EFFECTS OF DIETARY SUPPLEMENTATION OF DIFFERENT VEGETABLE OILS VARYING IN POLYUNSATURATED FATTY ACID CONTENT ON REPRODUCTIVE PERFORMANCE AND OVARIAN FOLLICULAR CHARACTERISTICS OF MURRAH BUFFALO HEIFERS

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ABSTRACT

To compare the effects of dietary supplementation of different vegetable oils varying in polyunsaturated fatty acid content on reproductive performance and ovarian follicular characteristics, twenty Murrah buffalo heifers randomly divided into four groups (T_0, T_1, T_2 and T_3) of five animals in each on the basis of age and body weight (BW). All the animals were given concentrate, green fodder and wheat straw in 50:40:10 ratios, respectively, as per Kearl (1982) feeding standard. In addition, mustard oil, soybean oil and rice bran oil 3.5% of concentrate mixture were supplemented in groups T_1, T_2 and T_3 respectively. The average age at puberty reduced in all the treatments groups as compared to control, however, BW at puberty remain uninfluenced ($P>0.05$) in all the groups. The number of small size follicles (<3 mm) were significantly higher ($P<0.05$) in T_2 as compared to T_1 and T_0 group but at par with T_3. Number of medium size follicles (3 to 6 mm) differ significantly ($P<0.05$) among treatment groups and control group. Total number of ovarian follicles followed a similar trend as shown by number of small follicles. Overall, the findings of the present study concluded that dietary supplementation of vegetable oils reduced the age at puberty, increased the number of small size of follicles, diameter of the large follicle and improve the conception rate in Murrah buffalo heifers.

Keywords: buffalo, *Bubalus bubalis*, Murrah buffalo, supplementation, vegetable oils, characteristics

INTRODUCTION

India has 57% of the world's buffalo population and half of the total milk produced comes from buffaloes (FAOSTAT, 20130). Hence, buffalo has assumed place of pride in the Indian dairy farming but poor reproductive performance is serious limitation in exploiting the production potential of buffaloes (CIRB, 2013). Heifers are an important asset to the dairy farmer because they are the future replacement stock and key determinants of the economic future of dairy farm (Anjum *et al*., 2013). Heifer production is most expensive part of the dairy farm operation (Heinrichs, 1993) as it does not generate any income to the farm directly but require inputs like feeding, housing and veterinary expenses with no visible returns earliest (Anjum *et al*., 2013).

Delayed puberty is one major limiting

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factor in buffalo husbandry which results in increased age at first calving thereby causing heavy economic losses to the dairy farmers. The onset of puberty is the result of a series of complex developmental events involving many tissues, cell types and regulatory systems. Nutrition influences reproduction through changes in metabolic substrates and circulating concentrations of metabolic hormones rather than direct effects on reproductive hormones including gonadotropins (Samadi *et al*., 2014). Puberty is usually obtained in buffaloes at the age of 26 to 30 months and body weight of 250 to 400 kg (Ghuman, 2014).

Research conducted in other species has clearly indicated that better nutrition reduced the age at puberty. In buffaloes also, it is possible to bring the animal into puberty at age of 18 to 24 months (Anjum *et al.,* 2012; Jabbar, 2004) through strategic nutritional interventions. In this regard, one of the strategies to enhance reproduction efficiency is inclusion of nutrients such as fats; seems to be related to an extra-caloric effect of fat, more specifically of certain fatty acids. Originally, it was believed that the improvement in reproduction due to the addition of fat was solely a result of an increase in energy availability to the animal.

