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Concentrations of Some Blood Metabolites, Some Hormones and Reproductive Performance of Delayed Pubertal Barki Ewe Lambs after Various Fat Supplementation.

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ABSTRACT

This study was carried out to investigate the impact of magnapac dry fat and vegetable oil supplementation on some blood parameters, some hormones and reproductive performance in non-pubertal ewe-lambs during the summer season. In this investigation fifteen delayed pubertal ewe lambs (age >16 month) were used after being assigned randomly into three groups. First group [Magnapac® supplemented group (DFM), n=5], each lamb was fed the basal diet supplemented with 50 g of DFM for forty five days starting from 1st June while the second group [dry fat derived from vegetable oil treated group (DFV), n=5), each lamb was fed as previous group with the exception of using 50 g DFV instead of DFM. A third group (n=4) served as control. Lambs were exposed to mature, fertile rams daily and blood samples were collected twice weekly. Results elaborated that the addition of dietary fat (DFM and DFV) to the diets of barki ewe lambs led to a significant ($p<0.05$) increase in glucose, cholesterol and total lipids in both 1st and 2nd experimental groups compared with their levels in control group. In the meantime, fat administration resulted in a non-significant increase in the concentrations of triglyceride and leptin among the three groups. Results also showed that the progesterone concentrations (P_4) were significantly higher ($p<0.05$) in treated groups than control one. Reproductive performance of ewe lambs was improved where ovarian cyclicity %, fertility %, lambing%, fecundity and prolificacy rates reached 100% and 80% in 1st and 2nd experimental groups respectively compared to 0 % in control one. In conclusion, this study confirms that the oral administration of dry fat played an essential physiological roles to induce cyclicity in delayed pubertal ewe lambs and improved their reproduction.

Keywords: dry fat; glucose; leptin,; progesterone, delayed puberty.

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INTRODUCTION

Barki breed sheep is one of the subtropical breeds that are raised mostly under unfavorable management and environmental conditions including less availability and seasonal fluctuations in feed resources and heat stress which affect their fecundity and productivity (Aboul-Ela and Aboul-Naga, 1987).

In sheep, the timing of puberty depends on the level of nutrition and photoperiod. If both are satisfactory, the frequency of GnRH and LH pulses increase and stimulate the activity of the gonads leading to puberty (Adam and Robinson 1994). Many hormones are involved in the regulation of the reproductive axis at the onset of puberty by nutrition and leptin is thought to be among the important ones (Shirley et al. 2001). Peripheral administration of leptin advances puberty in normal rodents (Chehab et al.1997) and stimulates the frequency of LH pulses in sheep (Henry et al.2001).

Feeding supplemental dietary fat to the cows increased serum progesterone, serum and increased plasma cholesterol and cholesterol content in follicular fluid and in the CL (Hawkins et al.1995; Lammoglia et al.1996). Cholesterol is a precursor for the synthesis of progesterone by ovarian cells and both high and low density lipoproteins deliver cholesterol to ovarian tissues for steroidogenesis (Grummer and Carroll, 1991). Cows supplemented with fat had increased number and size of large follicles (Lucy et al., 1991), luteal activity (Wehrman et al., 1991), serum progesterone concentrations after the first postpartum ovulation (Hightshoe et al., 1991), and pregnancy rates (Bellows et al., 1999). Thus, study on the effects of feeding supplemental fat on puberty in ewe lambs is warranted.

Few information have been reported in post-pubertal ewe lambs' reproduction so the objective of the present investigation was to elucidate the influence of DFM and DFV supplementation on some blood parameters and reproductive performance in non-pubertal ewe-lambs during the summer season.

MATERIALS AND METHODS

This investigation was done at private Farm in Wadi Elnatroon in summer seasons (May – December). A total of fifteen non pubertal barki ewe-lambs of an age more than 16 months and weight 35-45 kg were used in the present study. Fertile rams were left free with animals twice daily for estrus detection and insemination of ewe-lambs exhibited estrus.

