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Immune Response and Productive Performance of Dairy Buffaloes and their Offspring Supplemented with Black Seed Oil

Research Article

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ABSTRACT

Twenty pregnant buffaloes, eight weeks before the expected calving date, were assigned into two homogeneous groups of 10 animals each, according to their season of lactation (2nd to 8th season of lactation); The first group (G1) were fed on a ration consisting of concentrate feed mixture (22% yellow corn, 35% cotton seed meal, 33% wheat bran, 4% rice bran, 3% molasses, 2% limestone and 1% sodium chloride), rice straw and berseem (in winter) berseem hay or corn silage (in summer) according to the feeding regime of the traditional farm system. The second group (G2) was fed the control ration plus 10 ml black seed oil (BSO)/head/day. After calving, the offspring of buffaloes of each group were divided into two similar groups of five animals each, according to their birth weight. Calves of T1 and T2 represented the offspring of G1 buffaloes (the control group) while those of T3 and T4 belonged to buffaloes of G2. The results showed that nutrients digestibility were nearly similar for both G1 and G2 (P>0.05). Total plasma protein, albumin, GPT, GOT, plasma immunoglobulin and blood hematocrit for lactating buffaloes tended to be higher (P<0.05) in G2 than those of G1. Four days after parturition showed insignificant differences regarding total solid, fat, solid not fat, total protein, lactose and ash. The treated group (G2) showed higher (P<0.05) total solid, fat, solid not fat, total protein and ash content than those of G1, while lactose value was nearly similar. The results of buffalo calves showed that the highest (P<0.05) values of dry matter, organic matter, crude protein, crude fiber, nitrogen free extract (except that of ether extract; P>0.05) digestibility were recorded for T2 compared to those of the other groups (T1, T3 and T4), but those in T3 showed the lowest values. Calves fed supplemented ration with BSO (T2 and T4) had higher nutrient digestibility values than those of the non supplemented groups (T1 and T3). No significant differences (P>0.05) were observed in plasma total protein, albumin, urea, creatin, total lipids, glucose, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, triglyceride, hematocrite and neutrophil to lymphocyte ratio (NLR). On the contrary, plasma cholesterol concentration was decreased in treated groups (T2 and T4), while plasma immunoglobulin concentration was increased, regarding to calves' performance during the suckling period.

KEY WORDS black seed oil, buffalo calves, immune response.

INTRODUCTION

Buffaloes are considered the main milk producing animal in Egypt. They produce about 60% of the total milk production, and share with large portion in meat production, for that, great attention must be paid to their new-born health and their viability.

The economic losses associated with the neonatal diseases in calves are alarmingly high. Gastro-enteritis and respiratory infections are the most serious pathological dis-

orders implicated with such losses, particularly during the first period of life, when the immune system is incompatible enough against the vast array of causative pathogens (El-Bordeny, 2006). Some medicinal herbs and plants have some properties as antiseptic, antibacterial activities against harmful microorganisms, antispasmodic, treatment gastrointestinal complaints, tonic and anti-inflammatory, which attribute to the active materials.

Using medicinal herbs and plants (MH&P) with humans has been known since the old civilizations. Old drugs industry depended upon the raw material of MH&P and their extracts, which proved safe. Inversely many synthesized chemicals caused many hazards to animals, plants and humans. The world health organization (WHO) encourages using MH&P to substitute or minimize the use of chemicals through the global trend to go back to nature, (Allam *et al.* 1999). Some studies indicated that such plants had beneficial effects on digestibility, live weight gain and feed efficiency with cow. Black seed (*Nigella sativa*) is recently used for medical purpose, where, it is well known as antibacterial, antifungal, antidiabetic and immune enhancing (Khodary *et al*. 1996).

Therefore, the object of this study was to investigate the effect of using black seed oil in dairy buffaloes (at last stage of pregnancy) and in their offspring diets on some blood parameters, immune response and productive performances of buffaloes and their new-born calves.

