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Effects of Flushing and Selenium-Vitamin E Administration on Reproductive Performance, some Hematological, and Biochemical Parameters in Gezira Ecotype Ewes During the Non-Breeding Season

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Abstract

This study investigated the effects of flushing and intramuscular administration of selenium (Se) and vitamin E (Vit E) on reproductive performance (number of service conception rate, lambing and twining rate), some hematological parameters (white blood cells, lymphocyte, red blood cells), and some blood biochemical constituents (total protein, albumin, glucose, triglycerides, cholesterol, inorganic phosphorus, calcium and aminotransferase) in 24 adult Gezira ecotype ewes (Dubasi and Shugor; aged 2–3 years; mean body weight 36 kg) during the non-breeding season (March–May 2018). all ewes were kept on basal diets for adaptation period for three weeks and randomly assigned to six equal groups (n=3/group): (A) control (basal diet only), (B) 50% concentrate supplementation, (C) 100% concentrate supplementation, (D) half-dose Se+Vit E, (E) full-dose Se+Vit E, and (F) 100% concentrate + full-dose Se+Vit E. Treatments were administered for eight weeks (four weeks pre- and four weeks post-mating). Breeding was done on 15 days post treatment with the sires of proven fertility. Reproductive performance (conception rate, lambing rate, twinning rate) was significantly higher ($P<0.01$) in all treated groups compared to controls. Hematological analysis revealed significant increases ($P<0.01$) in white blood cell and lymphocyte counts in treated ewes, while red blood cell indices showed no significant differences. Serum total protein, albumin, glucose, cholesterol, triglycerides, calcium, inorganic phosphorus, and aminotransferase activities were elevated in treated groups relative to controls. In conclusion, concentrate supplementation alone or combined with Se+Vit E administration effectively improved reproductive efficiency and health status in Gezira ecotype ewes during the non-breeding season.

Key words: Flushing, Selenium, Vitamin E, Ewes, fertility, Sheep

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Introduction

Four types of Sudanese sheep (Desert, Nilotic, Arid upland and Equatorial upland) and seventeen breeds were found (El-Hag, 2001). Sudan desert sheep breed is one of the most important meat and milk producing sheep in Sudan and represent more than 65 percent of the total sheep in Sudan and nearly 100 percent of Sudanese sheep exports (El-Hag et al., 2001). Nomadic desert sheep are raised under open rangeland and obtain adequate feed from grazing during rainy season, but are on the verge of starvation during the dry season. Dry season pasture does not meet the maintenance requirement of sheep and may lead to loss of weight and mortality in young animals. Transhumant's and sedentary farmers raise desert sheep (Dubasi and Shugor ecotypes) to produce meat, milk and to a lesser extend skin (Abdelgadir et al., 1998).Flushing is the feeding of extra grain or lush pasture two to three weeks prior to the breeding season for the purpose of increasing the number of ova shed from the ovary for yielding twins (Banerjee, 1999). This is aimed for improving the lambing rate (to reduce barrenness otherwise), through increased proportion of twinning (Owen, 1976). Flushing the ewes with good feeding for a short period prior to breeding season has been used to improve litter size (Smith et al., 1983; Leury et al., 1990). Increased twinning percentage due to flushing has been reported in Nilgiri (Anilkumar et al., 2003) and Horro ewes (Galmessa and Prasad, 2002), whereas discouraging result has been reported in Bharat Merino ewes (Narayanan et al., 2003).The adverse effect of nutritional stress on follicular development, embryo quality and embryo-mother signaling has been documented (Abecia, etal, 2006). Vitamin E acts as an intra cellular antioxidant thereby protecting cellular membranes from oxidative damage (Surai, 1999). Selenium is an essential component of glutathione peroxidase an enzyme involved in detoxification of free radicals (Smith, and Akinbamijo, 2000). Selenium supplementation has been reported to improve reproductive performance of sheep. Imbalances in trace, minerals, may occur in farm animals, especially sheep, whose intake of minerals depend largely on the content in the forage and thereby on the soil where lay grows. Sudan as many other countries in the world has low content of Selenium (Se) in the soil. Animals that mainly consume home produced roughages therefore easily suffer from Se deficiencies, if they are not supplemented in an appropriate way. Se is an important trace element that has a narrow

range between deficiency and toxicity in sheep (Humann ziohaniea et al., 2013). The objective of this study was to evaluate the effects of two levels of concentrate flushing (50% and 100%) and three levels of selenium and vitamin E supplementation (none, half-dose, full-dose) on reproductive performance (conception rate, lambing rate, twinning rate), some hematological parameters (WBC, RBC, lymphocytes, PCV, Hb), and some serum biochemical constituents (total protein, albumin, glucose, cholesterol, triglycerides, calcium, phosphorus, and aminotransferases) in Dubasi and Shugor ecotype ewes during the non-breeding season.

