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EFFECT OF DIETARY FAT ON OVARIAN AND METABOLIC RESPONSE OF HEIFERS SUFFERING FROM OVARIAN INACTIVITY

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SUMMARY

Eleven Holstein Friesian heifers suffering from ovarian inactivity were allocated according to their age and weight to either a normal lipid (NL; n=6, 2.3% ether extract) or high lipid (HL; n=5, 5.8% ether extract) diet. Diets were fed for 55 days and formulated to be almost isonitrogenous. The number and percentage of heifers that showed regular changes in progesterone concentrations consistent with normal cyclic ovarian activity were 1/5 (20%), 2/5 (40%) and 4/5 (80%) up to 10, 20 and 55 days of the treatment period, respectively, in non-cycling heifers receiving HL diet. The comparable results in NL-fed heifers were 0/6 (0%), 1/6 (16.6%) and 2/6 (33.3%), respectively. The differences between the two groups were significant ($p< 0.1$-0.6). Three heifers in HL (60%) and no heifer (0%) in NL showed continuously elevated serum progesterone for at least 35 days after the last recorded estrus and confirmed to be pregnant by rectal palpation 45 days post service. The difference in pregnancy rate between the two groups was highly significant ($p<0.01$). Concentrations of serum total lipids, triglycerides, total cholesterol and high density lipoprotein-cholesterol were 1.26 (10.64±0.79 Vs 8.42±0.41 g/dl; $p<0.03$), 2.12 (218.90±18.53 Vs 103.23±9.3mg/dl; $p<0.001$), 1.66 (141±11.20 Vs 85.60±4.61 mg/dl; $p<0.06$) and 1.27 (38.10±3.71 Vs 29.96±1.16 mg/dl; $p<0.06$) times greater in HL when compared to NL-fed heifers. Moreover, serum progesterone levels during the mid-luteal phase of oestrous cycle tended to be higher ($P<0.19$) in HL than NL (4.97±0.819 Vs 3.61±0.728 ng/ml). Insulin concentration was significantly ($p<0.03$) elevated in heifers receiving HL (14.70±1.75 μμ/ml) than those receiving NL (4.07±1.27 μμ/ml). However, increasing dietary lipids (HL) in the diets did not significantly ($P<0.05$) affect serum concentrations of albumin (3.03±0.16 Vs 2.77±0.19 g/dl), urea (43.30±2.47 Vs 40.93±4.38 mg/dl), Ca (7.17±0.90 Vs 6.33±0.86mg/dl), Mg (2.22±0.08 Vs 2.02±0.18mg/dl), Zinc (0.61±0.11 Vs 0.91±0.14mg/l) Cu (0.73 ±0.04 Vs 0.61±0.1mg/l) or selenium (1.42±0.07 mg/l Vs 1.64±0.12 mg/l). From this study, it can be recommended that dietary fat can be sucessively used as a non

hormonal treatment for inactive ovaries in heifers and to improvize their pregnancy rate.

INTRODUCTION

One of the goals of many dairy management programs is to develop replacement heifers that would conceive at 14 to 16 mo. of age and calve at approximately 2 yr. In heifers, once cyclical activity has commenced at puberty it should continue uninterrupted, apart from pregnancy, throughout the animal life. However, dietary insufficiency or, in some circumstances, dietary excess can have profound effects upon reproductive function during the immediate postpubertal period. The most deleterious effect of inadequate nutrition is the cessation of cyclical activity (ovarian inactivity), although other less severe manifestations could be observed; including silent estrus, ovulatory defects, coception failure as well as fetal and embryonic death (Arthur et al., 1989). In female cattle, a complex array of endocrine, autocrine and paracrine interactions drive the establishment of ovarian steriodogenic capability and the development of a dominant follicle to ovulatory competence (Greenwald and Terranova, 1988). The final stages of this process are linked ultimately to the ability of the hypothalamus-hypophyseal axis to generate the appropriate pattern of gonadotropine release (Goodman 1988), a process that can be attenuated by several intrinsic and extrinsic factors. In spite of the need for sufficient gonadotropic support, Wehrman et al. (1991) hypothesized that the metabolic environment within the follicle itself must be considered as a potentially independent modulator of steroidogenic and maturational capacity. It is well recognized that gonadal activity is indirectly influenced by overall energy balance of the animal through regulation of gonadotropin secretion (Randel, 1990). However, to what extent selected changes in rumen fermentation products (Mason and Randel, 1983), metabolic hormone secretions (Rutter et al., 1989) or available precursor in the metabolic pools (Williams, 1989) act directly on the ovarian physiological processes and activities remain obscure.