Experimental Materials

Two types of protected fats imported and locally produced were used.

1. Dry rumen protected fat derived from palm oil [Magnapac® imported from Notel, Spain (DFM)]. It contains 84% brute fat and 16% ash.
2. Dry fat derived from vegetable oils [soya bean, sun flower and palm, (DFV)] locally produced by Ibex Co., Egypt. It contains 82.5% brute fat, 2.5 %methionine and 15% Ash.

Experimental design

Experimental animals proved to be non pubertal by repeated progesterone measurements during the previous 3 weeks prior to the experiment (twice weekly, 0.5 ng/ml) were used. Those animals were allocated randomly into three groups (5 ewe-lambs per each group) as follows:

a- 1st Experimental Group

Animals at this group were fed the basal diet supplemented with about 50 g (3% of the diet DM) of DFM for each animal daily according to Funston (2002). That amount correspond a daily intake of 3.56 Mcal ME/head/day and it was fed for forty five days starting from 1st June.

b- 2 nd Experimental Group

Animals at this group were fed as previous group with the exception of using 50 g DFV instead of DFM.

c- 3rd Group

Animals at this group were fed a diet containing concentrate mixture and rice straw. Each animal was fed concentrate mixture at the rate of 0.75 kg/head daily with offering water twice daily and served as control.

Sampling

Regular blood samples were collected twice weekly along the experimental period to monitor progesterone profiles and consequently to assess the ovarian activity throughout the experimental period. Immediately after collection, the samples were centrifuged at 3000 x g for 10 minutes and the plasma was separated and stored at –20°C till analysis.

Blood metabolites' determinations

Determination of plasma cholesterol and triglycerides were done according to Allain et al., (1974) and Bucolo and David (1973) respectively using commercial kits of BioSystems S.A (Spain). Total lipids were assayed according to Frings and Dun (1970) using Cal test Diagnostics kits while glucose levels was determined according to Bergmeyer et al.,(1974) using Bio-Analytics kit.

Hormonal determinations

Measurement of leptin concentrations was done using DRG® leptin ELISA commercial kit purchased from Germany and based on the sandwich principle according to Guillaume and Bjorrntorp (1996). Intra- and inter-assay of coefficients of variation were 3.1 and 9.7% respectively. Sensitivity of the assay was 1.0 ng/ml.

P_4 was measured using ELISA commercial kit for quantitative determination of progesterone in serum or plasma with inter and intra-run precision and had a coefficient of variation of 2.9% and 4.8 % respectively and a sensitivity of 0.05ng/ as the method done by Arakawa et al., (1982).

Statistical Analysis

Statistical significance was assessed using multiple analysis of variance (ANOVA, Statgraphics version 5), followed by multiple comparison LSD range test. Probability values < 0.05 were considered significant. The statistical analysis was computed using SPSS software.

RESULTS

The addition of dietary fat (magnapac and vegetable oil) to the diets of non-pubertal barki ewe lambs led to a significant ($p<0.05$) increase in glucose, cholesterol and total lipids in both 1st and 2nd experimental groups compared with their levels in control group as shown in Fig 1.. In the meantime, fat administration resulted in a non-significant increase in the concentrations of triglyceride among the three groups (fig 1).