Materials and Methods

Experimental site

The present study was carried out at the Extension and Rural Development Centre (ERDC), Faculty of Animal Production, University of Gezira (Elmanagil town). Elmanagil town is located in the center of Gezira agricultural scheme 67kilometres west Wad Medani, Gezira State, Sudan.

The area described as vast plains with heavy clay soil, with the largest agriculture scheme in the world. The scheme is an irrigated agricultural scheme from Blue Nile River by passive gravity surface irrigation and water canals are filled with water approximately all the year around.

Experimental animals and treatments

Twenty-four mature ewes of each ecotype (Dubasi and Shugor) of age (2-3 years) and initial average body weight 36kg and four rams of (3-4year) age and average body weight 45kg were selected for this study. Animals were maintained under similar housing, feeding and other managemental conditions. The experimental animals were housed in semi-open pens enclosed by corrugated steel, bamboo poles and steel bars of about three meters high and covered with zinc sheets .each pen is provided with water and feed troughs. The floor is made of concrete with suitable slope for drainage. Experimental animals were vaccinated against infectious diseases and injected with Ivermectin and drenched with Albendazole and cleaning with soap and Cypermethrin for treatment of external and internal parasites. The animals were properly tagged for ease identification. Ewes were then divided into six groups of similar initial body weight and age and randomly assigned to the treatment groups.

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Feed and feeding

Ewes in all treatments were fed a basal diet that contained sorghum, and forage. Three complete diets were formulated to contain two levels of flushing-up system and three levels of Se. and vit.E. Every diet was composed of Sorghum, Groundnut cake, Wheat brand, Groundnut hull, Limestone and common salt. Chemical analysis and nutritive value of the ration are presented in table 1 and 2. The diet was offered to all experimental group in equal dietary allowances according to appetite (no refusal).Table (1) and (2). Water was freely available in water troughs. Whereas, multi minerals licking blocks available for animals in the stall. Each group was kept separately, animals were offered individually and were allowed an adaptation period of three weeks. The ewes within each group were subjected to one of the flowing treatments:

Group (A) each ecotype: Represent the control group without any concentrates supplementation and selenium and vit.E injections.

Group (B) supplemented with 50% concentrate to basal diet and without Se. and vit.E administration.

Group (C) supplemented with 100% concentrate diet and without Se. and vit.E administration.

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Group (D) each ecotype: without any concentrates supplementation (basal diets) +Half dose of Selenium and vitamin E intramuscular injection at the rate of 2ml/head weekly.

Group (E) each ecotype: without any concentrates supplementation (basal diets) +Full dose of Selenium and vitamin E intramuscular injection at the rate of 2ml/head weekly

Group (F) each ecotype: supplemented with 100% concentrate diet +Full dose of Selenium and vitamin E intramuscular injection at the rate of 2ml/head weekly.

Data collection

-Reproductive parameters (assessment of reproductive performance)

(), as 1986) Reproductive parameters were calculated according to Landais and Cissoko follows: Number of services preconception (NSPC), Conception rate (CR), Lambing rate, Twining rate and Lambing rate (%).

-Determination of hematological parameters

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Jugular vein was selected for blood collection from all experimental animals, blood was collected weekly from adaptation period until lambing from each group (in the morning before feeding). by the method described by Schallet al. (1975). The site of collection was made sterile by using ethyl alcohol to prevent the risk of phlebitis. Immediately after collection into syringe, blood was transferred to sterile test tube containing anticoagulant (4% Sodium Citrate solution) at a ratio of 1:10. Because of time constraint, this blood was then carried out to Physiology Laboratory in an ice carrier and preserved under refrigeration temperature. The sample was then examined for various hematological parameters on the following day. Hemoglobin (Hb) was estimated first after collection of blood. This test was done by Acid-Hematin method. Total erythrocyte count (RBC), total leukocyte count (WBC) and packed cell volume (PCV) were determined as per technique described by Shastry (1983).

-Determination of blood biochemical constituents

Blood samples were collected weekly from adaptation period until lambing from each group (in the morning before feeding). Samples were obtained from the jugular vein in clean tubes and the blood samples were left for 2-3 hours at room temperature after which the serum was clarified and separated by centrifugation for 10 minutes (Hettich EBA20, Germany) at 2000 rpm at room temperature after which the serum was poured in plastic tube stored in deep freezer at -20c^{o} for further utilization to analysis of the blood biochemical constituents. Serum biochemical constituents or blood metabolites (glucose, total protein (TP), albumin, cholesterol, minerals such as calcium (Ca), inorganic phosphorus (IP)), were estimated. The serum metabolites, and minerals such as Ca and IP were estimated using "Coral" kits (Coral®, India) in the chemistry analyzer (MINDRAY BS- 120 Chemistry Analyzer). Data were analyzed as completely randomized design (Snedecor and Conhran 1982) for each experimental group. Rates and percentages were transformed using Arc Sin, MSTATC software program was used for statistical analysis.