Recent works suggest that the change in dietary lipid intake and thus lipid metabolic status may offer a noval approach for exploiting the link between lipoprotein-cholesterol metabolism and ovarian function (Williams, 1989 and Ryan et al., 1992). A cascade of metabolic, morphologic and hormonal changes were reported at the ovarian level through increments of dietary fat intake. Fat supplementation increases serum and follicular fluid cholesterol concentrations and the area occupied by lipids in small and large luteal cells, shortens postpartum anoestrum period, enhances production and / or clearnce of steroid hormones coupled with enhanced follicular development followed by normal luteal function during the first postpartum estrous cycle and increases the life span of induced corpora lutea (Deluna et al.,

1982, Williams, 1989, Hightshoe et al., 1991, Wehrman et al., 1991, Ryan et al., 1992 and Lammoglia et al., 1996). Therefore, on the light of these data, the objective of this study was to evaluate the ovarian and metabolic responses of dairy heifers suffering from ovarian inactivity to dietary fat and to testify if fat supplementation can be used as a non hormonal treatment for anoestrum heifers.

MATERIALS AND METHODS

This work was carried out at EL-Gemeza experimental farm (belonging to Animal Production Research Institute) close to Tanta city.

Animals:

In this farm, routine rectal palpation of the ovaries of Holstein-Friesian heifers were carreid out for diagnosing the cows suffering from ovarian inactivity. The diagnosis depended on the absence of any ovarian structures as proved from two successive examinations with two weeks interval. Such diagnosis was also verified by serum progesterone profile (<1.0 ng/ml) in samples taken during this period. The age and weight range of the examined heifers varied between 2-3 yrs old and 320-380 kg, respectively. All selected heifers were examined rectally twice weekly during the experimental period to detect both the ovarian and uterine changes as well as for pregnancy diagnosis (45 days post-mating).

Dietary treatment:

Eleven Holstein-Friesian heifers suffering from ovarian inactivity were allocated according to their age and weight to either a normal lipid (NL; n=6, 2.3% ether extract (EE), control group) or high lipid (HL; n=5, 5.8% EE, treated group) diet. Elevation of ether extract in the HL was affected by using Hydrogenated Vegetable (palm) Oil (HVO). Formulation, chemical composition as well as daily nutrient intake of the used diets are shown in table 1. Diets were fed for 55 days and formulated to be almost isonitrogenous. They were balanced to meet NRC (1989) requirement for heifers weighing 350 Kg to gain 0.300 or 0.450 Kg/day in NL or HL, respectively.

Hormonal and Metabolic assay:

To monitor ovarian activity and for early pregnancy diagnosis, blood samples for serum progesterone assay were taken at 3-4 days intervals for two weeks prior to the start of dietary treatment and for 75 days later. Progesterone concentrations were determined by radioimmunoassay (RIA) according to Abraham (1981). Intra- and inter-assay coefficients of variation were 10 and 14%, respectively. Heifers exhibiting serum progesterone on at least two consecutive occasions were considered to have initial ovarian luteal activity. Heifers were observed for estrus two times daily and those came in estrus were naturally serviced by a proven bull.

Table 1: Formulation, chemical composition as well as daily nutrient intake of the experimental diets.

Item	Normal lipid (NL)	High lipid (HL)
1- Ingredients (%):		
Cocentrate mixture*	58.82	57.36
Wheat straw	41.18	40.15
Fat (HVO)	---	2.49
2- Chemical composition (DM)%:		
DM	92.00	92.12
TDN	52.84	57.90
CP	12.64	12.34
RUCP**	4.83	4.71
RDCP	7.81	7.63
EE	2.30	5.80
CF	18./47	18.02
Ca	0.60	0.59
P	0.32	0.32
Mg	0.24	0.24
3- Daily intake/head/day:		
DM(kg)	7.80	8.00
TDN (kg)	4.13	4.62
CP (g)	985.92	989.67
EE (g)	179.86	465.16
Ca (g)	46.92	47.31
P (g)	25.02	25.43

HVO-Hydrogenated Vegetable Oil, DM=Dry Matter, TDN=Total Digestible Nutrients, CP=Crude Protein, RUCP=Rumen Undegradable Crude Protein, RDCP=Rumen degradable Crude Protein, EE=Ether Extract, CF=Crude Fiber, Ca=Calcium, P=Phosphorus & Mg=Mg=Magnesium.
*Cosists of 65%undecorticated cotion seed cake, 9% wheat bran, 20 rice polish, 3% rice polish, 3% molasses, 2% CaCo3 and 1% NaCl.
**Calculated according to NRC (1989).

