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Estimation of certain blood metabolites between normal, delayed sexual maturity and low growth rate Karan fries crossbred heifers in different seasons

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Abstract
The present study reports the seasonal variation of certain plasma metabolites in normal and delayed sexual maturity Karen Fries crossbred heifers at Livestock Research Center, ICAR-National Dairy Research Institute, Karnal, Haryana over a period of one year. Total 160 heifers; eight healthy heifers in each month [24, 16, 32, 24 healthy heifers in rainy, autumn, winter and summer, respectively and 8 heifers from each group (delayed sexual maturity and low growth rate) per season were selected for the study. Plasma metabolite like glucose, urea nitrogen, cholesterol and non-esterified fatty acid (NEFA) was estimated. There was significant ($P \leq 0.05$) seasonal variation of plasma metabolites like glucose, urea nitrogen, cholesterol and NEFA was observed. In delayed sexual maturity and low growth rate heifers mean plasma glucose, cholesterol and NEFA concentration differed significantly ($P \leq 0.05$) from healthy heifers and and also did not follow a similar pattern. It might be due to change or variation in the fodder other management effects. Herd status regarding metabolic parameters need to be evaluated regularly in different seasons to achieve set targets in terms of growth performance of heifers by adjusting feeding management.

Keywords: Karen Fries normal heifers, delayed sexual maturity, low growth rate, plasma metabolites

Introduction
Growth is influenced by genetic potential, nutrition and environmental factors. Generally, heifers do not have constant growth rate. In fact, the most usual pattern is a faster growth between the birth and onset of puberty, followed by a slower growth period. Puberty and sexual maturity are closely related stages of life which have an enormous effect on the performance of dairy animals in production and reproduction. Onset of puberty is credited to achieving target weight at a particular age rather than age alone [25, 15]. Delayed puberty has been reported as one of the major reproductive disorder in dairy farming. Such conditions result in great economic losses in form of reduced conception rate, delayed conception and pregnancy, reduced lactation yield and decreased no of calves per animal. The prevalence of delayed puberty in heifers was observed in 20.81% and highest in crossbred cattle 95.02% as compared to local cattle 4.98% [4].
Most of the blood constituents fluctuate with level of feeding, diet composition, age of the animal and change in relative body condition. Nutritional status of animal is reflected by plasma biochemical profiling [28]. The tropical areas significantly affect the quality and quantity of forages in different seasons [1]. Most of the nutrients are involved in proper growth and reproductive maturation [26]. Serum constituents are good indicators of the physiological condition of animals [17]. Among them, nutritional well-being of animal is best reflected by plasma glucose, plasma cholesterol are some of the key determinants affecting the fertility and cyclicity in dairy animals [25, 27]. Thus, the present investigation was planned to study the effect of season and blood metabolites to evaluate the health status of f Karen Fries heifers.

Materials and Methods
Location of experiment
Present study was conducted at livestock research center, ICAR-National Dairy Research Institute, Karnal, India over a period of one year (June, 2008 - May, 2009) on crossbred calves (Holstein Friesian × Tharparkar).

Karnal is located on 29^0 42' N latitude and 72^0 02' E longitudes, with an altitude of 250 meters above the mean sea level in the bed of Indo-Gangetic alluvial plain. Depending major seasons like rainy (July-September), autumn (October-November) winter (December-March) and summer (April-June).

Experimental animals

The nutrient requirements of all the animals were met from concentrates (33% on DM basis) and *ad libitum* green fodder. During winter, green fodders like berseem, oat, mustard and winter maize were fed; whereas in summer and rainy seasons green fodders (predominantly maize, sorghum and cowpea) were given.

Feedings were spread in 3 to 4 feeding during day and night. The composition of concentrate mixture was same in terms of DCP and TDN content though ingredients varied depending on season.

Karen Fries heifers above 18 - 22 months of age were selected in this group.

To study the metabolic status in normal heifers, a total number of 8 heifers were selected every month and blood samples were collected at monthly interval. To study the effect of season on age at sexual maturity in each season 8 animals were selected on the basis of low growth rate (<400g/day) as "low growth rate heifers" and heifers attained sexual maturity after 24 months were classified as "delayed sexual maturity heifers".