MATERIALS AND METHODS

The experimental animals and rations

Twenty pregnant buffaloes, 8 weeks before the expected calving date were assigned to two homogeneous groups, (10 animals each; G1 and G2), according to their season of lactation. Animals of G1 were fed on a ration containing concentrate feed mixture (CFM) consisting of (22% yellow corn, 35% cotton seed meal, 33% wheat bran, 4% rice bran, 3% molasses, 2% limestone and 1% sodium chloride), rice straw (RS), and berseem (in winter), berseem hay (BH) or corn silage (in summer) which offered to cover the daily requirements of pregnant buffaloes according to the traditional allowances of the experimental farm station (NRC, 2001). While those of G2 were fed the same ration plus 10 ml black seed oil (BSO)/head/day.

After calving, the buffalo's offspring of each group were divided randomly into two similar groups (five animals each) according to their birth weight. Calves of T1 and T2 are the offspring of buffaloes of G1 (the control group) and those of T3 and T4 are the offspring of buffaloes of G2. Calves of each sub group were suckled artificially (10% of LBW) and fed on calf starter *ad lib* (T1 and T3) or plus 5 mL (BSO)/head/day (T2 and T4).

Buffalo calves were introduced to the treatments at 7 days-old after colostrum feeding, up to the weaning (105 days old). Average initial weights of the experimental calves were 38.2, 37.75, 39.75 and 42.2 kg for T1, T2, T3 and T4, respectively. The chemical compositions of the experimental feedstuffs are presented in Table (1).

Digestibility trials

All animals (Buffaloes and calves) in each treatment were used in each trial. A grab sample method was applied at which acid insoluble ash (AIA) was used as an internal marker (according to Forbes and Garrigus, 1948) for determining the nutrients digestibility. The digestibility coefficient of a certain nutrient was calculated according to the following formula:

Digestibility (%)= 100 – ((% indicator in feed/% indicator in feces) x (% nutrient in feces/% nutrient in feed) x 100)

Analytical methods

The chemical composition of the different feedstuffs and feces were analyzed according to the A.O.A.C, (1995) methods to determine moisture content, dry matter DM, organic matter OM, crude protein CP, crude fiber CF, ether extract EE, and ash, while nitrogen free extract NFE content was calculated by difference. Chemical composition of milk (fat, protein, lactose, total solids and solids not fat) were determined by using Milko-Scan Analyzer while ash was calculated by difference.

Blood samples

Blood samples of pregnant buffaloes were taken from all animals at 45**,** 30 and 15 days prepartum and at 7th day postpartum. The blood samples were taken from the jugular vein at three hours post morning feeding. While those of buffalo calves were taken from all the experimental calves monthly until weaning. The blood was directly collected into a clean dried glass culture tubes after adding EDTA and sodium floride. Blood plasma was obtained by centrifuging the blood samples soon after collection at 4000 rpm for 15 minutes and then stored in deep freezer at -20 ºC for subsequent chemical analysis. Blood smears were also obtained by separating 2 ml of the whole blood into Ependorf tubes to determine the leucocytes differential count. For the hematological evaluation, about 0.5 mL of whole blood was obtained into heparinized tubes.

Hematocrite (Ht) was determined using heparinized capillary tubes and microhematocrite centrifuge. Leucocytes differential count was obtained by staining the blood smears using Wright's stain and were differentially counted according to Heckner *et al*. (1988).