However, it soon became apparent that individual fatty acids themselves can play a pivotal role in influencing reproductive parameters (Santos *et al*., 2008) by acting as a precursor of progesterone via cholesterol and prostaglandins (Staples *et al*., 1998). Dietary fat supplementation improved conception rate (Cerri *et al*., 2009; Moriel *et al*., 2009), reduced service period (Lopes *et al*., 2009), increased number and size of large follicles (Lucy *et al*., 1991), improved follicle (Zachut *et al*., 2008), and embryo (Thangavelu *et al*., 2007) development and increased expression of genes involved in reproduction (Mattos *et al*., 2000). Most of the data available on influence of fat supplementation on reproductive performance emanates from studies having nutrition and lactation related objectives rather than those of reproduction. Therefore, the aim of the present experiment was to investigate the effect of dietary supplementation of different vegetable oils varying in their polyunsaturated fatty acid (PUFA) content on reproductive performance and ovarian follicular characteristics in Murrah buffalo heifers.

MATERIALS AND METHODS

Animals and experimental design

The present experiment was conducted in the experimental cattle shed of National Dairy Research Institute, Karnal, India. Twenty Murrah buffalo heifers (15 to 17 months age, 240 to 245 kg body weight) were selected from the Livestock Research Centre, National Dairy Research Institute, Karnal andrandomly dividedinto four groups (T_0, T_1, T_2 and T_3) of five animals in eachon the basis of age and body weight (BW). The total mixed rations were prepered from concentrate mixture, green fodder and wheat straw in the ratio of 50:40:10 and were offered to animals twice daily to meet the requirements as per Kearl, (1992). The composition of concentrate mixture fed to control and treatment groups is given in Table 1. Heifers in T_1, T_2 and T_3 were supplemented with mustard oil, soyabean oil and rice bran oil 3.5% of concentrate mixture, respectively, while heifers in T_0 acted as control. All the heifers were given an adaptation period of 3 weeks. Feed offered was weekly adjusted according to their body weight and to achieve 5 to 10% orts daily.

Table 1. Ingredient composition (%) of concentrate mixture.

Ingredients	Control	Treatment
Maize	33	38
Bajra	05	00
Deoiled mustard cake	00	35
Mustard oil cake (MOC)	12	00
Soyabean meal (SBM)	21	00
Deoiled rice bran (DORB)	06	0
Wheat bran	20	24
Mineral mixture	02	02
Salt	01	01

Feed chemical composition and analysis

All the diets were formulated to be isonitrogenous and isocaloric and were fed for entire experimental period. Feeds and fodder samples were dried in a hot air oven at 60°C till a constant weight was attained and ground in a Wiley mill to pass a 1 mm sieve. The samples were analyzed for proximate principles; cell wall constituents, total digestible nutrient (TDN), digestible energy (DE) and metabolizable energy (ME) content of rations were calculated. The standard macro-Kjeldahl procedure was used to determine nitrogen in feeds and fodder samples. Neutral detergent fibre (NDF) was determined as per McQueen, (1979), Ether extract, Acid detergent fibre (ADF) and lignin by AOAC, (2005).

Fatty acid analysis of feed, fodder and oils

Fatty acids profile of oils, feeds and fodder are analysed as per direct trans-esterification method (O'Fallon *et al*., 2007). In brief, nonadecanoic acid (C19:0) was used as an internal standard (0.5 mg/ml of methanol). Total lipids were extracted from 1 g of dried feed sample and 40 µl of oil. GC was calibrated using a range of commercial fatty acid standards (Supelco, Sigma-Aldrich, USA). The GC was fitted with a flame ionization detector and helium (205 kpa) was used as the carrier gas. The injector temperature was held isothermally at 260°C and the detector temperature was 270°C. The column oven was held at an initial temperature of 100°C for 5 minutes and then programmed to increase 5°C per minute to 220°C, 2°C per minute to 230°C and 1°C to a final temperature of 240°C, which was held for 74 minutes. Individual fatty acid was identified by comparing the area and retention time with that of standard mixture and concentration of individual fatty acids were calculated from their peak area.