One blood sample was also collected from all heifers on day 20 post-feeding for metabolic assay. Sera were enzymatically assayed for total lipid (TL), magnesium (Mg), urea, albumin (BioMerieux, France), total cholesterol (TCH), triglycerides (TG), high density lipoprotein-cholesterol (HDL-CH) and calcium (Ca) STANBIO, San Antonio, Texas). Serum insulin hormone was measured by RIA according to Taylor (1976). Intra- and inter-assay coefficients of variation were 6.2 and 6.6%, respectively. Serum zinc, copper and selenium were also determined using atomic absorption spectrophotometer (Mod. 3300, Perkin Elemer, USA).

All the obtained data were statistically analyzed using PCSTAT computer program according to Snedecor and Cochran (1982).

RESULTS

Data presented in table 2 indicated that, the number and percentage of heifers that showed regular changes in progesterone concentrations coincided with normal cyclic ovarian activity were 1/5 (20%) 2/5 (40%) and 4/5 (80%) at 10, 20 and 55 days of the treatment period, respectively in non-cycling heifers receiving HL diet. The comparable results in NL-fed heifers were 0/6 (0%), 1/6 (16.6%) and 2/6 (33.3%),

Table 2: Percentage of non-cycling heifers fed either a NL or HL diet for 55 das that showed ovarian response on the basis of serum progesterone profile and their prgnancy rate.

Treatment		% of cyclic heifers up to day:		Pregnancy rate %	
	n	10	10	55	55
Normal lipid (NL)	6	0/6 (0%)*	1/6(16.6%)*	2/6 (33.3%)**	0/6 (0%)***
High lipid (HL)	5	1/5 (20%)	2/5 (40.0%)	4/5 (80.0%)	3/5 (60.0%)

* P<0.10 **P<0.06 ***P<0.01

respectively. The differences between the two groups were significant (p<0.1- 0.6).

One heifer in HL and 4 heifers in NL groups showed continuous basal progesterone concentration indicative of ovarian inactivity.

Data presented in table 2 also revealed that, 3 heifers in HL (60%) and no heifers (0%) in NL dietary treatment showed continuously elevated serum progesterone for at least 25 days after the last recorded estrus and were confirmed to be pregnant by rectal palpation 45 days post service. The difference in pregnancy rate between the two groups was highly significant (p<0.01).

As shown in Fig. 1&2., increasing dietary lipid intake (HL) significantly increased serum concentrations of all lipid profile (hyperlipidemia). Concentrations of serum TL, TG, TCH and HDL-CH were 1.26 (10.64 ± 0.79 Vs 8.42 ± 0.41 g/dl; p < 0.03), 2.12 (218.90 ± 18.53 Vs 103.23 ± 9.3 mg/dl; p < 0.001), 1.66 (141 ± 11.20 Vs 85.60 ± 4.61 mg/dl; p<0.06) and 1.27 (38.10 ± 3.71 Vs 29.96 ± 1.16 mg/dl; p < 0.06) times greater in HL, when compared with NL-fed heifers.

Concentrations of progesterone (P4, Fig. 3) calculated from samples that were collected from cyclic heifers in NL (2 heifers with 3 samples) and HL (4 heifers with 6 samples) during mid luteal phase (day 7-12 of oestrus) of estrous cycle (onset of estrus = day 0) indicated that P4 tended to be higher in HL than NL (4.97 ± 0.819 Vs 3.61

Fig.1. Serum concentrations of TG, TCH and HDL-CH observed on day 25 after feeding heifers on NL or HL diet

