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POSSIBLE EFFECTS OF SUPPLEMENTATION WITH *NIGELLA SATIVA* CAKE ON REPRODUCTIVE PERFORMANCE, OVARIAN RESPONSE, AND EMBRYO RECOVARY OF FEMALE BALADI GOATS

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SUMMARY

This study is one of many recent attempts that search for economic feed resourses with the aim to improve reproductive effeciency of the local breeds of farm animals in Egypt. Thirty female baladi goats were used in two experiments. In experiment 1, twelve immature females wieghed 8-11 kg and aged 4-5 months were alloted into two groups : control (n = 4) fed on basal control ration and NSc group (n = 8) fed on the same basal ration supplemented with 100gm nigella sativa cake. All females were fed till maturity and run continuously with fertile bucks. Jagular blood samples were collected towice weekly for progesterone assay to detect sexual maturity, ovarian cyclicity, conception and pregnancy. The parameters of the reproductive characterestics in the pattern of continuous/total, lambed/continuous, conception, fertility, and prolificacy were calculated. Initial and final body weights were individually recorded and body weight gain was calculated. Five months postfeeding , three females from each group were slaughtered and blood samples were collected for determination of plasma protein and lipid indicators. Samples of rumen contents were taken for determination of pH, ammonia, volatile fatty acids (total and individual) and protozoal activity. Right and left avaries were desiccated immediately after slaughtering and ovarian weights and structures were recorded. In experiment 2, eighteen mature female baladi goats of 1.5-2 years were allocated into two groups: control (n=8) and NSc (n=10) receiving

the same rations as in the experiment 1. The oestrus was synchronized by withdrawing progestagen-impregnated pessaries 14 days after their intravaginal insertion. Two days prior to pessaries removal, each doe received 800-1000 i.u. equine Chorionic Gonadotrophin. Twenty four hours, does were observed for heat, then naturally mated with fertile buck for 2-3 times during the estrus period. Assessment of the superovulatory response was carried out on day 6 following estrus. Both ovaries were examined morphologically and the number of corpora lutae and follicles >5 were recorded. The uterine horns were flushed and the recovered embryos / ova were counted, evaluated, and clasiified according to thier stage and morphology. Plasma total protein, albumin, total lipids, and triglycrides were not significantly changed. However, plasma urea and cholesterol were significantly decreased on supplementation with Nigella sativa cake. Analysis of rumen juice revealed that the total volatile fatty acids, the molar proportion of propionate were increased, while the molar proportion of acetate, and accordingly the A/P ratio were decreased in (NSc) group. However, the values of pH, ammonia, the molar proportion of butyrate and the protozoal activity were not affected by the addition of Nigella sativa cake. There was a marked increase in the reproductive indexes of does supplemented with Nigella sativa cake, where the percentages of conception, fertility and prolificacy were 37.5, 37.5, and 100 % versus Zero in control does. The final body weight was increased significantly in does of (NSc) group 15.04 ± 0.82 Kg than controls. Consequently the weight gain is significantly higher in (NSc) groups than control. The number of corpora lutea was significantly increase in (NSc) group than the control one. Similarly, the total ovarian responses (TOR) was also significantly increased in (NSc) group than the controls. However, the ovulation rates among the groups were not significantly different. The total percentage of transferable embryos was significantly higher in (NSc) group compared to control one. Meanwhile the percentage of non-transferable embryos (Fair) was significantly ($P<0.001$) lower in treated group (38.98 %) compared to control one (80.00 %). In addition the recovery rate for transferable embryos was significantly higher in treated group (28.57 %) than in control one (11.76 %). The total embryo recovered was not significantly affected by dietary treatment

Kay wards: Nigella sativa , goats , Reproductive performance , blood and rumen profiles

INTRODUCTION

Energy availability influences reproduction fitness. Changes in an animal's energetic status can result in modulation of the hypothalamic-pituitary - gonadal axis (Cunningham et al., 1999). Suppression of pulsatile LH secretion has been documented after fasting or feed restriction in myriad species, including sheep and goats (Thomas et al.,

1990). In addition to its effect on reproductive hormonal profiles, feed restriction can delay the timing of puberty onset (Foster and Olster, 1985 and Hall et al., 1990) and directly affect the other reproductive processes (Dickerman et al., 1993 and Gill and Rissman, 1997).