Blood sampling and analysis

Blood samples of the animals were collected fortnightly via jugular venipuncture using 9 ml vacutainer tubes with heparin as anticoagulant (Vacuette®, Greiner Bio-one Gmbh, Austria) during morning hours before feeding and watering and kept in ice box. Immediately after the collection of blood, each blood sample was centrifuged at 4°C at the rate of 3000 rpm for 20 minutes to separate the plasma. The separated plasma samples were stored in cryovials at -20°C till the further assay for the hormone. Plasma progesterone concentration was

estimated using bovine progesterone hormone (P4) ELISA Kit (Endocrine Technologies, Inc. Newark, CA). The progesterone ELISA kit was based on the principle of solid phase enzyme-linked immunosorbent assay.

Age at puberty and ovarian ultrasonography

Puberty was identified by estimating peripheral level of progesterone. The age at puberty (first ovulation) was calculated as the midpoint between two consecutive blood samples in which the progesterone level was <1 ng/ml in first sample and >1 ng/ml in second sample (Halder and Prakash, 2005). Additionally, the heifers were also subjected to ultrasonography at 15 days interval for assessing the presence or absence of a corpus luteum. The age and body weight at puberty for each animal was recorded. Ultrasonography was performed transrectaly by an expert with an Aloka1 500 V ultrasound scanner equipped with a 7.5 MHz linear array transducer in each animal at 15 days interval and on the day of oestrus to examine the presence of corpus luteum and ovarian follicular characteristics, respectively. Based on diameter, the follicles were counted and classified as: small size (<3 mm), medium size (3 to 6 mm) and large size (>6 mm).

Breeding and conception rate

A teaser bull was used in the morning and evening for the detection of heat. The heifers detected in estrus were bred by artificial insemination. The age and BW at insemination for each animal was recorded. Heifers not returning to oestrus after insemination were examined for pregnancy through ultrasonography at 45 days post breeding. Numbers of services per conception were also recorded.

Statistical analysis

The experimental data were analysed by ANOVA for completely randomised design using SAS version 9.3 programme using the linear equation model with interaction.

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$$

Where Y is the parameter, μ is population mean, α_i is interval, β_j is treatment, $\alpha\beta$ is interval treatment interaction and e_{ij} is the residual error. Comparison with $P<0.05$ declared significant.

RESULTS AND DISCUSSION

Chemical composition and fatty acids profile of the experimental diets

The chemical composition of the concentrate mixture offered is presented in Table 2 and major fatty acids of oils in Table 3. Mustard oil was rich in oleic acid, erucic acid, lioleic acid, soybean oil in oleic acid and linoleic acid and rice bran oil has high content of palmitic acid, Linoleic acid and oleic acid respectively.

Age and body weight at puberty and sexual maturity

The data pertaining to age and BW at puberty and sexual maturity are shown in Table 4. Dietary supplementation of the vegetable oils reduced ($P<0.05$) the average age at puberty in treatments groups as compared to control group. Additionally, among the treatment groups, the animals in soyabean oil supplemented group (T_2) and ricebran oil supplemented group (T_3) attained puberty at an earlier age (21.79±0.75 and 22.11±0.52 months respectively) followed by mustard oil fed group (24.19±0.50). The BW at

Table 2. Chemical composition of feed ingredients offered (% DM basis).

Attributes	Concentrate		Wheat straw	Green fodder
	Control	Treatment		
DM	91.20	90.97	91.22	19.23
OM	92.04	91.68	92.13	90.95
CP	20.71	20.84	3.10	8.78
EE	4.48	2.56	0.92	2.14
TA	7.96	8.32	7.87	9.05
NDF	27.32	26.48	75.45	64.72
ADF	12.31	11.25	53.14	40.84
TDN %	72.10	70.32	45.34	55.65
ME (Mcal/kg)	2.76	2.68	1.56	2.02

puberty remain uninfluenced (P>0.05) in all the groups as a result of dietary supplementation of vegetable oils.