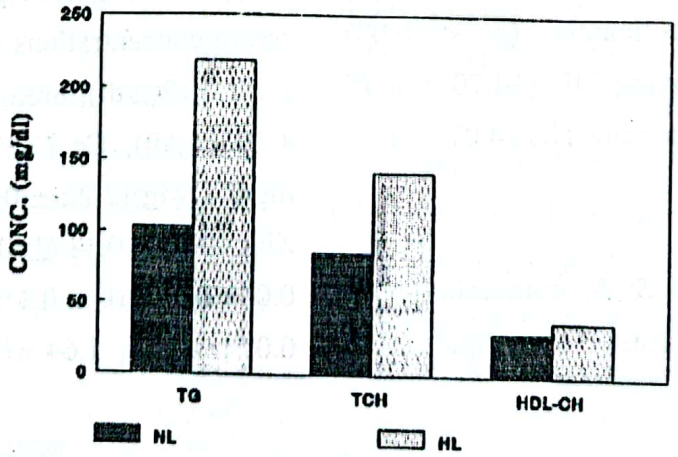


Fig.2. Serum concentrations of TL and Albumin observed on day 25 after feeding heifers on NL or HL diet

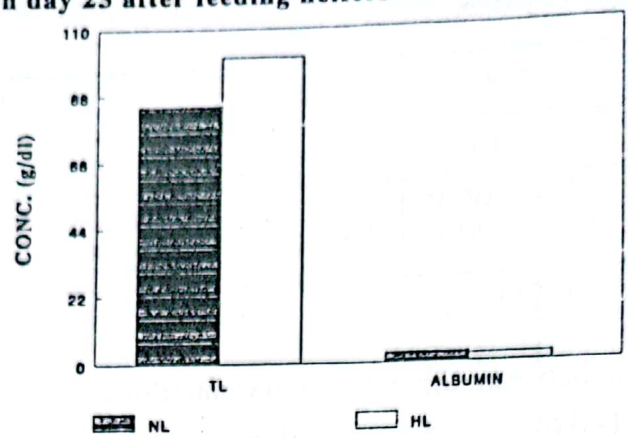
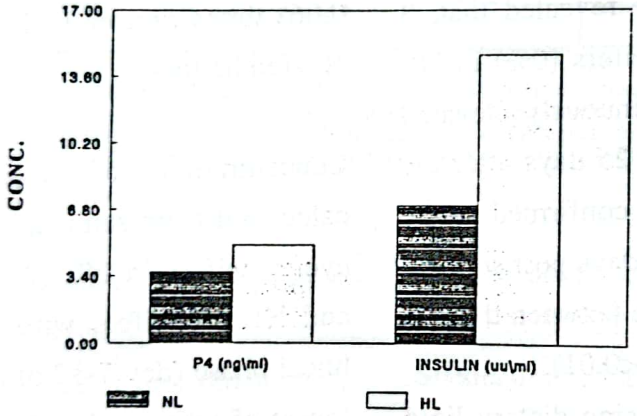


Fig.3. Serum concentrations of P4 (mid luteal phase) and insulin (on day 25 after feeding) observed in heifers fed a NL or HL diet



± 0.728 ng/ml, P<0.91). Moreover, insulin concentration was significantly (p < 0.03) elevated in heifers receiving HL (14.70 ± 1.75 μμ/ml) than in those receiving NL (4.07 ± 1.27 μμ/ml).

As shown in Fig. 2, 4 & 5, comparing with normal lipids (NL), increasing dietary lipids (HL) in the diets did not significantly affect (P < 0.05) serum concentrations of albumin (3.03 ± 0.16 Vs 2.77±0.19 g/dl), urea (43.30 ± 2.47 Vs 40.93 ± 4.38 mg/dl), Ca (7.17 ± 0.90 Vs 6.33 ± 0.86 mg/dl), Mg (2.22 ± 0.08 Vs 2.02 ± 0.18 mg/dl), Zinc (0.61 ± 0.11 Vs 0.91 ± 0.14mg/1) Cu (0.73 ± 0.04 Vs 0.61 ± 0.1 mg/1) or selenium (1.42 ± 0.07 mg/1 Vs 1.64 ± 0.12 mg/1).

