

EFFECT OF SOME NATURAL IMMUNE POTENTIATORS ON THE PERFORMANCE OF BALADI DOES

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ABSTARACT

Fifteen Baladi does aging 8-10 months old and weighing 15-16 Kg were used as experimental animals. Does were reared at the National Research Center Experimental Farm (Abo Rawash, Giza, Egypt). Does were kept free and away from bucks and were randomly allocated into ~~three~~ equal groups for performing feeding trials. Does were fed on the farm ration alone (R1), or the farm ration and injected subcutaneously by propolis (1 ml/head) every 15 days (R2) or fed on the farm ration contained 100 g of black cumin cake / animal/day (R3). Immediately at the end of the feeding period, the effect of supplementation on digestibility, nutritive values, rumen activity of rations were investigated and does were injected with 2 doses of prostaglandins and were allowed to be mated by bucks and followed up till kidding and some reproductive and kidding parameters were recorded.

Results indicated that supplementation improved the average daily gain by 21.4% and 28.5% for propolis group and Nigella group, respectively. The results of digestibility trials indicated that total dry matter intake, g/kg BW increased with *Nigella Sativa* than that of propolis. Nigella supplementation improved the digestibility of DM, OM, CP and CF in contrast to NFE digestibility which was lower for nigella than that of propolis group. Adding nigella tended to improve the nutritive value of basal diets. Ruminal pH value significantly decreased 3 hours post feeding while, NH₃-N and TVFA'S concentration significantly increased. From the reproductive point of view, conception and twinning rate as well as kids vigor improved after supplementation with propolis and nigella. Also, general health status and liver function improved after supplementation as shown by the cellular and biochemical changes in the blood, especially the increased gamma globulins and the hepatoprotective effect.

It could be concluded that propolis and nigella improved the productive and reproductive performance of Baladi does.

Keywords : Does-Propolis-Nigella Sativa cake-production-reproductive performance

INTROUDCTION

Goats are an important cheap source of good quality animal protein. However, the limited experimental work that has been carried out indicated the inefficient productive and reproductive performance of this species under the Egyptian condition (Ahmed, 2004). This problem is mainly related to nutritional factors. This obligate researchers to search for new non-conventional feed sources to fulfill the nutrient requirement and energy demand.

Nowadays there are new trend to improve the reproductive performance of farm animals using herbal life style and dietary choices that can help the whole body and improve the immunological status of the animal and consequently improve the productive and reproductive performance.

Propolis (Bonomi *et al.*, 2002-b) and *Nigella Sativa* (Black cumin seed), (Saleh *et al.*, 2002) gave satisfactory results when used to improve the reproductive performance of farm animals.

Propolis is a resinous material collected by bees from different plant exudates (Bankova *et al.*, 1996). It increased the total volatile fatty acids concentration without affecting the dry matter intake, rumen pH, ammonia nitrogen and microbial protein concentration in steers (Stradiotti *et al.*, 2004). It improves the general health status and body weight gain and feed utilization of calves at weaning (Bonomi *et al.*, 2002-a) and promotes both cellular and humoral immune response in domestic animals and poultry industry (Kong *et al.*, 2004). Also, it has an anti inflammatory (Wang *et al.*, 1993), antimicrobial (Koo *et al.*, 2000; Hegazi and Abdel-Hady, 2002), antioxidative (Neiva *et al.*, 2000 and Hegazi and Abdel-Hady, 2003) and antiviral (Hegazi *et al.*, 1993) and antifungal (Kujumgiev *et al.*, 1999) effects. As well as an immuno-stimulating effect (Hegazi *et al.*, 2004) and hepatoprotective activity (Popoca, 2004).