Early sexual maturity was achieved (P<0.05) in T_2 and T_3 as compared to T_0 and T_1. The mean BW at sexual maturity was higher (P<0.05) in T_2 and T_3 as compared to T_0 but at par with T_1. The average age at first conception was significant (P<0.05) reduced in T_2 and T_3 as compared to T_0 while the value in T_1 group was comparable to rest of the groups. The heifers of treatment groups conceived earlier than the heifers in control, indicating positive influence of dietary supplementation of vegetable oils.

The heifers in soyabean oil supplemented group (T_2) and ricebran oil supplemented group (T_3) attained puberty at an earlier age followed by mustard oil fed group (T_1) attributable to the more content of PUFA in soyabean and rice bran oil. The heifers of treatment groups attained earlier puberty than in control, indicating positive influence of dietary supplementation of vegetable oils. Garcia *et al.* (2003) observed little or no effect on age at puberty in heifers fed high fat diets while Lammoglia *et al.* (2000); Whitney *et al*. (2000) found greater percentage of heifers reaching puberty fed with vegetable oils.

No significant difference (P>0.05) was reported in the BW at puberty as a result of supplementation. A close look in to the data had shown that buffalo heifers supplemented with different vegetable oils attained puberty at an average of 374 kg BW (68% of mature BW), while the control heifers attained puberty at 350 kg BW (63% of mature BW), where mature BW of Murrah buffaloes is considered to be 550 kg. It is generally recognized that when heifers attain 55 to 65% of mature body weight, there is no risk of complexity in breeding and calving percentage (Freetly *et al*., 2001). Results of present study are in tune with Anjum *et al*. (2012), who reported buffalo heifers raised on stair step nutritional regimen attained puberty at 382 kg BW (69.45% of mature BW), while the control heifers attained puberty at 364 kg BW (66.21% of mature BW). Halder and Prakash, (2005) reported that Murrah buffalo heifers attained puberty at BW of 380.67 kg. A lower growth rate is associated with a delayed onset of puberty and thus higher age at first calving in heifers (Bhatti *et al*., 2007; Maquivar *et al*., 2006). Early sexual maturity

was achieved ($P<0.05$) in T_3 and T_2 as compared to T_0 and T_1. Age at sexual maturity is a function of BW rather than age; it is highly dependent on growth rate of the animals. Similar trend had been reflected in the present study. Heifers that grow at faster rate, show short prepubertal period and calve at younger age, have greater lifetime production than those heifers exhibiting slow growth rate, long prepubertal period and calving at older age (Short and Bellows, 1971).

Plasma progesterone concentration and reproductive performance

Plasma progesterone (P4) concentration (ng/ml) levels were estimated in blood plasma samples of Murrah buffalo heifers collected at fortnight interval. The plasma progesterone levels at the beginning of the experiment remained similar ($P>0.05$) among all the groups and exhibited a continuous basal level ranging from 0.11 to 3.49 ng/ml. Progesterone concentration remain less than 1.00 ng/ml during prepubertal

Table 3. Fatty acid profile of different oils (g/100 g FA).

Fatty acid	Oil			Concentrate		Green fodder	Wheat straw
	Mustard	Soyabean	Rice bran	Control	Treatment		
Myristic acid (C14:0)	0.19	0.08	0.49	0.14	0.17	1.94	1.05
Palmitic acid (C16:0)	8.10	9.06	17.36	14.18	11.95	13.20	20.28
Palmitoleic acid (C16:1, Cis-9)	0.02	0.04	0.04	0.37	0.34	0.97	0.24
Stearic acid (C18:0)	3.17	3.65	1.83	4.53	2.39	2.11	31.40
Elaidic acid (C18:1, Trans-9)	0.08	0.03	0.12	0.46	0.59	1.38	0.00
Oleic acid (C18:1, Cis-9)	21.22	19.89	40.91	26.47	19.81	8.53	13.42
Linoleic acid (C18:2, Cis-9,12)	19.71	54.44	35.73	34.61	40.48	14.39	6.31
Arachidic acid (C:20)	0.06	0.12	0.36	0.92	0.95	0.58	3.29
α-Linolenic acid (C18:3, Cis-9,12,15)	3.82	5.17	1.81	3.56	3.79	48.27	0.00
Behenic acid (C22:0)	0.40	0.19	0.20	0.00	0.00	0.00	0.00
Erucic acid (C22:1)	20.05	0.00	0.00	9.74	15.72	0.00	0.00
Eicosapentaenoic acid (C20:5,Cis-5,8,11,14,17)	0.32	0.04	0.33	0.00	0.00	0.00	0.00
Nervonic acid (C24:1, Cis-15)	0.50	0.00	0.01	0.00	0.00	0.00	0.00
Total SFA	11.92	13.1	20.24	19.77	15.46	17.83	56.02
Total MUFA	41.87	19.96	41.08	37.04	36.46	10.88	13.66
Total PUFA	23.85	59.65	37.87	38.17	44.27	62.66	6.31