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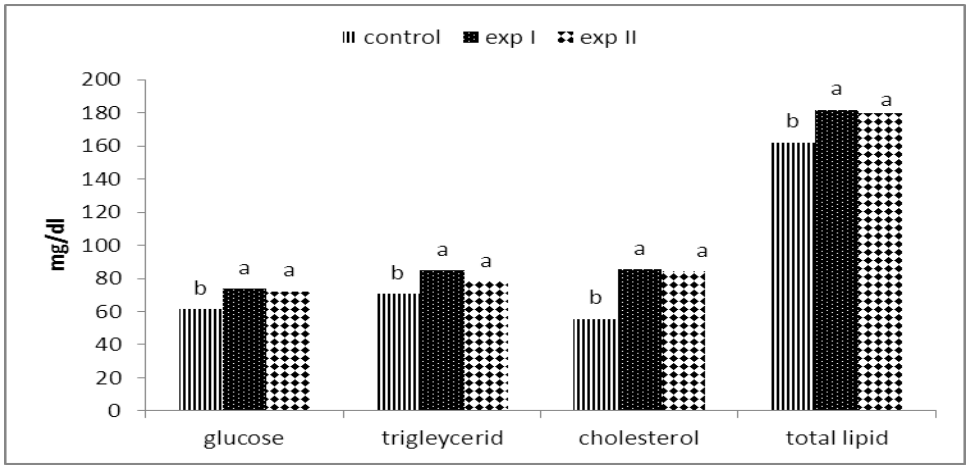

Fig 1: Concentrations of some blood metabolites of barki ewe lambs submitted to different fat supplements

Different superscript letters (a b and c) in the same colum indicate a significant (P<0.05) difference

Leptin concentrations showed a non-significant (p<0.05) increase among 1st , 2nd experimental and control groups throughout the treatment period from 1st day to the 17th day as shown in Fig 2. Starting from the 21st day of fat administration there was a significant (p<0.05) increase of leptin levels (0.66±0.03 and 0.6±0.02) in the 1st and 2nd exp. groups compared with control group (0.33±0.05). Leptin concentrations among the experimental groups and the control ones kept remained elevated till the end of experiment.

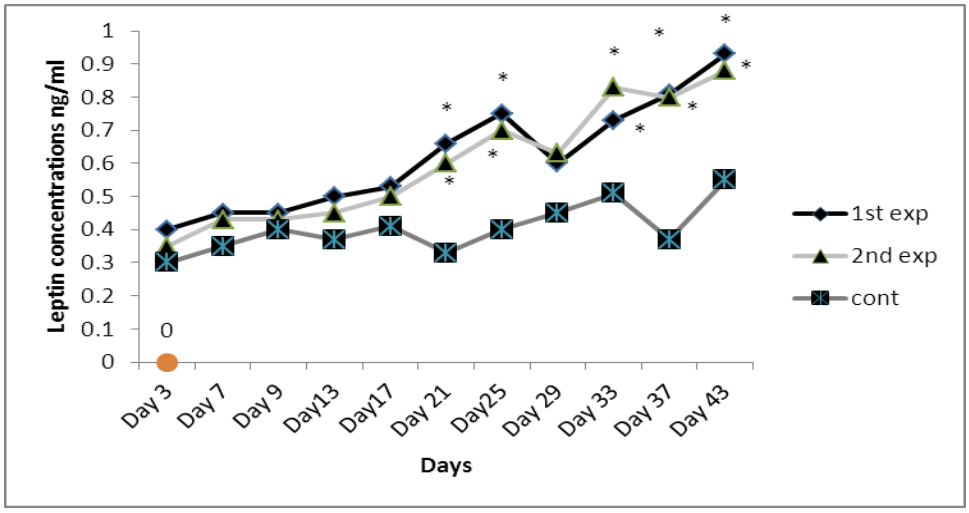

Fig 2. Average leptin concentrations (ng/ml) in experimental and control ewe-lambs

*Value indicates a significant difference (P<0.05) among groups.

It was observed from fig 3 that the plasma progesterone levels (ng/ml) showed a non-significant difference among 1st, 2nd experimental and control groups throughout the treatment period from 1st day to the 17th day as these values were within the basal levels. Starting from the 21st day of administration there was an elevation in plasma progesterone concentrations (0.83±0.26 and 0.51±0.27) in the 1st and 2nd exp. groups compared with control group (0.12±0.05) which indicated that the treated ewe-lambs in the 1st and 2nd groups began to resume their ovarian activities. On the 23[2rd]day of administration, the progesterone levels has increased progressively in the 1st and 2nd groups and remained elevated during the following days till achieving its maximal level on the 43rd day of treatment (2.97±0.65 and 1.93±0.41) which indicated that these animals became pregnant. In the meantime, the mean value of plasma progesterone levels in the 2nd group toward the end of the experimental period was non-significantly (p<0.05) lower than that of 1st exp. Group. Meanwhile in the control group, the plasma progesterone values were within their basal levels (below 0.5 ng/ml) throughout the experimental period indicating that the control animals did not regain their ovarian activity (Fig 3).