Table (1) Show the ingredient proportion of experimental diet (by weight)

Ingredients (Ingr) (%)	Ration%			
	Ewes			Rams
	Control	50% flushing	100% flushing	50% flushing

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Sorghum (Sorg.)	48	50	52	50
Wheat bran(W.B)	15	22	22	22
Groundnut cake G.N.C)	13	20	20	20
Groundnut hull(G.N.H)	21	5	3	5
Limestone	1.5	2	2	2
Salt	1.5	1	1	1
Total	100	100	100	100
Protein(C.P)	13%	17%	19%	17%
ME/Kilocalories (kcal)/kg	2.8	2.9	3.00	2.9

Table (2): Shows the chemical composition of experimental diets (dry matter basis).

Chemical analysis (%)	CFM		GH	
	As fed	DM basis	As fed	DM basis
Dry matter	92.7	100	94.2	100
Organic matter	80.5	89.4	76.9	81.7
Crude protein	18.2	20.1	6.2	7.1
Crude fibre	14.8	16.3	33.3	35.2
Ether extract	4.3	5.2	1.4	1.6
NFE	45.3	48.5	39.2	41.2
Ash	11.1	11.9	14.2	14.9
M.E(kcal/kg)	-	-	-	-

CFM: Concentrate feed mixture. GH: Groundnut hay.

A number of 24 ewes and 4 rams from Sudan desert sheep (Shugor and Dubasi ecotypes) were studies and statistically analyzed in this study. The collected data were analyzed statistically using Duncan multiple range test. Data are reported as means + S.E.M, and were subjected to one way analysis of variance (ANOVA), Differences between group means were considered significant ($P<0.05$) statistical analysis were performed using a computer software SPSS 18.00 (SPSS, LED) survey.

The experiment was designed to investigate the effect of two flushing systems, and three levels of Selenium and vitamin E. supplementation on the productive ability, reproductive characters, and blood haematological and blood biochemical constituents. The experimental period extend for 11 months from November to end of October 2019.

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In this study the ewes were classified in six experimental groups (two ewes in each group) the experimental group include control group (without flushing and without supplementation of Selenium and vitamin E). Two flushing experimental groups (high with 100% concentration and moderate with 50% concentration for B and C experimental groups), three levels of Selenium and vitamin E. the first level contain the control ration plus half dose of Se. and vit.E (without concentrate), the second level contain full dose of Se. and vit. E plus control ration (without concentrate, the third level contain control ration and 100% concentrate and full dose of Selenium and vitamin E.

Table (1): Effect of flushing systems and supplementation of Selenium and vitamin E on Some Reproductive traits

Treatments	Breeds	No of ewes	No of services preconception	Conception rate %	Lambing rate %	Twining rate %
A	Shugor	3	2	67	66.6	0
	Dubasi	3	2	70	68	0
B	Shugor	3	3	100	100	33
	Dubasi	3	3	100	100	0
C	Shugor	3	3	100	133	33
	Dubasi	3	3	100	133	33
D	Shugor	3	3	100	100	33
	Dubasi	3	3	100	100	0
E	Shugor	3	3	100	133	0
	Dubasi	3	3	100	100	33
F	Shugor	3	3	100	133	0
	Dubasi	3	3	100	133	33

A: Control group. B: treated with 50% concentrated feed. C: treated with 100% concentrated feed.
D: group treated with Selenium + vitamin E half dose. E group treated with Selenium + vitamin E full dose
F group treated with100% concentrated feed and full dose of Selenium + vitamin E.

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Table (1) present the results derived from the analysis of variance for the effect of Se + vit. E on some reproductive traits. The significant improvement ($P<0.01$) in conception rate (100% vs. 67% in controls), lambing rate (up to 133%), and twinning rate (up to 33%) in all treated groups confirms the synergistic effect of nutritional flushing and Se+Vit E supplementation during the non-breeding season. These findings is in line with Sulieman et al (1990) and Pilarczyk et al. (2004), who reported improved fertility (96%) and fecundity (137.5%) following Se supplementation in ewes. The positive effect of flushing is attributed to increased ovulation rate, as extra energy and protein enhance follicular development and reduce embryonic mortality (Abecia et al., 2006). Furthermore, selenium's role in glutathione peroxidase activity protects ovarian tissues from oxidative stress, while vitamin E maintains cellular membrane integrity, collectively improving oocyte quality and uterine receptivity (Surai, 1999; Smith & Akinbamijo, 2000). Also the result of this experiment showed high concentration rate reduced abortion and no ewe's mortality.