Many factors affect the ovarian response to superovulatory treatments, recovery rate and embryo quality. The level of dietary energy pre and postmating play an important role in ovarian response to gonadotrophins. Mani et al. (1992 & 1993) concluded that low level of feeding in goats pre and postmating resulted in the delay and suppression of estrus, reduced ovulation rate, decreased incidence of multiple ovulation and increased loss of embryos.

The mechanisms by which ovulation and ovulation rate are modulated by nutrition is a matter of speculation. Increased nutritional levels may result in increased hepatic mixed function oxidase activity, thereby increasing the rate of degradation of steroids and lessening the inhibition of the hypothalamic-pituitary axis and resulting in increased gonadotrophin secretion (Kirkwood and Aherne, 1985 and Thomas and Williams, 1996)

Poor reproductive efficiency of local breeds of farm animals in Egypt is mainly due to nutritional factors. Large shortage in animal's feedstuffs obligate workers to search for new cheaper uncommon feed sources to fulfill the nutrients requirements and energy demands.

Nigella sative seed is one of the most important medicinal plants. After oil extraction, considerable amounts of Nigella sativa cake become available. This cake is a suitable meal to be included in the diets of farm animals as it contains some remained oil and more than 30% protein (Abdel-Aal and Attia, 1993; Khalifah, 1995; and Zeweil, 1996).

Previous studies investigated the effects of feeding diets containing Nigella sativa cake on productive performance of sheep (Awadalla, 1997 and Gabr et al., 1998), buffaloes (Youssef et al., 1998), and poultry (El-Ghamry et al., 1997). They recorded improved feed conversion, quantity and quality of produced meat, and increased economic efficiency.

These beneficial possitive effects make it important to fully investigate the possible effects of Nigella sativa cake on reproductive potentials of farm animals. The present study was carried out with the aim to clarify the effect of Nigella sativa cake as a supplement on: (1) Plasma and Rumen metabolic profiles, and subsequent reproductive performance of growing female baladi goats (2) superovulatory responses of baladi does treated with equine Chorionic Gonadotrophin (eCG) with special focus on ovarian responses and embryo quality.

MATERIALS AND METHODS

A total number of thirty female baladi goats were used in two experiments, carried out at April in the experimental farm of National Research Center, in Abi-Rawash.

Experiment 1

Twilve immature females aged 4-5 months and weighed 8-12 kg were randomly alloted into two groups, control (N=4) fed on 750 g maintenance ration /head daily recommended by NRC (1989), and treated one (N=8) given the same basal ration supplemented with 100 g / animal of Nigella sative cake. The chemical composition, ingredients, and daily intake of experimental rations are illustrated in table 1. All females were fed in an open shed till maturity and run continuously with the bucks. Jagular blood samples were collected twice weekly for progesterone assay to detect sexual maturity and ensure ovarian cyclicity, conception and pregnancy (Continious P4 profile > 1 ng/ml over at least 25 days post mating as recorded by Pope et al., 1989). The parameters of the reproductive performance and frequency of cyclicity in the pattern of continuous/total, lambed/ continuous, conception , fertility, and prolificacy were calculated. Initial and final body weights of all animals were individually recorded and body weight change (gain) was calculated. Five months postfeeding, three females from each group were slaughtered and blood samples were collected for determination of plasma protein and lipid indicators. Samples of rumen contents were taken for determination of pH value, ammonia production, volatile fatty acids (total and individual) and protozoal activity. Right and left avaries were dessicated immediately after slaughtering and ovarian weights and structures were recorded.