Blood samples were collected from all these heifers during each season for analysis of metabolic profile. Experimental procedures were approved by the Institutional Animal Ethics Committee.

Blood sampling, plasma metabolites estimation

Blood samples were collected from jugular venepuncture using 9 ml blood collection tubes with Heparin as anticoagulant (Vacutte®, Griner Bio-one Gmbh, Austria) from the heifers in the early morning. Samples were centrifuged at @3000 rpm for 20 minutes at 4ºC to separate plasma and separated plasma samples were stored in 2 ml cryovials (Tarson, India) at -20ºC until further analysis. Plasma metabolites; glucose (O-Toluidine end point method, [16], urea nitrogen [31] cholesterol (ferric chloride-sulphuric acid method), non-esterified fatty acid (NEFA) (modified copper soap extraction method [36], were estimated.

Statistical analysis

The statistical analysis was done using Sigmaplot version 11.0 (Systat Software Inc., USA). Data sets were first tested for normality by Shapiro-Wilk's normality test and analysed by two way analysis of variance (ANOVA) with general linear model (GLM). All pair-wise differences in mean were compared by Tukey post hoc test. Difference between means was considered statistically significant when $P \leq 0.05$ and plasma metabolites concentrations were presented as mean ± SE.

Table 1: Monthly meteorological variable during the study period

Seasons	Meteorological variables (Mean±SE)			
	Temperature (ºC)	Relative Humidity (%)	Rain fall (mm)	Wind speed (km/hr)
Rainy	29.28±2.27	80.17±5.46	140.90±27.59	4.20±0.26
Autumn	22.57±4.51	67.00±13.31	7.60±6.6	2.25±0.35
Winter	16.48±2.82	71.12±8.21	4.40±1.8	3.32±0.33
Summer	29.02±3.24	54.00±9.53	62.13±42.37	7.23±0.78

Table 2: The blood metabolites in normal, delayed sexual maturity and low growth rate Karen Fries crossbred heifers during different seasons

Parameters	Rainy (mean± SE) (n=24)	Autumn (mean± SE) (n=16)	Winter (mean± SE) (n=32)	Summer (mean± SE) (n=24)
Glucose (mg/dl)				
Normal pubertal heifers	66.34^{bB}±1.45	56.72^{aB}±1.81	55.04^{aC}±1.28	65.70^{bB}±1.48
Delayed sexual maturity	64.09^{cB}±3.36	60.37^{bB}±0.96	42.15^{aB}±0.57	62.06^{bA}±1.60
Low growth rate	51.42^{bA}±3.15	50.85^{bA}±1.08	32.22^{aA}±1.34	59.84^{cA}±1.48
BUN (mg/dl)				
Normal pubertal heifers	28.85^{bA}±0.90	23.47^{aA}±1.10	30.83^{cC}±0.78	23.46^{aA}±0.90
Delayed sexual maturity	31.29^{bB}±1.8	28.93^{bB}±0.44	28.73^{bB}±0.86	23.35^{aA}±0.60
Low growth rate	30.41^{bB}±1.35	29.73^{bB}±1.64	27.26^{bA}±0.71	23.86^{aA}±0.21
Cholesterol (mg/dl)				
Normal pubertal heifers	88.26^{aB}±5.08	120.35^{cC}±6.21	140.02^{dA}±4.40	112.18^{bB}±5.08
Delayed sexual maturity	69.98^{aA}±1.26	71.66^{aA}±1.38	160.76^{cB}±3.63	145.82^{bC}±7.67
Low growth rate	97.27^{bC}±3.98	91.38^{bB}±1.39	160.68^{cB}±3.64	76.07^{aA}±3.67
NEFA (µmol/L)				
Normal pubertal heifers	259.23^{bB}±9.47	205.14^{aA}±11.60	334.31^{dB}±8.20	275.37^{cA}±9.47
Delayed sexual maturity	213.66^{aA}±7.78	377.56^{cB}±7.78	317.12^{bA}±2.67	282.30^{bB}±3.86
Low growth rate	206.27^{aA}±2.47	370.17^{dB}±2.47	316.10^{cA}±28.67	273.7^{bA}±7.65

Means with different superscript in a row (a, b, c, d) and column (A, B, C) differ significantly ($P<0.05$)

Results and Discussion

Plasma Glucose

The blood biochemical parameters plasma glucose were within the normal range in all the season [18]. The concentration of plasma glucose in healthy heifers differs significantly $P<0.05$) among the four seasons and also did not follow a similar pattern. Glucose level was within the reference range of buffalo heifers given by Abd Ellah *et al.* [1] (glucose reference range 35.45–92.47 mg/dl).

