rumen-protected niacin and choline also promotes efficient utilization of available nutrients in the diet of the heifers under an experimental diet (Al-Abbasy, 2013) [2]. Niacin effectively inhibits lipolysis through the binding and stimulation of the G-protein-coupled receptor (Gille *et al.*, 2008) and also increases the growth rate by 4.5%/ gm/ day/ animal (Department of Animal Husbandry and Dairying, Basic Animal Husbandry Statistics, 2015, 2019). It has been recorded that oral administration of niacin results in the increased microbial synthesis of protein and greater weight gain (Flachowsky, 1993) [14]. On the contrary, Kumar and Das, (2006) [25] reported that niacin supplementation @ 100 and 200 ppm in buffalo calves' diet had no significant effect on their growth and nutrient utilization.

**Table 3:** Body weight of Heifers fed an experimental diet in comparison to the Control Heifers in the field and controlled conditions

Duration from initiation feeding	Field conditions			Controlled conditions
	T <sub>0</sub> Group	T <sub>1</sub> Group	T <sub>2</sub> Group	T <sub>3</sub> Group
	Heifers Control (n=20) (kg)	Heifers fed 3 months (n=20) (kg)	Heifers fed 5 months (n=20) (kg)	IDF Heifers fed 3 months (n=10) (kg)
Pre-Treatment	168.32 ± 6.32 <sup>x</sup> (154.91-174.38)	171.11 ± 6.54 <sup>x</sup> (142.85-189.27)	160.50 ± 9.91 <sup>x</sup> (154.83-167.43)	178.32 ± 8.32 <sup>x</sup> (154.91-174.38)
1 month Post-treatment	185.32 ± 7.59 <sup>w</sup> (177.45-199.74)	214.65 ± 8.12 <sup>w</sup> (193.43-225.74)	199.77 ± 10.32 <sup>w</sup> (189.62-212.43)	215.72 ± 9.75 <sup>w</sup> (177.45-199.74)
2 months Post-treatment	208.85 ± 9.98 <sup>a</sup> (194.16-219.58)	256.31 ± 9.18 <sup>b,y</sup> (244.28-265.96)	238.01 ± 7.54 <sup>m,y</sup> (239.73-257.82)	256.85 ± 10.98 <sup>y</sup> (194.16-219.58)
3 months Post-treatment	221.38 ± 12.68 <sup>a,m,y</sup> (205.48-232.93)	277.63 ± 11.86 <sup>b,v,y</sup> (265.35-291.79)	287.58 ± 11.43 <sup>b,n,v,y</sup> (296.37-321.86)	312.38 ± 14.68 <sup>b,v,y</sup> (205.48-232.93)
4 months Post-treatment	238.21 ± 10.73 <sup>a,v,y</sup> (214.77-251.95)	*	312.18 ± 9.43 <sup>b,n,v,y</sup> (297.47-325.42)	*
5 months Post-treatment	251.08 ± 11.49 <sup>a,n,v,y</sup> (238.51-267.08)	*	347.84 ± 14.29 <sup>b,n,v,y</sup> (332.05-359.71)	*

Figures with different superscripts (x, y) within a column for a particular parameter differ significantly ( $P < 0.01$ )  
 Figures with different superscripts (w, v) within a column for a particular parameter differ significantly ( $P < 0.01$ )  
 Figures with different superscripts (m, n) within a column for a particular parameter differ significantly ( $P < 0.01$ )  
 Figures with different superscripts (a, b) within a row for a particular parameter differ significantly ( $P < 0.01$ )



**Fig 3:** Body weight of Heifers fed an experimental diet in comparison to the Control Heifers in the field and controlled conditions

Similarly, choline is a major lipotropic compound in dairy cows (Zenobi *et al.*, 2018) [48]. Thus, their addition promotes efficient growth and early sexual development. Choline is also involved in the fatty acid metabolism in the liver (Shahsavari *et al.*, 2016) [40]. Diets supplemented by choline have recorded an increase in overall productivity under long and short trials (Elek *et al.*, 2008) [10] and reduced incidence of fatty liver, especially common in over-conditioned cows (Xu *et al.*, 2006) [46].

Estrous expression with the feeding of the experimental diet to heifers it was recorded that the average age in months of first estrous expression is reduced to 20.35 months in the T1 group, 21.33 months in the T2 group and 20.14 months in the T3 group which is appreciably lesser than the control group with estrus expression at an average age of 22.29 months. The average age of estrus expression for experimentally fed animals was 20.60 months which is remarkably lesser than the control group at 22.29 months (Table 4). Thus, with the feeding of experimental supplements to heifers, it reduced the age at first estrus by 2 months. It was also recorded that the estrus induction rate in the experimental group was significantly higher as compared to the control group by the end of the experiment. Simultaneously there was early induction of estrus and overall higher estrus induction rate.