Fig.4. Serum concentrations of urea, calcium and Mg observed on day 25 after feeding heifers on NL or HL diet

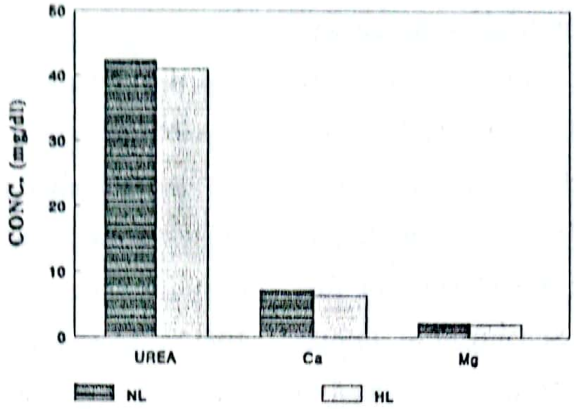
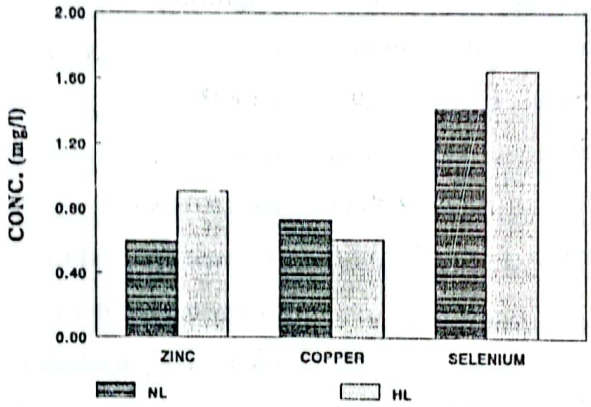


Fig.5. Serum concentrations of zinc, copper and selenium observed on day 25 after feeding heifers on NL or HL diet



DISCUSSION

It is worth to mention that the inclusion rate of HVO in the current study (2.49%) did not exceed the maximum rate of dietary fat (3%) recommended by NRC (1989). Moreover, El-Badawy et al. (1994) did not find any deleterious effect on organic matter (OM), CP, EE and CF digestibility or on rumen fermentation pattern when they used HVO (up to 5%) in sheep diets.

Results of the current experiment support previous data which showed that diets high in lipid content (5-13%) increased the concentration of TL, TCH, TG, and HDL-CL (Park et al., 1983, Talavera et al., 1985, Morgan and Williams, 1989; Williams, 1989, Hightshoe et al., 1991, Wehrman et al., 1991, El-Badawy et al., 1994 and Lammoglia et al., 1996). The increase in serum lipid might be attributed to the depression in lipogenic enzymes activities by the liver and adipose tissues associated with feeding

adipose tissues associated with feeding supplementary fat (Starry, 1981). Feeding long chain fatty acids were reported to induce shifting in the balance from active protomeric to inactive polymeric form of acetyle CoA carboxylase in bovine adipose tissues (Bauman and Davis, 1975). On the other hand, the higher serum cholesterol associated with HVO feeding despite the fact that vegetable oils are cholesterol free agreed with the findings of Schauff et al. (1992) and El-Badawy et al. (1994). It may be related to the dietary unsaturated long chain fatty acids in vegetable oils which may stimulate the *de Novo* cholesterol synthesis (Kritchevsky and Tepper, 1965). Moreover, Wehrman et al. (1991) added that the increase in plasma total lipids and cholesterol was associated with an improvement in steroidogenesis which led to normal cyclic animal.

The significant enhancement in the percentage of heifers that had ovarian activity during the feeding period in HL compared with NL group (80 Vs 33.3%) could be supported by the data of Deluna et al. (1982), Hightshoe et al. (1991) and Williams (1989). This enhancement may be attributed to the improvement of folliculogenesis and/or to the enhacement of LH secretion (Grummer and Carroll, 1991). Observations of altered follicular development in beef (Hightshoe et al., 1991, Wehrman et al., 1991 and Lammoglia et al., 1996) and dairy cows (Lucy et al., 1989) as well as in prepubertal heifers (Ryan et al., 1992) reflect the ability of hyperlipidemic diet to enhance follicular development during prepubertal or postpartum periods by increasing the number of medium sized follicles from which the preovulatory follicles were selected. One possible explanation for such improvement is that the hyperlipidemic diet stimulates androgen biosynthesis that plays a critical role in preovulatory developement (Tonetta and Dizerga, 1989). Meanwhile, the enhancement of LH secretion responsible for ovulation was reported by Deluna et al. (1982), Lucy et al. (1989) and Hightshoe et al. (1991) who observed higher concentration of LH with or without exogenous GnRH during postpartum period in cows-fed fat. Moreover, Ryan et al. (1995) found that anoestrous cows supplemented with dietary fat for 3 weeks and/or treated with a synthetic progesterone before inducing ovulation exhibit an increased tendency to ovulate ($P < 0.09$) compared with non-treated control as well as the duration of the induced luteal phase was also enhanced ($P < 0.04$).