In delayed sexual maturity and low growth rate heifers significant ($P<0.05$) difference was observed among the season; and winter season, low blood glucose level was observed in delayed sexual maturity and low growth rate heifers. It might be due to change or variation in the fodder and other management effects. The blood glucose level gives an indicator of the energy status of an animal. These levels indicated a low energy status probably affecting the follicular development resulting follicular atresia, inactive ovaries and anoestrus.

In winter season, low blood glucose level was observed in delayed sexual maturity and low growth rate heifers. These levels indicated a low energy status probably affecting the

follicular development resulting follicular atresia, inactive ovaries and anoestrus. Our results for glucose concentration for such heifers are in agreement with [2] and [13]. The low conception rate associated with hypoglycemia due to energy deficiency [20, 3]. reported that pituitary function might be particularly influenced by blood glucose level.

The results are associated with [9] observed the low blood glucose may be associated with infertility [21] and [11] reported significantly lower serum glucose level in anoestrus than normally cycling animals. Energy deficient diets have been associated with delayed sexual maturity, anestrum in heifers and impaired cyclicity postpartum [23].

All the three groups higher glucose level observed in summer and rainy season. The higher level of glucose might be due to increased glucose synthesis from propionate a volatile fatty acid produced in rumen because of feeding of more amount of green fodder as compared to dry fodder. In agreement with our result [25], in Kankrej heifers [35], also reported higher glucose level in crossbred heifers fed on different roughages. High levels of glucose, amino acids and metabolic hormones in cow heifers are likely to serve as metabolic signals transmitting nutritional status information to the hypothalamic neurons, which leads to increased LH secretion and early onset of puberty [39].

Blood Urea nitrogen

The normal pubertal heifers the plasma urea nitrogen was within the normal range(10-30 mg/dl) in all the season. The concentration of blood urea nitrogen in normlpubertal heifers differs significantly $P<0.05$) among the four seasons and there was no significant ($P<0.05$) difference was observed autumn and summer season. However, the BUN concentration was high as compared to that reported by [18]. The rise in BUN level may denote imbalance of protein and energy levels in the diet. The higher level of BUN may also be because of high availability of leguminous fodder berseem fed in winter. The concentration of urea in the blood reflects the degree of protein catabolism and is synthesized in the liver from CO_2 and NH_3. The values of serum blood urea has been considered to be an indicator of total protein intake and its determination with creatinine is considered important in order to exclude renal damage, which is fairly frequent in this species.

The higher intake of protein, mostly degradable, leads to formation of ammonia which is converted into urea in the liver which is an energy consuming process. This, in the long run, may cause hepatic failure and degeneration causing infertility, puerperal collapse, lameness and steatosis [12, 5]. So, balancing the protein and energy is the major concern in the herd feeding strategies.

In heifers with delayed sexual maturity and low growth rate was significant ($P<0.05$) difference was observed among the season; however, there was no difference between rainy, autumn and winter season. But in summer low level was observed in the present findings. The BUN concentration is an indicator of energy protein balance [7] and is typically increased in cows deficient in energy. The rise in BUN level may denote imbalance of protein and energy levels in the diet. While high protein diet fed to cows has been observed to cause increased blood urea and reduced fertility [33, 7]. The higher blood urea level reported in anoestrus cow [29].