period in all the groups. The overall average plasma progesterone concentrations (ng/ml) were 2.80±0.18, 2.96±0.20, 3.14±0.15 and 3.05±0.14 in T_0, T_1, T_2 and T_3, respectively, (Table 5) which did not differ significantly (P>0.05) between the groups but numerically higher values was reported in dietary vegetable oils supplemented groups than control. Perusal of the data in Table 5 also highlighted that number of animals pregnant in 1st artificial insemination (AI) was higher in T_2 group followed by T_1 and T_3 then T_0 and number of animals pregnant in 3rdAI was more in T_1, T_3 and T_0 than T_2. No. of AI per conception remained similar (P>0.05) in all the groups.

The lack of effect on plasma progesterone concentration in the present study is in agreement with Phillipe *et al*. (2014) who reported that dietary supplementation of calcium salts of PUFA did not affect the overall serum P4 concentrations in treatment and control groups. Other studies conducted by Fuentes *et al*. (2008); Llyod *et al.* (2002); Mandebvu *et al.* (2003); Childs *et al.*

Table 4. Effect of different vegetable oils on reproductive performance in buffalo heifers.

Attributes	C	T1	T2	T3
Puberty age (months)	27.35^{c}±0.37	24.19^{b}±0.50	21.79^{a}±0.75	22.11^{a}±0.52
Sexual maturity age (months)	29.27^{c}±0.44	27.33^{bc}±1.39	24.72^{a}±1.53	24.45^{a}±1.67
Conception age (months)	32.23^{b}±3.81	29.03^{ab}±1.71	27.25^{a}±1.30	27.48^{a}±1.26
No. of AI	2.80±0.37	2.60±0.50	2.20±0.37	2.40±0.60
BW at puberty (kg)	350.6±14.27	366.6±14.45	376.6±14.79	380^{a}±28.72
BW at sexual maturity (kg)	404.60^{a}±9.76	426.40^{ab}±10.41	445.40^{b}±12.09	437.20^{b}±17.42

C: no supplementation served as control, T_1: supplemented with mustard oil;
T_2: supplemented with soyabean oil; T_3: supplemented with rice bran oil.

^{abc}Values bearing different superscripts in a row differ significantly (P<0.05).

Table 5. Effect of different vegetable oils on conception rate in buffalo heifers.

Attributes	C	T1	T2	T3
P4 concentration (ng/ml)	2.80±0.18	2.96±0.20	3.14±0.15	3.05±0.14
No. of animals attain puberty	5	5	5	5
No. of animals inseminated	5	5	5	5
No. of animals pregnant at 1st AI	1	2	3	2
No. of animals pregnant at 3rd AI	3	3	2	3
Conception rate (%) at 1st AI	25	40	60	40
Conception rate (%) at 3rd AI	75	60	40	60

C: no supplementation served as control, T_1: supplemented with mustard oil;
T_2: supplemented with soyabean oil; T_3: supplemented with rice bran oil.