Nigella sativa is a herbaceous plant that contain high protein content, crude fat and major minerals such as calcium, phosphorus, potassium, magnesium and sodium (Abdel-Aal and Attia, 1993). When undecorticated cotton seed meal was substituted by *Nigella sativa* cake and given for calves it increased the daily weight gain by about 8.8% and improves almost all nutrient digestibilities and feeding values as TDN and DCP. *Nigella* contains materials known as Nigellon, Thymoquinone and Thymohydroquinone that known to possess antimicrobial effect and was found to enhance production of interleukin-3 and 1 beta by lymphocytes suggesting it has an effect on macrophages (Haq *et al.*, 1995). Also, it influence the general health condition represented by increased hematocrit and hemoglobin values (Zacui *et al.* 2002). The hepatoprotective effect of *Nigella sativa* was recorded in some models of bucks as it succeeded to correct the changes in enzymatic activities (ALT, GGT, AP) and albumin content in serum, while it decreased cholesterol level and elevated the thyroid function and improved the reproductive performance of treated animals (Daghash *et al.*, 1999).

The current study aimed to evaluate some productive and reproductive aspects of baladi does supplemented with propolis or *Nigella Sativa*.

MATERIAL AND METHODS

The present study was carried out in the National Research Center Experimental Farm (Abou Rawash, Giza) during the period from July-2004 to April-2005.

Experimental animals:

Fifteen post pubertal healthy Baladi does (8-10 months old and 15-16 Kg live body weight) were used in this study. Estrous activity was observed for at least 3 cycles and all the does have normal cycle duration (18-21 days). Does were kept free in an open shed away from bucks and fed on ration contained complete feed mixture (3% of body weight). Wheat straw (*ad-lib*) and clean drinking water were available at all times.

Propolis:

Ethanol extract of propolis was prepared as outlined by Liu et al.(2004)

Nigella sativa cake:

Nigella seed cake was obtained from the market after squeezing the seeds and getting rid of oil to be used in other medical purposes

Experimental design:

A feeding trial, lasted for 120 days followed by 10 days digestibility trial and one day for rumen sample collection was carried out. Does were randomly allocated into three comparable groups (5 animals each), housed in separate pens to determine the feeding value of the experimental rations and were subject to one of the following treatments.

- 1- Fed on the farm ration in addition to 100 g. of undecorticated cotton seed meal and injected subcutaneously (S.C) by 1 ml /head each 15 days saline and kept as the control group (R1)
- 2- Fed on the farm ration in addition to 100 g. of undecorticated cotton seed meal and injected S.C by 1ml propolis each 15 days (R2)
- 3- Fed on the farm ration in addition to 100 g of *Nigella Sativa* cake / animal/day and injected S.C by 1ml saline /head each 15 days (R3).

Does were continuously observed for appearance of any clinical symptoms of discomfort as well as for estrous activity. The chemical composition of all feed ingredients and the constituents of the experimental rations are shown in table (1). Feed residues were daily collected and weekly weighed. The development of body weight was recorded fortnightly to adjust the daily amount of feed intake. Immediately, at the end of the feeding period, three animals were randomly chosen from each group and placed in individual place for 7 days preliminary period followed by 3 successive days for manual collection of rectal fecal samples to avoid soil contamination, to assess the nutrient digestibility and nutritive values of the rations using lignin as an internal indicator (Crampton and Harris, 1969).

The average daily voluntary intake during the last three days of the digestibility trial was recorded for each doe and representative samples of the offered and refused food and feces were collected, dried at 65°C for a constant weight and ground through 1 mm screen for proximate analysis followed by one day for rumen sample collection

Samples from ruminal fluid were collected using stomach tube and filtered through two layers of cheese cloth before and three hours post feeding for determination of pH immediately using EIL digital combination electrode pH meter. The rest of filtrate was kept frozen for further analysis of ammonia nitrogen and total volatile fatty acid concentration.