Plasma Cholesterol

The serum cholesterol level in the overall population of Karen Fries heifers ranges from (76.07±3.67 to 160.76±3.63mg/dl). The normal healthy heifers the mean plasma cholesterol was within the normal range in all the season [18]. Cholesterol level was within the reference range of buffalo heifers given by Abd Ellah *et al.* [1] (cholesterol reference range 34.92–76.82 mg/dl). Though there was significant ($P<0.05$) difference of cholesterol level was observed between all the four seasons. The values of serum cholesterol are usually considered as an indicator of good hepatic lipoproteins production used as carriers of triglycerides, synthesized from NEFA. Total cholesterol indirectly reflects the degree of exogenous energy availability and the hepatic functionality; its levels rise owing to a moderate negative energy balance, low temperatures and high thermal ranges [6]. In the present study heifers in autumn and winter season showed higher cholesterol level which could be due to negative energy balance as *ad lib.* Berseem was supplied during these periods. The higher concentration of cholesterol with the advancement of age is probably a physiological adjustment to meet growth requirements. The positive correlation of cholesterol with age of the cow is very important and the cholesterol level increased only up to 4 years of age and then decreased due to acclimatization to stress from lactation and gestation [34]. This continued increase of cholesterol with increasing age in present study is also reflected to get acclaimed to the environmental stress.

Heifers with delayed sexual maturity and low growth rate animals significant ($P<0.05$) difference in cholesterol level was observed among summer and winter; however, there was no difference between rainy and autumn. In heifers delayed sexual maturity showed low level of cholesterol in rainy and autumn season. In low growth rate heifers lower cholesterol observed in summer season. Many authors reported the relation of cholesterol with estrus induction [22]. Reported the low cholesterol level in anoestrus cows and high cholesterol level in normal cycling cows. Dynamics of serum cholesterol values was similar to [37] and [22, 8]. The cholesterol value was higher in present findings which are similar to [38] who observed cholesterol values were higher in farm animals than in village animals which could be responsible for exhibiting prominent sign of estrus because higher cholesterol level is responsible for higher oestradiol production. The present findings agreement with [40] reported higher cholesterol content in estrogen dominant phase of estrus cycle. [19] regarded cholesterol to be widely distributed precursor in blood in free and esterified forms. Hydrolysis of cholesterol ester is essential for positive synthesis of steroid based sex hormones.

Non-esterified fatty acid (NEFA)

The Non-esterified fatty acid (NEFA) level in normal healthy heifer significant ($P< 0.05$) difference was observed among four seasons; however, there was highest level observed winter followed by summer season. In delayed sexual maturity and low growth rate heifer significant (($P< 0.05$) difference was observed among all season; however, there was highest level observed autumn followed by winter and summer season.

The normal values of NEFA for cows in positive energy balance were less than 200 µM. The energy balance has been recognized as the main determinant of plasma NEFA concentration but other factors *viz.* impending parturition, stress, poor nutritional, etc., have an important influence on plasma NEFA concentration. The concentration of NEFA in blood reflects the degree of adipose tissue mobilization,

therefore, the greater the extent of negative energy balance, the more NEFA are released from body fat and the higher the concentration of NEFA in the blood. Animals adapt to negative energy balance by mobilizing energy from adipose tissue in the form of NEFA, metabolic and endocrine factors regulate the rate of NEFA release but with a low degree of sensitivity. Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and changes in tissue responsiveness to those hormones [10].

Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and changes in tissue responsiveness to those hormones. The results are agreement with [14] highest values were obtained in both summer and winter seasons (55.38 and 33.81 mg/ 100ml) which declined consistently with number of days in both the seasons. However in our study NEFA was highest in winter and autumn compared to summer and the levels are very high in all three groups normal, delayed sexual maturity and low growth rate heifers and in winter the concentration of NEFA was higher than other season could be due to high protein in the fodder *i.e*. from berseem, which is supplied *ad lib* to the animals. Consequently, stressors and poor nutritional management causing reduction in voluntary DMI results in large increase in NEFA in availability of fodder in different seasons. These findings are associated with [32] the degree of negative energy balance is critical for growth rate, delayed sexual maturity, health status and productivity of animals.

Conclusion

In the present study low blood glucose level was observed in delayed sexual maturity and low growth rate heifers in winter season might be due to change or variation in the fodder. The higher level of blood urea nitrogen and non-esterified fatty acid in winter season may also be because of high availability of leguminous fodder berseem which is supplied ad lib to the animals. Delayed puberty attainment caused due to malnourishment during pre pubertal growth phase caused delayed maturation of hypothalamic-hypophyseal-ovario-uterine axis. Herd status regarding metabolic parameters need to be evaluated regularly in different seasons to achieve set targets in terms of growth performance of heifers by adjusting feeding management.

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