Feeding of carbohydrates and lipids such as bypass fats, that predominantly escape rumen fermentation and are digested and absorbed in the small intestines are novel methods aimed at reducing ruminal fermentation losses and improving nutrient utilization (Noel, 2000) [30]. Apart from the energy source, fat also supplies essential fatty acids such as oleic and linoleic fatty acids (Cerri *et al.*, 2009) [6], that act as a precursor of progesterone synthesis via the cholesterol and prostaglandins pathways (Staples *et al.*, 1998) [43]. Similarly,

with the feeding of by-pass protein in the heifer's diet, an essential nutrient for growth and development is provided in terms of the amino acids (Promkot *et al.*, 2007) [33] which are the building blocks for the body and reproduction. Plasma or serum proteins are closely related to tissue proteins and are recorded to be essential for the development of endocrine glands, such as pituitary and sex organs (Tiwari *et al.*, 2018) [44]. This is related to poor growth and poor availability of nutrients such as essential fatty acids and amino acids from physical and sexual maturation. Feeding of bypass proteins also leads to better digestibility of dry matter, organic matter, and crude protein

in ruminants (Preston and Leng, 1987) [32]. It is directed to various macro-micro minerals and bypass fat that positively affect steroidogenesis and follicular growth, and promote early symptoms of ovulatory estrus (Singh *et al.*, 2011; Dhama *et al.* 2019) [8].

Gille *et al.* (2008) [19] reported that the supplementation of niacin effectively inhibits lipolysis, thereby helping in attaining the weight required to express puberty, this relates to the time of estrus expression and body weight goals. Similarly, Niacin supplementation has been recorded to increase the growth rate by 4.5%/ 1gm/ day/ animal (Department of Animal Husbandary and Dairying, Basic Animal Husbandry Statistics, 2015, 2019). Choline supplementation in the diet of heifers reported by Guretzky *et al.* (2006) [21], stated that it improved ovulation in both heifers, thus promoting the expression of fertile estrus. Similarly, Choline supplementation had a non-significant reduction in a heifer to days to first estrus and conception in cows (Ardalan *et al.*, 2010) [3]. With the supplementation of experimental feed, the age at first estrous has significantly reduced close to 19-20 months of age.

**Table 4:** Average age at first Estrous for heifers fed experimental supplement under field conditions and controlled conditions

Age in months for estrous expression	Field conditions			Controlled conditions
	T <sub>0</sub> Group	T <sub>1</sub> Group	T <sub>2</sub> Group	T <sub>3</sub> Group
	Heifers Control (n=20)	Heifers fed 3 months (n=20)	Heifers fed 5 months (n=20)	IDF Heifers fed 3 months (n=10)
19 months (1 month Post-treatment)	0	2	1	2
20 months (2 month Post-treatment)	0	7	4	2
21 months (3 month Post-treatment)	1	8	5	3
22 months (4 month Post-treatment)	3	*	4	*
23 months (5 month Post-treatment)	3	*	4	*
Avg. age in months	22.29	20.35	21.33	20.14
Average age in months	22.29	20.60		

\*Could not be sampled

## Conclusion

The supplementation of the experimental diet composed of bypass protein, bypass fat, rumen protected choline, rumen protected niacin and probiotics, could significantly reduce the age at first estrus by 2 months. This will significantly improve the lifetime productivity of the animal and will support the farmer's income through early production.

## Acknowledgment

This study was supported and funded by All India Coordinated Research Project (AICRP, ICAR) project number 174, Principal Investigator- Dr. C.B. Singh, Professor, Department of Veterinary Animal Genetics and Breeding, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, Uttarakhand 263145

## References

1. Shankare Gowda AJ, Devaraj M. The body condition of periparturient dairy cattle fed with propylene glycol,

bypass fat-and protein. J Pharm. Innov. 2019;8(9):438-442.

2. Al-Abbasy EG. Effect of Adding Two Levels of Niacin in Milk Production and Controlling Indicators of Ketosis in Friesian Cows Postpartum. Br. J Dairy Sci. 2013;3(1):1-4.
3. Ardalan M, Rezayazdi K, Dehghan-Banadaky M. Effect of rumen-protected choline and methionine on physiological and metabolic disorders and reproductive indices of dairy cows. J Anim. Physiol. Anim. Nutr. (Berl). 2010;94(6):259-e265.
4. Berry D, Macdonals K, Penno J, Roche R. Association between body condition score and live weight in pasture-based Holstein Friesian dairy cows. J. Dairy Res. 2006;73:487-491.
5. Cargile B, Tracy D. Interaction of nutrition and reproduction in the dairy cow. Bov Repro. 2021, 389-398.