After the end of the feeding trial, animals were injected S.C with bovine serum albumin in the right side and with saline in the left side of neck. After 48 hours, the skin thickness was measured using caliber and the difference between left and right side for each animal was recorded as a marker for immunological status of does in different groups

The external genitalia were examined for cyclical changes and the does were teased by bucks painted by colored greasy material on the

breasket for detection of estrous activity. Thenafter, animals were intramuscularly injected with 2 doses 11 days apart of prostaglandin F_{2α} (Lutalyse Upjon, the Netherlands). At the time of the second dose, proven bucks were painted with colored material and kept with females, signs of estrus and buck marks were recorded. Animals were followed up until kidding. Parameters relevant to fertility as the conception rate and kidding characteristics were recorded for different groups.

Sampling

At the end of the experiment and before mating, rumen liquor samples were collected using stomach tube for analysis of ammonia -N and total volatile fatty acids (TVFA's) concentration before and 3 hours post feeding.

Blood samples were collected by jugular vein puncture into two tubes one of them was heparinized for performing complete blood picture (Jain, 1997) and the other for separation of serum which was kept in eppendorff vials at -20 °C until biochemical analysis

Analysis :

For feed stuff and fecal samples, Dry matter(DM) , crude protein (CP), ether extract (EE) crude fiber(CF) and ash were determined according to AOAC (1995). The nitrogen free extract (NFE) was calculated by subtracting CP,EE and CF out of OM . Ammonia nitrogen was determined according to Conway (1962) and total volatile fatty acid concentration were determined by steam distillation as described by Warner (1964) .

Serum T3 and T4 were assayed by ELIZA according to DEA(1995) and NCCLS(1998), respectively. Total protein , cholesterol , creatinine and gama glutamyl transferase(GGT) were colorimetrically assayed (Henery,1981) using commercial chemical kits.

Statistical analysis

Statistical analysis was carried out using one way analysis of variance as outlined by Sedecor and Cochran (1980)

RESULTS AND DISCUSSION

Baladi goats accepted and consumed rations containing *Nigella sativa* cake from the first day of the experiment without any apparent digestive troubles. Also feeding on *Nigella sativa* and injection of propolis induced no hazardous effect on the metabolism or the health status of the experimental does. Similar results were reported by Awdallah (1997); El Ayek *et al* (1999);; Badawy *et al* (2001) ; El- kady *et al* (2001) and Abdel- Ghani ,(2003) Regarding nigella . Also, Heavy *et al.*(1997 and 2004) found no adverse affect due to supplementation with propolis.

I- Effect of experimental rations on some nutritional aspects :

1-The chemical composition of the ration

The chemical analysis of feed stuffs in the experimental rations are presented in table (1). It indicates that the CP and EE content of *Nigella sativa* cake were higher than that of the concentrate feed mixture, while the later showed higher content of CF and NFE and coincide with the results of El- Kady *et al.*, (2001) and Abdel-Ghani (2003).

Table (1): Chemical composition of feed stuffs used in the farm and experimental rations .

Item	DM	Chemical composition (DM% basis)					
		OM	CP	CF	EE	NFE	ASH
Concentrate feed mixture(CFM)*	91.06	91.70	14.03	16.98	3.28	57.41	8.30
Wheat straw (W S)	93.60	90.70	4.22	40.47	1.20	44.81	9.30
<i>Nigella sativa</i> cake NSC	92.23	90.90	29.50	9.20	15.10	37.10	9.10
Undecorticated cotton seed meal(UCSM)	90.00	92.90	27.30	27.10	2.70	35.80	7.10
Rations for the three experimental groups (calculated)							
Control ration (R1)	91.50	91.58	13.00	22.99	2.89	52.79	8.42
Propolis ration (R2)	91.57	91.54	12.68	23.56	2.72	52.56	8.46
<i>Nigella sativa</i> cake (R3)	91.74	91.39	12.93	21.96	4.10	52.73	8.61

*CFM was formulated from 38% undecorticated cotton seed ,33% wheat bran , 12% yellow corn ,9% rice brane ,4% molasses , 3% lime stone and 1% salt