One hundred cells were counted and differentiated into

Table 1 Chemical composition of the experimental feed stuffs (% on DM basis)

Feed stuff	As DM basis								As fed	
	DM	OM	CF	CP	EE	NFE	Ash	*** AIA	**** TDN	****DP
Milk	16.36	95.37	0.00	22.83	41.24	31.30	4.63	0.00	23.40	3.30
Berseem	16.16	87.30	27.91	19.35	3.54	36.50	12.70	4.55	11.97	2.00
Corn silage	26.57	94.30	27.82	12.80	2.81	50.87	5.70	1.49	66.10	8.00
Rice straw	91.07	81.93	34.01	3.57	1.82	42.53	18.07	12.35	40.00	0.00
** Calf starter	91.63	89.35	8.85	17.05	3.84	59.61	10.65	3.27	72.97	10.32
* CFM	91.36	89.93	14.23	18.06	2.95	54.69	10.07	4.27	63.70	9.80
Berseem hay	89.00	85.82	35.91	15.40	1.31	33.20	14.18	2.25	48.0	9.00

DM: dry matter; OM: organic matter CP: crude protein; CF: crude fiber; EE: ether extract; NFE: nitrogen free extract; TDN: total digestible nutrients; DP: digestive protein.
*CFM: Concentrate feed mixture: consisted of 22% yellow corn, 35% cotton seed meal, 33% wheat bran, 4% rice bran, 3% molasses, 2% limestone and 1% sodium chloride.
**Calf starter consisted of 40% yellow corn, 15% soybean meal, 10% rice bran, 32% wheat bran, 1% sodium chloride, 0.5% mineral salts and 1.5% lime stone.
***AIA: Acid insoluble ash.
****Calculated according to Abou Raya (1967).

lymphocytes, neutrophils, eosinophils, basophils, and monocytes, the neutrophil/lymphocyte ratio (NLR) was calculated according to the following formula:

neutrophil/lymphocyte ratio (NLR)= (number of neutrophils/number of lymphocytes)

Hematocrite (Ht) was determined according to Rodak (1995) using heparinized capillary tubes and microhematocrite centrifuge, in which the blood samples were pipetted in the microhematocrite tubes, centrifuged and the Ht was calculated according to the following formula:

Hematocrite value (Ht)= (Volume of formed elements/ Volume of blood) x 100

Blood plasma was analyzed for glucose, total protein (Armstrong and Carr, 1964), Albumin (Doumas *et al.* 1971), total lipids (Frings *et al.* 1972), urea, creatinine, glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) (Reitman and Frankel, 1957), cholesterol, triglyceride (Fassati, 1982), and total bovine plasma immunoglobulins (Nancy *et al.* 1977).

Statistical analysis

The data were analyzed according to statistical analysis system (SAS) User' s Guide (1995). Separation among means was carried out using Duncan' s multiple range tests, (1955).

RESULTS AND DISCUSSION

1. Pregnant buffaloes

Nutrients digestibility

Adding BSO not significantly affected nutrients digestibility (Table 2). Similar results were obtained when they fed sheep on different levels of *Nigella sativa* cake (NSC). Indicated that substituting undecorticated cotton seed meal (UCSM) of concentrate feed mixture (CFM) protein by *Nigella sativa* meal (NSM) protein at 0 and 100% in the rations improved DM ,OM, CP and NFE digestibility, but with no effect on the feeding value. Also, found that digestibility of DM, OM and CF in two diets supplemented with NSC for growing lambs were not different when compared to the control diet. Moreover, reported that rations containing (NSM)/(0, 10, 20, 30 and 40 %) in lambs' rations significantly improved DM, OM and CF digestibility and didn' t significantly increase CP and EE digestibilities.

Table 2 Effect of BSO supplementation on the nutrients digestibility coefficient of the pregnant buffaloes

Item	G1	G2
DM	56.38±2.86	58.61±4.01
OM	60.27±2.25	62.02±4.62
CP	59.66±7.70	63.79±6.48
CF	51.25±8.13	53.30±9.24
EE	60.20±8.88	61.85±8.59
NFE	67.03±1.45	66.10±2.31

Blood parameters

Values of plasma protein concentration (Table 3) for animals fed on BSO, (G2) were slightly ($P<0.05$) higher than those of the control animals (G) during the entire experimental period (pre- and post-partum).