6. Cerri RLA, Juchem SO, Chebel RC, Rutigliano HM, Bruno RGS, Alvão KNG, *et al.* Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *J Dairy Sci.* 2009;92:1520-1531.
7. CIRB. (Central Institute for research on Buffalo). Buffalo performance at CIRB. Annual Report, 1999-2000, 14PP.
8. Dhama AJ, Patel JA, Hadiya KK, Parmar SC, Chaudhari DV. Nutritional infertility and ameliorative measures in dairy animals of middle Gujarat. *IJVSBT.* 2019;14(03):05-09.
9. Dransfield MBG, Nebel RL, Pearson RE, Warnick LD. Timing of insemination for dairy cows identified in estrus by a radio telemetric estrus detection system. *J Dairy Sci.* 1998;81(7):1874-1882.
10. Elek P, Newbold JR, Gaal T, Wagner L, Husveth F. Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. *Animal.* 2008;2(11):1595-1601.
11. Endecott RL, Funston RN, Mulliniks JT, Roberts AJ. Implications of beef heifer development systems and lifetime productivity. *J Anim. Sci.* 2013;91:1329-1335.
12. Evans AO, Davis FJ, Nasser LF, Bowman P, Rawlings NC. Differences in early patterns of gonadotrophin secretion between Early and Late Maturing bulls, and changes in semen characteristics at puberty. *Theriogenology.* 1995;43:569-78.
13. Ferguson JD, Galligan DT, Thomsen N. Principal Description of Body Condition Score in Holstein Cows. *J Dairy Sci.* 1994;77:2695-2703.
14. Flachowsky G. Niacin in dairy and beef cattle nutrition. *Arch. Anim. Nutr.* 1993;43:195-213.
15. Galina CS, Arthur GH. Review of Cattle Reproduction in the Tropics. 2. Parturition and Calving Intervals. *Animal Breeding Abstracts.* 1998;57:679-686.
16. Garg MR, Bhandari BM, Sherasia PL. Effect of supplementing bypass fat with rumen protected choline chloride on milk yield, milk composition and metabolic profile in crossbred cows. *Indian J Dairy Sci.* 2012;65:319-323.
17. Garnsworthy PC, Sinclair KD, Webb R. Integration of physiological mechanisms that influence fertility in dairy cows. *Anim.* 2008;2(8):1144-1152.
18. Gasser CL, Behlke EJ, Grum DE, Day ML. Effect of timing of feeding a high concentrate diet on growth and attainment of puberty in early weaned heifers. *J Anim. Sci.* 2006;84:3118-3122.
19. Gille A, Bodor E, Ahmed K, Offermanns S. Nicotinic acid: pharmacological effects and mechanism of action. *Annu. Rev. Pharmacol. Toxicol.* 2008;48:79-106.
20. Gupta SK, Singh P, Shinde KP, Lone SA, Kumar N, Kumar A. Strategies for attaining early puberty in cattle and buffalo: A review. *Agric. Rev.* 2016;37(2):160-167.
21. Guretzky NJ, Carlson DB, Garrett JE, Drackley JK. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *J Dairy Sci.* 2006;89(1):188-200.
22. Heinrichs AJ, Heinrichs BS, Harel OG, Rogers W, Place NT. A prospective study of calf factors affecting age, body size and body condition score at first calving of Holstein dairy heifers. *J Dairy Sci.* 2005;88:2828-2835.
23. Heinrichs A, Jones C, Ishler V. Body Condition Scoring as a Tool for Dairy Herd Management. Penn State College of Agricultural Sciences. 2017;1:1-2.
24. <https://ahd.uk.gov.in/pages/display/107-livestock-demography> Official Website of Department of Animal Husbandry, Govt. Of Uttarakhand, 04/08/2022.
25. Kumar R, Dass RS. Effect of niacin supplementation on growth, nutrient utilization and blood biochemical profile in male buffalo calves. *Asian-australas. J Anim. Sci.* 2006;19:1422-1428.
26. Kumar S, Alex R, Gaur GK, Mukherjee SS, Mandal DK, Singh U, *et al.* Evolution of Frieswal cattle: A crossbred dairy animal of India. *Indian J Anim. Sci.* 2018;88(3):265-275.
27. McGuffey RK. A 100-Year Review: Metabolic modifiers in dairy cattle nutrition. *J Dairy Sci.* 2017;100:10113-10142.
28. Moreira F, Risco C, Pires MFA, Ambrose JD, Drost M, DeLorenzo M. Effect of Body Condition on Reproductive Efficiency of Lactating Dairy Cows Receiving a Timed Insemination. *Theriogenology.* 2000;53:1305-1319.
29. Nisa MU, Javaid A, Shahzad MA, Sarwar M. Influence of varying ruminally degradable to undegradable protein ratio on nutrient intake, milk yield, nitrogen balance, conception rate and days open in early lactating Nili-Ravi buffaloes (*Bubalus bubalis*). *Asian-Australas. J Anim. Sci.* 2008;21(9):1303-1311.
30. Noel RJ. Official feed terms. In: Association of American Feed Control Officials. Official Publication. USA, 2000, 187-200.
31. Paul A, Mondal S, Kumar S, Kumari T. Body condition scoring in dairy cows-a conceptual and systematic review. *Indian J Anim. Res.* 2020;54(8):929-935.
32. Preston TR, Leng RA. Matching ruminant production system with available resources in the tropics and subtropics. Penambul Books, Armidale NSW, 1987, 1-1.
33. Promkot C, Wanapat M, Rowlinson P. Estimation of ruminal degradation and intestinal digestion of tropical protein resources using the nylon bag technique and the three-step *in vitro* procedure in dairy cattle on rice straw diets. *Asian-Australas. J Anim. Sci.* 2007;20:1849-1857.
34. Ranjan A, Sahoo B, Singh VK, Srivastava S, Singh SP, Pattanaik AK. Effect of bypass fat supplementation on productive performance and blood biochemical profile in lactating Murrah (*Bubalus bubalis*) buffaloes. *Trop. Anim. Health and Prod.* 2012;44:1615-1621.
35. Roche JR, Friggens NC, Kay JK, Fisher MW, Stafford KJ, Berry DP. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *J Dairy Sci.* 2009;92(12):5769-5801.
36. Roche JF. The Effect of Nutritional Management of the Dairy Cow on Reproductive Efficiency. *Anim. Reprod. Sci.* 2006;96:282-296.
37. Röttgen V, Becker F, Tuchscherer A, Wrenzycki C, Döpjan S, Schön PC, *et al.* Vocalization as an indicator of estrus climax in Holstein heifers during natural estrus and superovulation. *J Dairy Sci.* 2018;101(3):2383-2394.
38. Santos JE, Villasenor M, Depeters EJ, Robinson PH, Holmberg CH. Type of Cotton Seed and Gossypol in Diets of Lactating Dairy Cows: Plasma Gossypol, Reproduction and Health. *J Dairy Sci.* 2003;86:892-905.
39. Schroder UJ, Staufenbiel R. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *J Dairy Sci.* 2006;89(1):1-14.
40. Shahsavari A, D'Occhio MJ, Al Jassim R. The role of