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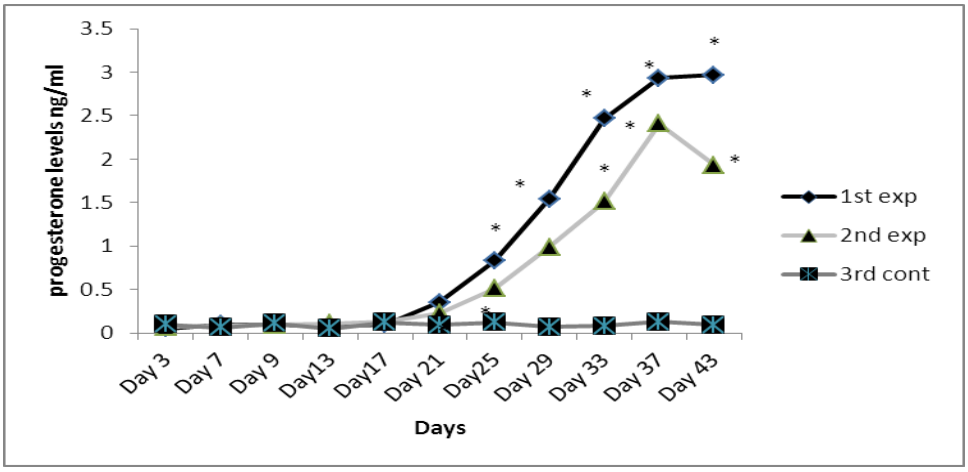

Fig 3.Average progesterone levels (ng/ml) in experimental and control ewe-lambs

*Value indicates a significant difference (P<0.05) among groups.

Reproductive characterestics

The reproductive characteristics of post pubertal ewe-lambs received DFM and DFV were summarized in table (1). Addition of fats to the diets of ewe lambs improved the reproductive performance in which ovarian cyclicity %, fertility %, lambing%, fecundity and prolificacy rates reached 100% and 80% in 1st and 2nd experimental groups compared to 0 % in control one.

Table 1. Reproductive performance of ewe-lambs received various fat supplements

Groups	Ovarian cyclicity %		Fertility %		Lambing %		Fecundity rate		Prolificacy %
	No.	%	No.	%	No.	%	No.	%	
Magnapac received group	5	100	5/5	100	5	100	5	100	100
Vegetable oil received group	5	80	4/5	80	4	80	4	80	80
Control group	5	0	0/5	0	0	0	0	0	0

DISCUSSION

To our knowledge, no controlled trials have been conducted to evaluate the potential for fat supplementation to impact cyclicity and resumption of ovarian activity in delayed pubertal ewes. The current study showed differential effects of fat-based diets on glucose, total lipids, cholesterol, leptin, progesterone levels and reproductive performance of delayed pubertal ewe- lambs. There was a significant increase in glucose values in experimental groups in comparison with control cones. These findings are in agreement with those found by Dicostanzo et al., (1999) who reported that a high glucose level is important if not crucial, for proper function of the reproductive processes. Moreover, Downing et al., (1995) found that infusion of glucose directly into the venous circulation in sheep would increase the ovulation rate.

The present investigation showed that fat supplementation resulted in significant higher levels of total lipids, cholesterol and triglyceride in experimental groups compared to control ones. The current results are in compatible with those recorded in cow by Lammoglia et al., (2000) who found that high fat diet increased cholesterol concentrations. Moreover, the concentration of cholesterol found in the groups of sheep supplemented with protected fat increased significantly in this study, similarly to what was seen by Ghoreishi et al., (2007). Elevated levels of triglycerides in the serum found in sheep supplemented with protected fat may be explained due to the higher amounts of fatty acids released in the abomasum because of the digestion of triglycerides contained in the palm oil. These triglycerides are digested into single fatty acids and a glycerol

molecule, which are absorbed in the small intestine, and they are again re-esterified into triglycerides in the blood (Palmiquist and Mattos, 2006).