In the same time, values of blood albumin were higher for animals fed on BSO than those of the control group both pre- and post-partum period ($P<0.05$).

However, these values are within the normal range of healthy buffaloes as reported by who found that values of plasma total protein concentration of healthy buffaloes ranged between 5.3 to 8.00 g/100mL.

Similar results were obtained when they replaced soybean meal protein by (NSM) protein at different levels in lamb rations.

Nonsignificant differences were observed in blood plasma urea, creatinine, total lipids, cholesterol, triglycerides, glucose, GOT and GPT concentration (Table 3) of pregnant buffaloes between the different experimental groups either during pre-or post-partum periods.

This means that there was no effect on such supplementation and had no negative efffects on either liver or kidney functions. In addition, values of the different blood parameters reported here were within the normal values which were reported by different authors (Merek, 199; Nazar, 1994; Khodary *et al.* 1996).

The values of GPT and GOT presented in this study are within the normal range as indicated by Merek (1991) who reported values from 6.9 to 36.3 for GPT and from 45.3 to 110.2 for GOT.

Table 3 Effect of BSO supplementation on some blood and blood plasma Parameters in buffalo calves during the entire experimental period

Item	Pre-partum		Post-partum	
	G1	G2	G1	G2
Total protein (g/dL)	7.55	7.68	7.88±0.59	8.14±0.48
Albumin (g/dL)	2.50[b]	3.38[a]	3.35±0.70	3.71±0.93
Urea (mg/dL)	29.50	26.39	25.23±2.68	25.54±2.50
Creatinine (g/dL)	1.79	1.58	2.03±0.47	2.37±0.28
Total lipid (mg /dL)	197.61	192.14	210.23±12.87	180.33±14.89
Cholesterol (mg/dL)	63.07	60.18	68.95±4.66	59.83±5.84
Triglyceride (mg/dL)	92.66	84.71	86.77±14.55	83.43±5.04
Glucose (mg/dL)	64.84	62.23	57.20±8.09	53.35±7.29
GOT (unit/L)	57.15	61.85	49.44±7.65	48.89±10.99
GPT (unit/L)	22.00	23.11	25.56±2.66	24.33±2.67
Immunoglobulin (mg/mL)	0.77	0.99	0.42±0.14	0.51±0.13
NLR	0.64	0.61	0.60±0.07	0.42±0.05
Hematocrite (%)	36.75	38.66	39.57±4.24	41.55±4.06

a and b Means of treatments within the same row with different superscript letters are significantly different ($P<0.05$).

The data of Table 3 clearly indicate that addition of BSO improved blood plasma immunoglobulin concentration (0.77 vs 0.99 and 0.42 vs 0.51 mg/mL for pre- and post-partum periods, respectively). These may be attributed to that, seed extracts and seed oil from *N. sativa*, exhibit immunostimulant properties (Aqel, 1993; El-Sayed, 2000) and exhibits immunopotentiating activities (Hailat *et al.* 1995). These results also agree with those obtained by El-Khenawy *et al.* (1999) who found that feeding the oil meal led to an increase in total globulins and delta globulins. The same results were obtained by El-Wafa *et al.* (2002). Moreover, Anwar *et al.* (2004) showed that *N. sativa* seeds have been widely used in traditional medicine and has been shown to have immunomodulatory properties. The addition of BS to feed of pigeons could act as an immunoprotective agent when chronic administration of anti biotics is considered (Al-Ankari, 2005).

Non significant differences were observed in blood hematocrit (Table 3) and neutrophil to lymphocyte ratio (NLR). Although, there was a slight increase of NLR value (P≥0.05) for control group compared to the treated group. The increase of blood hematocrit concentration of G2 may be due to improvement in immune system responsiveness. Black seed (*Nigella sativa*) contains Zn, Cu, Mn, Mg, Se, vit. C, vit. A, vit. E and folic acid which may have a role in enhancing immune system. Folic acid, Fe and vitamin C have roles in red blood cell formation, maturation, absorption and utilization. These results agree with those obtained by Ismail *et al*. (2003).