Experiment 2

Eighteen mature female baladi goats of 1.5-2 years were allocated into two groups and received the same rations as recorded in the experiment 1. The oestrus symptoms and duration of oestrus were recorded for all does. Synchronization of oestrus was achieved by withdrawing progestagen-impregnated pessaries (Containing 60 mg Medroxy progesterone acetate, veramix, Upjohn, USA) 14 days after their intravaginal insertion. Two days prior to pessaries removal, each doe received 800-1000 i.u. (eCG) equine chorionic gonadotrophin (Folligon, intervet, Holland). Twenty four hours, does were observed for heat, then naturally mated with fertile buck for 2-3 times during the estrus period. Assessment of the superovulatory response was carried out at laporotomy on day 6 following estrus (McKelvey et al., 1989). The animals were generally anaesthetized with xylazine (Rompn, Bayer), 0.2 mg/kg intramuscularly and followed by injection of sodium thiopental 15-20 mg/kg bw (Dorn and Kraemer, 1987). Both ovaries were examined morphologically and the number of corpora lutae (Functional and Regressed) and follicles >5 were recorded. The uterine horns were flushed with 40

ml Dulbecco's phosphate buffer saline (PBS) enriched with 2% Bovine Serum Albumin (BSA) using silicon two way folly catheter. The recovered embryos / ova were counted, evaluated, and clasiified according to thier stage and morphology under stereomicroscope.

Data Collection techniques

Plasma levels of total protein, albumin, urea, triglycerides, and cholesterol were determined using Stanbio kits (USA). Detrmination of plasma total lipids was performed using kits of CDI (Cal-Tech Diagnostics, INC, USA). Plasma Progesterone was measured in all samples (RIA) by the Coat-A - Count technique using kits of DPC (Diagnostic Products Corporation, Los Anglos, USA). Several parameters of rumen metabolic profile were determined as recorded by Badawy (1992). Reproductive efficiency indexes including conception rate (no. of does lambed/no. of does exposed) x 100, fertility percentage (no. of lambs born/no. of does exposed) x 100, and prolificacy percentage (no. of lambs born/ no. of does lambed) x 100 were calculated according to Doroliya et al. (1990)

Statistical analysis

One way analysis of variance (ANOVA) was used for comparing means of rumen metabolic profile, body weight, ovarian weight, and ovarian responses to superovulation in both control and treated groups according to steel and Torrie (1980) using the linear models procedure available in SAS software (SAS, 1986). Meanwhile the embryo recovery data was analysed using Microstate computer program, copyright (C) 1984 Escosoft, Inc., USA.

RESULTS

Experiment 1

The effect of the Nigella sativa cake supplementation on some plasma biochemical parameters are represented in Table 2. Plasma total protein and albumin are not significantly changed. However, plasma urea is significantly decreased in (NSc) group than controls. At the same time plasma total lipids and triglycrides are not affected, while plasma cholesterol is significantly decreased on supplementation with Nigella sativa cake.

Rumen metabolic profile of baladi goats supplemented with Nigella sativa cake is represented in Table 3. Analysis of rumen juice revealed that the total volatile fatty acids (TVFAs) is significantly increased in (NSc) group than control, while the values of pH and ammonia are not changed.Supplementation with Nigella sativa cake increase the molar proportion of propionate, decrease the molar proportion of acetate, and accordingly decrease the A/P ratio, but not affect the molar proportion of butyrate. The protozoal activity in the pattern of number, motility and viability are not affected by the addition of Nigella sativa cake.

The effect of Nigella sativa cake supplementation on the reproductive performance and cyclicity of female baladi goats during sexual maturity are presented in Table (4). Data of the reproductive characterestics accompanied with the progesterone profile indicated that the number and percentage of does that showed regular changes in progesterone concentration coincide with the ovarian activity, where three does of (NSc) group showed estrus behaviour and accepted the male. However, continuous basal progesterone level (below 1ng/ml) of control does indicated ovarian inactivity. These results were confirmed by the gross weight and appearance of the ovaries obtained from sloughtered females, where there is a significant increase in the ovarian weights and activities in (NSc) group compared to control. Moreover, percentage of does showed normal and continuous cycling is 75 % in (NSc) group and 50 % in control one.

There is a marked increase in the reproductive indexes of does supplemented with Nigella sativa cake, where the percentages of conception, fertility and prolificacy were 37.5, 37.5, and 100% versus Zero in control does (Table 4). Concerning the body weight gain the intial body weight is not significantly different between groups. However the final body weight is increased significantly in does of (NSc) group 15.04 ± 0.82 Kg than controls (12.5 ± 0.50 Kg). Consequently the weight gain is significantly higher in (NSc) groups than control (4.58 ± 0.8 and 1.50 ± 0.3 Kg respectively).