Intake of dietary fat in this study increased plasma leptin concentrations. These data coincide with those reported by Stricker-Krongrad et al. (1998) and Bahceci et al. (1999) who recorded high leptin secretion in rats fed high fat diet. However, it is difficult to compare our results with those obtained in laboratory rodents because of protocol differences. First, the dietary treatments were applied for a longer time in the studies in rats. Leptin injection was shown to advance puberty in normal mice (Chehab et al. 1997) rats (Cheung et al. 1997) and food-restricted rats (Gruaz et al. 1998). Additionally, in sheep, Nagatani et al. (2000) and Henry et al. (2001) also found a positive effect of leptin injection on pulsatile LH secretion. Thus, together with the data obtained in the current study, it appears that leptin conveys important information to the LH secretion mechanism about sufficiency of energy stores of body and body energy reserves are also taken into account for enrichment to the puberty.

Results of the current investigation indicated that animals in the 1st and 2nd experimental groups began to resume their ovarian activities starting from the 21st day of administration in the 1st and 2nd exp. groups compared with control group. On the 23rdday of fat administration, the progesterone levels has increased progressively in the 1st and 2nd groups and remained elevated during the following days till achieving its maximal level on the 43rd day of fat addition which indicated that these animals became pregnant. Meanwhile in the control group, the plasma progesterone values were within their basal levels (below 0.5 ng/ml) throughout the experimental period indicating that the control animals did not regain their ovarian activity (graph1).

Lambing percentages reached 100% and 80% in 1st and 2nd experimental groups compared to zero % in control one. The present results agreed with those obtained by McNamara et al., (2003) who found that fat supplements increased conception rate to first service of Holstein cows. Moreover, our current findings are in compatible with El-Banna et al., (2005) and Mehany et al., (2009) who recorded an improved reproductive performance in cows and lactating buffaloes&cows fed fat diets in comparison with the control ones. Grummer and Carroll (1991) reported greater serum progesterone concentrations in cows supplemented with fats, agreeing with the present findings found in this study. Hawkins et al. (1995) reported that elevated serum progesterone concentrations in cows supplemented with fats resulted from a decrease in progesterone clearance rate from the circulatory system rather than from an increase in progesterone secretion by the CL.

The positive influence of fat supplement on induction of puberty and maturity during this investigation may be accredited to one/all of the following physiological mechanisms:

- Intake of dietary fat in increased plasma leptin concentrations which were found to have a positive effect on pulsatile LH secretion and enrichment to puberty (Henry et al., 2001).
- The consumption of polyunsaturated plant oils increases basal serum insulin concentrations which may play a role in mediating increased follicular growth either directly or indirectly by modulating granulosa IGF-I (insulin-like growth factor – 1) production.
- Fat supplementation has also been shown to increase concentration of circulating growth hormone (Williams and Stanko, 1999).
- Energy provided by fat supplementation increases LH secretion in animals deficient in energy (Mattos et al., 2000).
- Feeding supplemental dietary fat also increased serum and follicular fluid cholesterol, serum progesterone, life span of induced corpus luteum (CL), the number of medium-sized follicles, and growth of the preovulatory follicle (Williams and Stanko, 1999).
- Feeding fat increases levels of cholesterol which serves as a precursor for the synthesis of progesterone by ovarian cells and both high and low density lipoproteins deliver cholesterol to ovarian tissues for steroidogenesis (Grummer and Carroll 1991).

In conclusion, this study confirms that the oral administration of fat played essential physiological roles to induce cyclicity in delayed pubertal ewe lambs and improved their reproduction.

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