2-Growth performance

Data of growth performance is illustrated in table (2). It shows a significant increase in the daily body weight gain by 21.4% and 28.5% in groups administered propolis and fed *Nigella*, respectively. This finding coincide with the previous studies that concluded that propolis improved weight gain and feed utilization by 16 and 13% in weaning (Bonomi *et al* 2002-b) and 17 and 15% in veal (Bonomi *et al* ,2002-a) calves, respectively. This improvement in the daily body gain could be explained in light of its biological activities. In this respect, it was recorded that propolis has antibacterial, antiviral and antifungal effects, so it improves the immune status of the animal. Moreover, these results were supported by the present increasing lymphocytic count and concentrations of serum gamma globulins as recorded in tables (7and 8). Improving of the health status make the animal directs the metabolism towards production due to the proper feed conversion rate . on the other hand, the present improving in the daily body weight gain following feeding on ration containing *Nigella sativa* agreed with the findings of Awdallah (1997); El Ayek *et al* (1999); Abdel- Ghani ,(2003) and El-Gendy,(2003) in sheep ; Badawy *et al* (2001) in does ; El- kady *et al.*, (2001)and El-Gaafarawy *et al.*, (2003) in calves. These authors substituted part of the ration protein (20 – 100%) with *Nigella* and they reported significant increase in average total feed intake, daily weight gain and feed conversion. This improvements may be related to the higher digestibility coefficient, especially for crude protein and increase total dry matter intake and consequently improved nutritive value.

Table 4: Effect of experimental rations on some rumen liquor parameter in baladi does

Item	Experimental groups			Overall Mean for time	LSD
	Control group (R ₁)	Propolis group (R ₂)	Nigella group (R ₃)		
Before feeding					
pH	7.10 ± 0.06	7.07 ± 0.07	7.03 ± 0.03	7.07 ^a	0.10
Ammonia-nitrogen (mg/dl)	12.57 ± 0.52	12.87 ± 0.75	13.73 ± 0.66	13.06 ^b	1.40
Total volatile fatty acid m.equ./dl	11.93 ± 0.86	12.53 ± 0.75	11.83 ± 0.35	12.10 ^b	1.82
After 3-hr feeding					
pH	6.63 ± 0.03	6.57 ± 0.07	6.70 ± 0.06	6.63 ^b	
Ammonia – nitrogen (mg/dl)	20.17 ± 0.87	20.40 ± 0.06	21.77 ± 1.16	20.78 ^a	
Total volatile fatty acid m. equ./dl	21.50 ± 0.55	20.53 ± 1.76	21.40 ± 1.21	21.14 ^a	
Overall mean for treatment					
pH	6.87 ^a	6.82 ^a	6.87 ^a		
Ammonia – nitrogen (mg/dl)	16.37 ^a	16.63 ^a	17.75 ^a		
Total volatile fatty acid m.equ./dl	16.71 ^a	16.53 ^a	16.62 ^a		

Means with different superscripts are different at least at ($p < 0.05$)

Table 5: some reproductive parameters in baladi does supplemented with propolis or *Nigella Sativa*

Parameter	Control(R1)	Propolis (R1)	Nigella(R3)
Conception rate(%)	70.00	75.00	80.00
No.services /conception	1.20 ± 0.20	1.33 ± 0.30	1.25 ± 0.25

Table 6: Some kidding parameters in baladi does supplemented with propolis or *Nigella Sativa*

Parameter	Control(R1)	Propolis (R1)	Nigella(R3)
Twining rate (%)	40.00	50.00	66.00
Ease of kidding(1easy-3 ystokia)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Kids vitality (1maximum vitality- 3 weak)	2.0 ± 0.57	1.25 ± 0.25	1.20 ± 0.48
Incidence of stillbirth (%)	0	0	0