Experiment 2

The data represented in the Table 5. revealed that the number of unovulated follicles was higher but not significantly different by feeding regimens ($P< 0.05$) between (NSc) and control groups (9.56 ± 2.43 and 3.86 ± 1.61 resp.). The number of corpora lutea was significantly increase ($P<0.05$) in (NSc) group (14.00 ± 2.43) than the control one (4.86 ± 1.88). Similarly, the total ovarian responses (TOR) was significantly ($P< 0.01$) increased in (NSc) group than the controls (23.56 ± 2.91 and 8.86 ± 2.89 resp.), however, the ovulation rates among the groups were not significantly different.

The total percentage of transferable embryos (Table 6) was significantly ($P<0.05$) higher in (NSc) group compared to control one (61.02 and 20.00 % resp.). Meanwhile the percentage of non-transferable embryos (Fair) was significantly ($P<0.001$) lower in treated group (38.98 %) compared to control one (80.00 %). In addition the recovery rate for transferable embryos was significantly ($P<0.001$) higher in treated group (28.57 %) than in control one (11.76 %). The total embryo recovered was not significantly affected by dietary treatment. Although the (NSc) group had lowest recovery rate (46.83 %), it had highest

percentage of transferable embryos (61.02 %) and lowest percentage of fair embryos (38.98 %). On the contrary, the control group had highest recovery rate (58 %) but it had highest percentage of fair embryos (80 %) and lowest percentage of transferable embryos (20%).

Table 1: Ingredients (%), chemical composition, and daily intake of basal (control) and Nigella sativa cake supplemented (NSc) rations.

Items	Control ration	(NSc) ration
Ingradient (%)		
Conc. Mix	42.86	40.54
Wheat straw	57.14	54.06
Nigella Sativa oil seed meal	----	5.40
Total	100	100
Chemical compositions		
(DN (%)	92.30	91.95
Crude protein (%)	8.47	9.34
TDN (%)	52.86	54.06
EE (%)	1.470	2.299
Daily intake/head/day		
DM intake (g)	1560	1660
Crude Protein (g)	132.13	155.04
TDN (Kg)	82.46	87.74
EE	22.93	38.16

Table 2: Plasma biochemical parameters of Baladi does in control (C) and Nigella sativa (NSc) groups.

Variables	Treatments	
	C	NSc
Total protein	8.85 ± 0.30	8.78 ± 0.15
Albumin	6.54 ± 0.19	6.21 ± 0.12
Urea	22.1 ± 3.1**	13.4 ± 1.3
Total lipids	386.5 ± 11.07	378.1 ± 6.1
Cholesterol	145.3 ± 4.5**	127.6 ± 4.2
Triglycerides	99.6 ± 5.5	99.8 ± 6.6

** Significant at P <0.05.

Table 3: Rumen metabolic profile of baladi goats in control (C) and Nigella sativa supplemented (NSc) groups.

Group	PH	Ammonia (mg/dL)	TVFAS (meq/dL)	Individual VFAs (%)					Protozoal Activity		
				Acetic	Propionic	A/P ratio	Butyric	Isovaleric	Number	Motility	Visibility
C	6.45 ± 0.40	14.35 ± 1.48	9.13 ± 5.16	48.73** ± 7.52	34.04 ± 3.16	1.43** ± 0.12	14.78 ± 2.11	2.45 ± 0.15	+++	+++	75%
NSc	6.23 ± 0.41	12.55 ± 1.76	11.57 ± 7.53	34.17 ± 5.89	42.27** ± 4.47	0.80 ± 0.15	20.99 ± 2.75	2.57 ± 0.11	+++	+++	80%

** $P < 0.01$

* $P < 0.05$

Table 4: Subsequent reproductive performance and Frequencies of Cyclicity of Baladi goats in control (C) and Nigella sativa (NSc) gorups.