The present study revealed a significant improvement in pregnency rate in heifers fed HL diet. Similar results were reported by Schneider et al. (1988) and Sklan et al. (1989) in cow-fed lipids. Such improvement in conception rate may be related to the enhancement in luteal steroidogensis as recorded in cows fed on hyperlipidemic diets (Grummer and Carroll, 1991). Improved fertility in cattle has been

associated with higher circulating progesterone concentration during luteal phase before (Fonseca et al., 1983) and after insemination (Bulman and Lambing, 1978). Grummer and Carroll, (1988) indicated that, cholesterol is a precursor for luteal cells progesterone synthesis and steroidogensis by luteal tissues from most species in vitro is dependent on the provision of lipoprotein cholesterol. In bovine, HDL-CH would be the major cholesterol source of ovarian steroidgenesis. Our results showed that feeding fat increase both cholesterol and HDL-CH. Moreover, the direct relationship between blood cholesterol and progesterone has been investigated by Talavera et al. (1985), Williams (1989). On the other hand, previous data indicate that, dietary fat may enhance pregnancy rate by reducing the number of subfunctional CL and increasing its life span in female cattle (Williams, 1989). It was suggested that, this phenomenon might result from the enhancement of thecal and/or granulosa cell development before ovulation or an increase in the pool of follicles from which a competent preovulatory follicle is selected from (Ryan et al., 1995). In the present study, serum progesterone was elevated during the luteal phase of estrous cycle in response to high dietary fat. Similar results were reported by Grummer and Carroll (1988), Hightshoe et al. (19991) and Spicer et al. (1993), Lammoglia et al., (1996) in cows and heifers. Wehrman et al. (1991) and Ryan et al. (1992) found that, cultured granulosa cells from preovulatory follicles fed HL diet exhibited an enhanced steroidogenesis capacity (high progesterone production). These may be attributed to increased availability of intracellular stores of cholesterol or increased number of lipoprotein receptors in granulosa cells (Wehrman et al., 1991).

An increase in the concentration of insulin hormone was observed in HL fed group. Similar results were reported by Cummins and Sortin (1987), Spoon et al. (1990) and Ryan et al. (1995). Given the important role of endocrine regulation of lipolysis, it is likely that the signal to reduce fatty acid mobilization from adipose tissues as a result of feeding fat would ultimately be hormonal in nature. Grummer and Carroll (1991) suggested that insulin is one of the metabolic hormones that acts as such a signal. Moreover, Ryan et al. (1995) suggested that, as insulin influences a number of ovarian cellular processes, it is possible that hyperinsulinemia is one mechanism through which high lipid-diets modify ovarian follicular and/or luteal processes.

Concerning serum urea and albumin, the non significant effect of HL on serum urea is supported by the observation of Palmquist and Conrad (1978) who found that feeding dietary fat did not reduce synthesis of microbial protein in rumen (i.e. did not enhance urea formation). On the contrary, Chalupa (1991) suggested that, because fat is minimally fermented in the rumen, it may not provide energy for growth of ruminal

micro-organisms. Thus ration containing supplemental fat may yield less ruminally synthesized microbial protein and should be formulated to contain more undegredable protein (RUCP). This did not seem to be true in the current study as both urea and albumin (indicator of protein status) were not affected by fat treatment. A matter that may be attributed to, uses of low fat% and/or the experimental diets had already higher percent of RUCP (4.71%, table 1) than those recommended by NRC, 1989 (2.1%).

The present results revealed non significant variations in serum Ca, Mg, Zinc, copper and selenium between heifers treated with or without fat supplementation. Similar results were reported by El-Badawy et al. (1994). Although, high fat may bind with calcium, reducing calcium, magnesium (Steele, 1984) and trace elements availability. In the current study, both Ca and Mg% in the used diets (0.7 and 0.24%, respectively, table 1) exceeded that recommended by NRC (1989) which may have prevented hypocalcimia and hypomagnesemia. Bock et al. (1991) found that, feeding of tallow or soybean oil soap with addition of Ca did not affect serum Ca or Mg levels. Moreover, the availability of the measured trace elements were not affected by fat supplementation.

From this study, it can be recommended that dietary fat can successfully be used as a non-hormonal treatment for inactive ovaries in heifers and to improvize their pregnancy rate.

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