Colostrum chemical analysis

Data of Table 4 showed that G2 tended to be higher (P≥0.05) overall means of TS, Fat, SNF, TP and ash content, while lactose mean was nearly similar. Values of TS, SNF, fat and protein content in colostrum of the experimental groups decreased gradually (P≤0.05) along the first four days after parturition.

2. Buffalo calves performance

Nutrient digestibility

Data of Table 5 clearly show that calves of T2 and T4 groups (fed on BSO) had higher (P≤0.05) values of nutrient digestibility (except that of EE) compared to T1 and T3 (fed BSO diet free). This may be attributed to either essential oils in BSO that enhanced nutrients digestibility (Allam *et al*. (1999) and/or the effect of BSO on rumen development (Nazer, 1983).

Blood parameters

Adding black seed oil (BSO) in calves' diet had no effect (P≥0.05) on plasma total protein, albumin concentration, urea, creatinine concentration or total lipid of new-born buffalo calves (Table 6). The present values are within the normal range obtained by many authors (El-Ashry *et al*. 1982; Youssef, 1992; Hilal *et al*. 1999; Weld Abd El-Kader, 2000; El-Wafa 2002; Ragheb 2003). Values of GOT and GPT obtained in this study were not affected by the BSO supplementation, that means no adverse effect on such addition on liver function where these values are in line with the normal values of those reported by Blinco and Dye (1958) and Merek (1991). The results of Table 6 showed that there was a significant decrease in plasma cholesterol due to BSO supplementation in (T2 and T4) compared to unsupplemented BSO groups (T1 and T3). It may attributable to high amounts of unsaturated fatty acids in black seeds which stimulate the cholesterol excretion into the int-

Table 4 Effect of (BSO) supplementation on colostrum chemical composition during the first four days after parturition

Item	Days after parturition								Overall mean					
	1		2		3		4		Groups		Days after parturition			
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	1	2	3	4
Total solids (%)	20.88± 0.62^{a}	19.92± 0.63^{a}	17.91± 0.48^{b}	17.05± 0.86^{bc}	16.28± 0.68^{bc}	17.62± 0.32^{b}	14.99± 0.43^{c}	17.21± 0.59^{b}	17.52	17.95	20.40^{a}	17.48^{b}	16.95^{c}	16.10^{c}
Fat (%)	5.01± 0.61^{bc}	3.41± 0.34^{d}	4.63± 0.54^{cd}	4.67± 0.58^{c}	5.39± 0.33^{bc}	5.98± 0.29^{bc}	6.18± 0.44^{b}	7.64± 0.54^{a}	5.30	5.43	4.21^{c}	4.65^{c}	5.69^{b}	6.91^{a}
Protein (%)	11.01± 0.40^{a}	11.93± 0.43^{a}	8.38± 0.67^{b}	7.50± 0.62^{b}	5.89± 0.41^{c}	6.86± 0.34^{bc}	4.23± 0.28^{d}	4.31± 0.22^{d}	7.38	7.65	11.47^{a}	7.94^{b}	6.38^{c}	4.27^{d}
Lactose (%)	3.63± 0.20	3.37± 0.10	3.70± 0.20	3.64± 0.56	3.98± 0.33	3.93± 0.30	3.88± 0.26	4.22± 0.47	3.80	3.79	3.5	3.67	3.96	4.05
Solids not fat (%)	15.87± 0.46^{a}	16.51± 0.40^{a}	13.28± 0.61^{b}	12.38± 0.74^{bc}	10.89± 0.66^{c}	11.64± 0.48^{bc}	8.81± 0.40^{d}	9.57± 0.60^{cd}	12.22	12.52	16.19^{a}	12.83^{b}	11.26^{c}	9.19^{d}
Ash (%)	1.23± 0.02^{a}	1.22± 0.02^{a}	1.20± 0.07^{a}	1.25± 0.07^{a}	1.02± 0.11^{ab}	0.85± 0.09^{bc}	0.70± 0.00^{c}	1.04± 0.12^{ab}	1.04	1.09	1.23^{a}	1.23^{a}	0.94^{b}	0.87^{b}
Moisture (%)	79.12± 0.62^{c}	80.08± 0.63^{c}	82.09± 0.48^{b}	82.95± 0.86^{ab}	83.72± 0.68^{ab}	82.38± 0.59^{ab}	85.01± 0.43^{a}	82.79± 0.82^{ab}	82.49	82.05	79.60^{c}	82.52^{b}	83.05^{a}	83.90^{a}