Variables	Treatments	
	C	NSc
No. of does exposed	4	8
Continuous/Total[1]	2/4 (50%)	6/8 (75%)
No. of does lambed	Zero	3
Lambed/continuous[2]	Zero	3/6 (50%)
Conception (%)[3]	Zero	37.5
Fertility (%)[4]	Zero	37.5
Prolificacy (%)[5]	Zero	100
Ovarian weight (g)		
Right ovary	0.30 ± 0.06	0.60 ± 0.05
Left ovary	0.23 ± 0.03	0.51 ± 0.04
Ovarian structures	Small smooth ovaries, however small follicles were seen	Multifollicles (3-4 large and mediem sized follicles)
Body wieght		
Initial B wt. (Kg)	11.0 ± 0.76	10.46 ± 0.59
Final B wt (kg)	12.5 ± 0.50	15.04 ± 0.82**
B wt change	1.50 ± 0.3	4.58 ± 0.8**

1: No. of does with contnuious cycling/total No. of does.
2: No. of does lambed/No. of does with continus cycling
3: No. of does lambed/No. of does exposed.
4: No. of lambed born/No. of does exposed.
5: No. of lambed born/No. of does lambed.
** Signivicant at P<0.05.

Table 5: Ovarian responses in superovulated Balady does in control and Nigella Sativa cake supplemented (NSc) rations.

Animal group	No. of does	Unovuated follicle	Corpora lutea (CL)	Total Ovarian Responses (TOR)	CL/TOR
NSc	9	9.56 ± 2.43^{a}	14.00 ± 2.43^{a}	23.56 ± 2.91^{a}	0.58 ± 0.09^{a}
Control	7	3.86 ± 1.61^{a}	4.86 ± 1.88^{b}	8.86 ± 2.89^{b}	0.55 ± 0.16^{a}
Ttoal	16	7.06 ± 1.66	10.00 ± 1.94	17.13 ± 2.75	0.57 ± 0.08

a,b: Values having the same letters in the same column are not significanlty different

Table 6: Embryo quality and quantity in superovulated Balady does in Control and Nigella Sativa cake supplemented (NSc) rations.

Animal group	Transferable embryo stages No. (%)			Recovery rate for transferable embryo (%)	Non-transferable embryo No. (%)	Total embryo recovered	Recovery rate* (%)
	Excellent	Good	Total		(Fair)		
NSc	24 $(40.68)^{a}$	12 $(20.34)^{a}$	36 $(16.02)^{a}$	28.57^{a}	23 $(38.98)^{a}$	59	46.83^{a}
Control	3 $(15.00)^{b}$	1 $(5.00)^{b}$	4 $(20.00)^{b}$	11.76^{b}	16 $(80.00)^{b}$	20	58.82^{a}
Ttoal	27 (34.18)	13 (16.46)	40 (50.63)	25.00	39 (49.37)	79	49.38
P value	P<0.02	P<0.05	P<0.001	P<0.02	P<0.001		P<0.12

a,b: Values having the same letters in the same column are not significanlty different

* Recovery rate = Number of recovered embryos/number of CL.

DISCUSSION

Experiment 1

Feeding of Nigella sativa cake to Baladi goats had no effect on plasma total proteins and albumin. This result agrees with those obtained by El-Ekhnawy et al. (1999). Other investigators (Abdel Aal and Attia, 1993, Khodary et al., 1996, Nassar, 1997, and Youssef et al., 1998) used Nigella sativa seeds and found a significant increase in the plasma total proteins, which might be due to the increase in the globulins level. Otherwise, Nassar (1997) reproted no significant changes in plasma albumin level on feeding of different forms of Nigella sativa in the ration of baladi cockerels. Plasma urea showed no significant difference between groups. Dissimilar result was obtained by El-Ekhnawy et al. (1999) who reported an increase in plasmsa urea with the supplementation of 150-250 g Nigella sativa cake. The present data showed a significant decrease in plasma cholesterol level, which was previously recorded by El-Dakhakhny et al. (1997), which might be attributed to the high content of unsaturated fatty acids, mainly linolinic (Barowiez et al., 1997)

Rumen juce analysis revealed that TVFAs had a significant increase in (NSc) group, which could be attributed to the high organic matter content (90.5 %) of Nigella sativa cake (Youssef et al., 1998). The low ammonia level due to Nigella sativa cake supplementation indicated slow release of protein degradation in spite of the relative high level of crude protein content (29.3%) of Nigella sativa cake (Gabr et al., 1998).