Table 7 : Heamgram of Baladi does supplemented with propolis or *Nigella Sativa*

Item	Control(R1)	Propolis (R2)	Nigella(R3)
RBCS(X10 ⁶ /UL)	9.9±0.48 ^a	10.53±0.34 ^a	10.89±0.57 ^a
HB(gm%)	11.64±0.38 ^a	12.70±0.34 ^b	13.06±0.21 ^b
PCV(%)	33.20±1.06 ^a	36.02±0.80 ^b	36.00±0.54 ^b
MCV(fl)	33.77±1.61 ^a	34.34±1.39 ^a	35.00±1.42 ^a
MCH(pg)	11.81±0.42 ^a	12.10±0.47 ^a	35.26±0.38A ^b
MCHC(%)	35.07±0.54 ^a	35.26±0.38A ^b	36.29±0.08 ^b
WBCS (x10 ³ /ul)	3.99±0.30 ^a	4.97±0.47 ^a	4.90±0.26 ^a
Lymphocytes(%)	57.80±1.28 ^a	66.40±2.22 ^b	63.00±1.18 ^b
Neutrophils(%)	41.20±1.01 ^a	30.60±2.13 ^b	32.60±1.43 ^b
Monocytes(%)	0.80±0.20 ^a	1.40±0.20 ^a	0.80±0.19 ^a
Eosinophiles(%)	0.40±0.24 ^a	4.00±0.31 ^b	0.60±0.40 ^a
Basophilles(%)	0.00±0.00 ^a	0.20±0.20 ^a	0.40±0.40 ^a

Means with different superscripts are different at least at (p<0.05)

Table 8 : Some metabolic profile tests in Baladi does supplemented with propolis or *Nigella Sativa*

Metabolic profile tests	Control(R1)	Propolis(R2)	Nigella(R3)
Total protein(gm/dl)	6.9±0.25 ^a	7.68±0.19 ^a	6.94±0.30 ^a
Albumin(gm/dl)	3.73±0.30 ^a	4.07±0.01 ^a	4.07±0.02 ^a
Total globulin(g/dl)	3.07±0.30 ^a	3.61±0.21 ^b	2.87±0.09 ^a
Alpha globulin(g/dl)	0.45±0.05 ^a	0.56±0.08 ^a	0.42±0.03 ^a
Beta globulin(g/dl)	0.49±0.01 ^a	0.56±0.03 ^a	0.44±0.03 ^a
Gamma globulin(g/dl)	0.42±0.03 ^a	0.57±0.03 ^b	0.48±0.03 ^a
Cholesterol(mg/dl)	78.00±3.47 ^a	67.00±5.21 ^a	39.40±8.42 ^b
GGT(u/l)	24.83±4.41 ^a	12.00±0.01 ^b	15.20±2.10 ^b
Creatinine (mg/dl)	1.25±0.29 ^a	1.84±0.12 ^a	1.86±0.29 ^a
T4(ug/dl)	4.58±0.26 ^a	3.78±0.36 ^a	6.04±0.27 ^a
T3 (ng/dl)	113.41±12.36 ^a	53.34±6.69 ^b	135.00±10.83 ^c
T. INDEX	5.17±0.61 ^a	2.11±0.46 ^b	6.71±1.32 ^a

Means with different superscripts are different at least at (p<0.05)

Table 9 :Skin sensitivity test in baladi does supplemented with propolis or *Nigella Sativa*

Group	The difference in Thickness of skin after treatment (mm)
Control(R1)	0.80±0.25 ^b
Propolis (R2)	2.90± 0.78 ^a
Nigella (R3)	1.32±0.69 ^{ab}

Means with different superscripts are different at least at (p<0.05)

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تأثير استخدام بعض محفزات المناعة الطبيعية على أداء إناث الماعز
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المركز القومي للبحوث - الدقي - الجيزة