- rumen-protected choline in hepatic function and performance of transition cows. *Br. J Nutr.* 2016;116:35-44.
41. Sharma R, George A, Kamble NM, Singh K, Panda SK, Chauhan MS, *et al.* Development of a culture system capable of long-term maintenance of buffalo (*Bubalus bubalis*) embryonic stem cells. *Reprod. Fertil. Develop.* 2011;23:251.
  42. Singh A, Sidhu S, Singh P. Bypass protein technology: A review. *Pharma. Innov. J.* 2019;8:150-153.
  43. Staples CR, Burke JM, Thatcher WW. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *J Dairy Sci.* 1998;81:856-871.
  44. Tiwari MR, Jha PK, Pant SR, Acharya MP, Thapa P, Shrestha BK. Effect of bypass protein supplement on milk production in Jersey cows. *BJAS.* 2018;47(2):98-104.
  45. Wangchuk K, Wangdi J, Mindu M. Comparison and reliability of techniques to estimate live cattle body weight. *J App. Anim. Res.* 2018;46(1):349-352.
  46. Xu G, Ye JA, Liu J, Yu Y. Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. *Asian-Australas. J Anim. Sci.* 2006;19(3):390-395.
  47. Yuan K, Shaver R, Bertics SJ, Espineira M, Grummer RR. Effect of rumen-protected niacin on lipid metabolism, oxidative stress, and performance of transition dairy cows. *J Dairy Sci.* 2012;95:2673-2679.
  48. Zenobi MG, Gardinal R, Zungia JE, Dias ALG, Nelson CD, Driver JP, *et al.* Effects of supplementing with ruminally protected choline on performance of multiparous Holstein cows did not depend upon prepartum caloric intake. *J Dairy Sci.* 2018;101:1088-1110.