The present study revealed that the most consestent action of Nigella sativa cake on rumen fermentation is its ability to increase the molar proportion of propionate at the expense of acetate, and thus theoretically increased the efficiency of converting feed energy available to the animal. The concept that the propionic acid fermentation was energetically more efficient than either the acetic acid or butyric acid fermentations based on many factors. Smith (1971) reproted that propionate was utilized by the ruminants tissue more efficienty than acetate. Another possible advantage of propionate was that it was more flexible as an energy source than acetate. Propionate enjoyed the luxury of having the potential to be used for glucineogenesis in addation to direct oxidation by the citric acid cycle (Badawy, 1992). Moreover, the efficiency of microbial protein synthesis was markedly higher with the propionate as opposed to the acetate fermentaion pattern as recorded by Ishaque et al. (1971).

The present data indicated that the supplementation with Nigella sativa cake enhance the sexual maturity and improve fertility parameters of growing baladi goats. Improved reproductive performance and fertility with supplementation of Nigella sativa cake was previously recorded in buffaloes by Youssef et al. (1998) and in Barki

ewes by El-Ekhnawy et al. (1999). Also, final body weight gain was significantly higher in does fed supplemented Nigella sativa cake ration. This result coincide with those obtained by Sharobeem (1996) in the albino, however, other investigators (Zeweil, 1996, El-Gohary, 1997, Awadalla, 1997) observed no effect of Nigella sativa cake on body weight and growth rate. Increased body weight and growth of (NSc) group might be attributed to high nutrient content and antimicrobial effect of Nigella sativa cake which act as a growth promotor agent (Rathee et al., 1982).

The current study in addition to the previous investigation, lead us to suspect that the positive reproductive impact of NS cake in growing female baladi goats may be attributed to :

1- Increased ruminal production of propionate due to feeding of Nigella sativa cake caused elevation of plasma insulin and glucose turnover which are important for the proper function of the reproductive processes (Ebling et al., 1990 and Pope and Hallford, 1991).
2- The higher content of unsaturated fatty acids especially linolinc acid originated from Nigella sativa (Al-Gaby, 1992), was recorded to improve reproductive response and fertility (Filley et al. 2000).
3- Increasing the energy content of the ration due to high content of crude protein and ether extract of NS cake, which raised the growth rate and body weight gain that enhance puberty and sexual maturity (Robinson, 1990 and Adam and Robinson, 1994).

Experiment 2

In the current study, the percentage of corpora lutea (CL) was significantly higher In (NSc) group which may explained by the higher ovulation rate. In this respect, Nigella sativa known to contain high amount of propionate which cause elevation of LH concentration and pulsation that lead to increase the ovulation rate (DiCostanzo et al. 1999). The total ovarian responses (TOR) was also significantly higher in (NSc) group than the control one, which may be attributed to high fat content of Nigella sativa cake. These results coincided with those obtained by Ryan et al. (1992) and Lammoglia et al. (1996) in dairy and beef cows, who found that the changes of follicular development associated with feeding of high fat ration reflected its ability to enhance follicular development. It could alter specific aspects of ovarian steroidogenetic potential and increase the population of medium sized follicular theoretically available to respond to gonadotrophin treatment in superovulation regimens (Wehrman et al., 1991).However, there is a significant elevation in the formed number of unovualted follicles. This result was confirmed by Houghton et al. (1995) who recorded that the treatment with high oil diet enhanced the fertility and conception rate. Moreover, Ryan et al. (1992) reproted that the unsaturated fatty acids constituted 84.82 % of total acids of Nigella sativa increased medium sized follicles in cattle.

The recovary rate of transferable embryos was higher in (NSc) supplemented does than the control which may be due to high fat content of NS cake and consequently elevation of the plasma progesterone level as well (Hawkins et al,1995). It was found that the level of progesterone at the day of recovary can affect the number of recovered embryos (Ismail et al., 1992 and Mansour, 1993) and embryo quality (Jensen et al., 1982, Lindsell et al., 1986). The percentage of fair (non-transferable) embryos was significantly lower in (NSc) group than control. This result was confirmed by Gunn (1983) who concluded that embryos of ewes kept in poor condition and low nutrition during breeding were to be detrimental to embryo survival.

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