أجريت هذه الدراسة في مزرعة ابو رواش التابعة للمركز القومي للبحوث بهدف دراسة تأثير الحقن بصمغ العسل أو إضافة كسب حبة البركة كمحفزات مناعية طبيعية على أداء إناث الماعز البلدية حيث استخدم عدد ١٥ أنثى ماعز متوسط أوزانها ١٥-١٦ كجم و عمرها من ٨-١٠ شهور تم تغذيتها على عليقة مكونة من العلف المركز (٣% من وزن الحيوان) + تين كمح بحرية و قسمت الماعز عشوائيا إلى ثلاث مجموعات متشابهة في العدد و الوزن لأجراء تجربة النمو (التي استمرت لمدة ١٢٠ يوما) و عزلت عن الذكور كالتالي :

- ١- المجموعة الأولى غذيت على العليقة السابقة محتوية على ١٠٠ جرام كسب قطن غير مقشور / رأس / اليوم واعتبرت كمجموعة ضابطة
- ٢- المجموعة الثانية غذيت على نفس العليقة بالإضافة إلى حقنها بصمغ العسل تحت الجلد (١ مل / للرأس) كل ١٥ يوم لمدة ٤ شهور
- ٣- غذيت على نفس العليقة السابقة محتوية على ١٠٠ جرام كسب حبة البركة / للرأس في اليوم .

و بعد الانتهاء من تجربة النمو تم دراسة تأثير تلك المعاملات على الهضم و القيمة الغذائية للعلائق المكونة و كذلك على سائل الكرش قبل و بعد التغذية بثلاث ساعات و تم حقن الإناث بجرعتين من البروستاجلاندين و تركت مع الذكور بعد الجرعة الثانية للتقيح و تم متابعة الإناث خلال فترة العسار و حتى الوضع و سجلت بعض المؤشرات الخاصة بالتساؤل كما تم تحليل بعض مكونات الدم الخلوية و الكيميائية بين المجموع المختلفة

كانت أهم النتائج المنحصل عليها كالآتي :

- ١- الزيادة الكلية في الوزن و كذلك الزيادة اليومية كانت افضل في المجموعة الثالثة وتلاها المجموعة الثانية بفارق معنوي عن المجموعة الأولى الضابطة
- ٢- تحسنت كفاءة التحويل الغذائي في كل من المجموعات الثانية و الثالثة عن المجموعة الضابطة
- ٣- تحسنت معاملات الهضم لمعظم العناصر الغذائية معنويا في المجموعة الثالثة و كذلك القيمة الغذائية عن المجموعة الثانية و الأولى
- ٤- أنخفض تركيز الأس الهيدروجيني معنويا بالمعاملات السابقة المختلفة بعد التغذية بثلاث ساعات عن قبل التغذية ، وقد صاحب ذلك الانخفاض ارتفاع معنوي في تركيز الأحماض الدهنية الطيارة و كذلك أمونيا نيتروجين سائل الكرش
- ٥- تحسنت الكفاءة التناسلية للحيوانات بعد هذه المعاملات متمثلة في زيادة معدل التوائم و معدل العشار
- ٦- أظهرت المعاملات السابقة تأثيرا إيجابيا و اضا في تحفيز مناعة الجسم متمثلة في زيادة الجاما جلوبيولينات و العد النوعي لخلايا الليمفاوية في الدم خاصة في المجموعة الثانية
- ٧- تحسنت الحالة الصحية العامة للحيوانات متمثلة في تحسن صورة الدم
- ٨- انخفضت أنزيمات الكبد انخفاضا معنويا مما يدل على أن لهذه المعاملات تأثير مفيد في حماية و تحسين وظائف الكبد
- ٩- تحسنت وظيفة الغدة الدرقية في المجموعة الثالثة بالمقارنة بالمجموعة الضابطة

ويستخلص من هذه الدراسة إمكانية استخدام بعض المواد الطبيعية الآمنة مثل صمغ العسل أو كسب حبة البركة كمحفزات مناعية لتحسين الأداء الإنتاجي و التناسلي في حيوانات المزرعة.