a, b, c and d means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Table 5 Effect of BSO supplementation on the nutrients digestibility coefficient of buffalo calves during suckling period

Item	T1	T2	T3	T4
DM	76.81±1.30^{ab}	85.41±2.68^{a}	71.59±4.07^{b}	78.99±3.61^{ab}
OM	78.78±0.97^{ab}	86.82±2.72^{a}	73.09±3.88^{b}	80.07±3.66^{ab}
CP	67.79±2.40^{b}	82.79±3.12^{a}	62.71±5.57^{b}	74.13±4.66^{ab}
CF	78.83±1.51^{ab}	86.51±4.62^{a}	71.07±3.51^{b}	77.10±4.58^{ab}
EE	92.93±1.25	95.45±1.09	91.25±2.34	94.32±1.59
NFE	77.21±2.33^{ab}	84.66±3.53^{a}	70.00±5.06^{b}	77.07±4.12^{ab}

a and b Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

estine and it can be oxidized to bile acids (Khodary *et al.* 1996).

Data of Table 6 indicated, nonsignificant differences between treatments in NLR and Ht in blood serum of the treated groups (T2 and T4). The highest nonsignificant NLR was recorded for T1 followed by T2 whereas the lowest one was observed for T3 (Table 6).

Concerning of blood serum hematocrite is of interest to notice that calves received BSO (T2 and T4) tended to have higher (P≥0.05) concentration than those BSO free (T1 and T3). The improvements of hematological traits observed in T2 and T4 may be due to improvement in immune system responsiveness. Black seed (*Nigella sativa*) contain Zn, Cu, Mn, Mg, Se, vit. C, vit. A, vit. E and folic acid which have a role in enhancing immune system. Folic acid, Fe and vitamin C have roles in red blood cell formation, maturation, and in hem biosynthesis, absorption and utilization. So, the Ht% increased in supplemented groups as reported here. These are in harmony with those reported by Ismail *et al.* (2003).

There was a significant effect of BSO supplementation on blood plasma immunoglobulin concentration, where the highest value (P<0.05) was recorded for T3 calves followed by those of T2 and T4 while the lowest value was recorded for calves of T1 fed and their dams on diet BSO free. It is noted that addition of black seed oil for dam and/or their offspring diet improved blood plasma immunoglobulin concentration. These results agree with those obtained by El-Khenawy *et al.* (1999), El-Wafa (2002) and El-Gaafarawy *et al.* (2003).

Calves performance

Data of Table 7 showed that calves fed on BSO diet grew faster than those fed on BSO free diet. Moreover, calves of T4 had significantly the highest weaning weight (117.49) and significant highest total gain (75.29 kg), followed by those of T2 (103.48 and 655.73 kg). It is clear that calves of T4 (BSO treated offspring of BSO treated buffaloes) showed the highest (P<0.05) daily gain. It may indicate that the effect of treating buffaloes before parturition was trans

Table 6 Effect of BSO supplementation on some blood and blood plasma parameters of buffalo calves during suckling period