(2008) also didn't observe any difference (P>0.05) in serum P4 concentrations by adding calcium salts of PUFA in the diet of heifers and cow. Contrast to our study, Lopes *et al*. (2009), Dirandeh *et al*. (2005), Reis *et al.* (2012) and Fauladi-Nashta *et al*. (2007) observed greater P4 concentrations in dairy cows fed with PUFA rich diets. However, Robinson *et al.* (2002) reported a reduced plasma progesterone concentration in the early luteal phase of cattle supplemented with n-3 and n-6 rich diets compared with control diet.

Fat supplementation may alter the onset of luteal activity by either improved energy balance or a direct effect on the ovary (Lucy *et al*., 1991). Fat supplementation has been used to manipulate progesterone synthesis by altering the concentration of plasma cholesterol in dairy cows (Carroll *et al*., 1990), But the relationship between dietary fat, fatty acid composition, and concentration of plasma progesterone is not fully understood (Petit and Twagiramungu, 2006). PUFA supplementation may increase the P4 concentration by increasing its synthesis via increase in the cholesterol uptake (Stocco and Clark, 1996) or by reducing the hepatic clearance of P4 (Hawkins *et al*., 1995). The lack of effect in present study may be due to fact that fat supplements used were unprotected and may have undergone biohydrogenation process thus reducing the impact on progesterone synthesis (Staples *et al*., 1998).

A tendency for soyabean oil to have greater overall pregnancy rates compared with ricebran oil and mustard oil was observed in the current experiment. In the similar line, Scholljegerdes *et al*. (2011); Bellows *et al*. (2001) reported increased pregnancy rates in heifers supplemented with soybean seeds as compared to controls receiving diets with equivalent energy. Mandebvu *et al.* (2003); Geary *et al*. (2002); Graham *et al.* (2001) also reported improved first service pregnancy rates in cows fed whole soybeans or soyabean oil. On the other hand, Whitney *et al*. (2001) reported no improvement in pregnancy rate among groups supplemented with soybean oil at 3% of the diet to pre-pubertal heifers. Conception rate did not differ in heifers fed lipid supplements (Lammoglia *et al*., 2000; Alexander *et al*., 2002; Whitney *et al*., 2001). The improvement in conception rate by fat supplementation may occur due to increase in size of follicle (Ambrose *et al*., 2006), increase in progesterone concentration favouring the maintenance of pregnancy (Mattos *et al*., 2002) or decrease in $PGF_2\alpha$ synthesis. The decrease in $PGF_2\alpha$ secreation would prevent the regression of the corpus luteum in the ovary and allow the continuous production of progesterone, favouring embryo survival (Bilby *et al*., 2006; Silvestre *et al*., 2011). This discrepancy in the response may be due to variation in the amount of fatty acid actually reaching specific tissues (Scholljegerdes *et al*., 2011) as 70 to 80% of polyunsaturated fatty acids get hydrogenated in the rumen if unprotected (Juchem *et al*., 2007).

Ovarian follicular characteristics

Ovarian follicular characteristics on the day of oestrus in Murrah buffalo heifers in different groups are presented in Table 6. The number of small size follicles (<3 mm) were significant higher (P<0.05) in T_2 as compared to T_1 and T_0 group but at par with T_3. The numbers of small size follicles were 33.33% and 27.27% more in T_2 and T_3 than T_0. Number of medium size follicles (3 to 6 mm) differ significantly (P<0.05) among treatment groups and control group. However, there was no difference in number of large follicles (P>0.05) among treatment and control groups. Total number of ovarian follicles followed a similar trend as shown by

Table 6. Effect of different vegetable oils on follicular characteristics in buffalo heifers.