Item	T1	T2	T3	T4
Total protein (g/dL)	6.37	6.19	6.47	7.21
Albumin (g/dL)	2.97	2.92	3.05	3.02
Globulin (g/dL)	3.40	3.27	3.42	4.19
Urea (mg/dL)	19.63	21.57	20.41	22.11
Creatinine (g/dL)	1.60	1.48	1.95	1.57
Total lipid (mg/dL)	173.10	181.38	219.69	191.17
Cholesterol (mg/dL)	64.96^{ab}	60.37^{ab}	72.22^{a}	53.66^{b}
Triglyceride (mg/dl)	66.34	87.60	100.13	74.30
Glucose (mg/dL)	76.46	84.64	72.62	84.00
GOT (unit/L)	19.20	19.00	25.42	19.90
GPT (unit/L)	24.05	19.94	24.13	25.35
Immunoglobulin (mg/mL)	0.31^{b}	0.46^{ab}	0.65^{a}	0.36^{b}
NLR	0.52	0.46	0.41	0.44
Hematocrite (%)	36.77	40.22	36.94	41.49

a and b Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

ferred to offspring and consequently led to more gain for calves received BSO treatment.

This may be explained by: 1- Black seed oil may be having a stimulating effect on the rumen proper functions and digestion (Allam *et al.* 1999; Nazer, 1983), 2- The higher digestibility that was recorded particularly for groups supplemented by BSO (T2 and T4, Table 5), which led to increase the absorbed nutrients from small intestine, consequently increased body weight gain, 3- BSO supplementati-

Table 7 Productive performance of calves in different groups

Item	T1	T2	T3	T4
		Animal weight		
Initial weight (kg)	38.20±2.27	37.75±2.50	39.75±3.64	42.20±1.43
Weaning weight (kg)	98.58^{b}±3.67	103.48^{ab}±1.49	102.33^{ab}±5.34	117.49^{a}±5.61
Total gain (kg)	60.38^{b}±1.86	65.73^{ab}±2.99	62.58^{b}±2.27	75.29^{a}±5.09
Period, d	105	105	105	105
Average daily gain (kg)	0.575^{b}	0.626^{ab}	0.596^{b}	0.717^{a}
		Feed conversion		
DM (kg/kg gain)	2.98^{a}	2.41^{ab}	2.59^{ab}	2.10^{b}
TDN (kg/kg gain)	2.56^{a}	2.18^{ab}	2.20^{ab}	1.85^{b}
DCP (kg/kg gain)	0.413^{a}	0.353^{ab}	0.353^{ab}	0.296^{b}

a and b Means of treatments within the same row with different superscript letters are significantly different ($P \leq 0.05$).
DM: dry matter; TDN: total digestible nutrients; DCP: digestible crude protein.

on increased efficiency of nutrient utilization and consequently led to more gain and 4- Increased protein anabolism due to higher protein digestibility which led to higher blood plasma total protein and albumin concentration (Table 6) that increase protein biosyntheses.

These results are in agreement with those of who stated that supplements of medicinal plant mixture (including BSO) improved daily body weight gain for different farm animals. Also, El-Khenawy *et al*. (1999) used Barki ewes to study the effect of diets supplemented by *Nigella sativa* oilseed meal. They found a marked increase in net live weight and body condition score (BCS) gain in both supplemental groups. Moreover, found that lambs fed on NSM had higher daily gain.

Feed conversion values (Table 7) expressed as DM, TDN and DCP intakes per gain were significantly better for groups supplemented by BSO (T2 and T4) than the other groups (T1 and T3). This may reflect the improvement of ADG as a result of BSO supplementation (Table 7). Similar results were obtained by Allam *et al*. (1999) on does of Zaraibi goats; on Friesian bull calves on lambs. From the obtained results it could be concluded that adding black seed oil to rations of pregnant buffalo and its offspring improved nutrient digestibility, enhanced the immune responses and productive performance of calves.

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