Attributes	C	T1	T2	T3
No. of small follicles (<3 mm)	1.6^{a}±0.24	1.8^{ab}±0.37	2.4^{c}±0.24	2.2^{bc}±0.2
No. of medium follicles (3-6 mm)	1.2^{a}±0.2	1.4^{ab}±0.24	1.8^{b}±0.2	1.6^{ab}±0.24
No. of large follicles (>6 mm)	1.0±0.00	1.2±0.2	1.4±0.24	1.4±0.24
Total no. of follicles	3.8^{a}±0.44	4.4^{ab}±0.81	5.6^{c}±0.68	5.2^{bc}±0.68
Diameter of large follicle	8.4^{a}±0.50	8.8^{ab}±0.58	9.6^{b}±0.50	9.4^{ab}±0.6

C: no supplementation served as control, T_1: supplemented with mustard oil;
T_2: supplemented with soyabean oil; T_3: supplemented with rice bran oil.
^{abc}Values bearing different superscripts in a row differ significantly (P<0.05).

number of small follicles. Total number of ovarian follicles in T_2 was higher (P<0.05) by 32.14 and 21.42% than T_0 and T_1. The average diameter of the large follicles was significantly (P<0.05) higher in T_2 as compared to control but comparable with other two treatments. Dietary fat has been shown to increase the number and size of follicles in cattle, independent of energy supply (Garnsworthy *et al*., 2008). In the present study numbers of small size follicles were higher in dietary oil supplemented group than control, however, number of large size follicles remained similar (P>0.05) in all the groups. This is independent of energy content of oils as all diets were isocalaoric. Ponter *et al*. (2006) reported dietary supplementation of soybean or linseed increased (P<0.05) the number of small follicles while, the number of medium and large sized follicles was not affected by dietary treatment. In other study Zachut *et al*. (2010) found that higher number of small sized ovarian follicles in linseed oil supplemented Holstein-Friesian cows, whereas numbers of larger follicles were higher in diets rich in sunflower oil group than linseed oil and control. There was no significant effect of rice bran supplementation on ovarian follicle number and progesterone concentration (Webb *et al*., 2001). A larger number of follicles may indicate a change in the process of follicular selection and an increased selection of structures for development which may be helpful in assisted reproductive technology (Nogueira *et al*., 2012).

The diameter of the larger follicle was greater in heifers fed the soybean oil. Ghasemzadeh-Nava *et al.* (2011), reported that dietary supplementation of polyunsaturated fatty acids have no significant effects on the mean number of follicles, however, the size of the largest follicle was significantly (P<0.05) greater in cows consumed a diet containing fish oil or soybean oil. Results of present study are corroborating with, Mattos *et al.* (2000); Robinson *et al.* (2002); Heravi Moussavi *et al.* (2007) who have reported the similar findings as a result of dietary supplementary of fat. The size of the preovulatory follicle has been positively related to improve the overall size of the corpus luteum (CL) and pregnancy rate in cattle (Lopes *et al*., 2009; Gulliver *et al*., 2012). Larger ovulating dominant follicles in heifers, non-lactating dairy cows, and lactating dairy cows resulted in larger CL (Funston, 2004). A large CL is known to produce more P4 which increases the rate of conception (Sartori *et al*., 2002). Dietary fat may enhance follicular development via metabolic hormones that act on the central nervous system

to stimulate GnRH secretion; lipid-supplemented cows have been found to have increased basal LH concentrations (Hightshoe *et al*., 1991) or may affect follicular development through metabolic hormones acting at the ovarian level. Plant oils such as soybean oil are rich in linoleic acid, which has been shown to increase ruminal propionate production, increasing gluconeogenesis and therefore insulin concentration (Chalupa *et al*., 1986). Fat supplements have been found to increase total and HDL-cholesterol concentrations (Thomas and Williams, 1996). As cholesterol is the precursor of all steroids, increased substrate availability may increase follicular steroid synthesis. Other studies by Carroll *et al.* (1990); Petit *et al.* (2002); Mandebvu *et al*. (2003); Ambrose *et al.* (2006) also showed that dietary supplementation of vegetable oil in dairy cows have a larger diameter of ovulatory follicle and larger CL.

CONCLUSION

It is concluded that dietary supplementation of vegetable oils reduced the age at puberty, increased the number of small size of follicles, diameter of the large follicle and improve the conception rate in Murrah buffalo heifers.

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