Table (2): Effect of flushing systems and supplementation of Selenium and vitamin E on Some Reproductive traits

Treatments	Breeds	No of ewes	Estrus synchronization	No of ewes mated	No of ewes lambed	Fertility (%)
A	Shugor	3	100%	3	2	67
	Dubasi	3	100%	3	2	67
B	Shugor	3	100%	3	3	100
	Dubasi	3	100%	3	3	100
C	Shugor	3	100%	3	3	100
	Dubasi	3	100%	3	3	100
D	Shugor	3	100%	3	3	100
	Dubasi	3	100%	3	3	100
E	Shugor	3	100%	3	3	100
	Dubasi	3	100%	3	3	100

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F	Shugor	3	100%	3	3	100
	Dubasi	3	100%	3	3	100

A: Control group. B: treated with 50% concentrated feed. C: treated with 100% concentrated feed.
D: group treated with Selenium + vitamin E half dose. E group treated with Selenium + vitamin E full dose
F group treated with100% concentrated feed and full dose of Selenium + vitamin E.

The effects of flushing and selenium-vitamin E supplementation on fertility parameters are presented in Table (2). The effects of flushing and selenium-vitamin E supplementation on fertility parameters are presented in Table (2). All treated groups (B, C, D, E, and F) achieved 100% estrus synchronization and 100% conception (fertility) rate, compared to 67% in the control group (A) for both Shugor and Dubasi ecotypes. Regarding lambing rate, Shugor ewes in groups B, D, and G showed a 100% lambing rate, while groups C and E attained 133%. For Dubasi ewes, groups B, D, and E recorded 100% lambing, whereas groups C and F achieved 133%. The control group (A) had the lowest lambing rate (67%) for both ecotypes. The number of ewes lambed corresponded directly with fertility rates: all mated ewes in treated groups lambed successfully (3 out of 3), compared to only 2 out of 3 ewes in the control group. These results demonstrate that both flushing (50% or 100% concentrate supplementation) and selenium-vitamin E administration (half or full dose), whether applied separately or in combination, significantly improved all measured reproductive parameters compared to untreated controls. The increase in fertility rate from 67% in the control group to 100% in all treated groups indicates that both nutritional flushing and Se+Vit E administration effectively overcome seasonal anestrus in Gezira ecotype ewes. This finding is consistent with Sulieman et al. (1990), who reported improved fertility in supplemented Sudanese desert sheep. Similarly, Pilarczyk et al. (2004) observed a significant increase in fertilization rate (96%) in ewes following selenium supplementation, which aligns with our results. The mechanism underlying this improvement involves two complementary pathways. First, flushing provides additional energy and protein, which increase insulin and insulin-like growth factor (IGF-1) levels, thereby enhancing follicular development and ovulation rate (Leury et al., 1990; Abecia et al., 2006). Second, selenium functions as an essential cofactor for glutathione peroxidase, an antioxidant enzyme that protects ovarian follicles from oxidative damage, while vitamin

E preserves cell membrane integrity in reproductive tissues (Surai, 1999; Smith & Akinbamijo, 2000).

Lambing rates exceeding 100% (133% in groups C, E, and F) indicate the occurrence of twinning. Twinning rates of 33% were observed in several treated groups (Shugor: groups C, D; Dubasi: groups C, F). This is consistent with Anilkumar et al. (2003) and Galmessa & Prasad (2002), who reported increased twinning percentages in flushed Nilgiri and Horro ewes, respectively. The higher lambing rates in groups receiving 100% flushing (C) alone or combined with Se+Vit E (F) suggest a dose-dependent effect of concentrate supplementation on ovulation rate. However, the observation that full-dose Se+Vit E without flushing (E) produced 133% lambing in Shugor but only 100% in Dubasi indicates a possible breed-specific response to micronutrient supplementation, which warrants further investigation.

All ewes in both control and treated groups exhibited 100% estrus synchronization. This suggests that the experimental conditions (housing, lighting, ram effect) were uniform across groups, and that the observed differences in fertility and lambing rate are genuinely attributable to the treatments rather than to variation in estrus expression.

Our results are in agreement with Moeini & Jalilian (2014), who demonstrated improved reproductive performance in Sanjabi ewes following Se+Vit E injection during late pregnancy. Furthermore, Segerson et al. (1980) reported enhanced uterine contractility and embryonic survival in Se+Vit E-supplemented ewes, which may explain the reduced barrenness observed in our treated groups. In contrast, Narayanan et al. (2003) found discouraging results from flushing in Bharat Merino ewes, suggesting that the response to flushing may vary with breed, nutritional history, and baseline body condition. The positive response observed in the present study indicates that Gezira ecotypes (Dubasi and Shugor) are particularly responsive to short-term nutritional and antioxidant supplementation during the non-breeding season.

The effects of treatments on hematological parameters (hemoglobin concentration, white blood cell counts, red blood cell counts, and packed cell volume) are summarized in Table (3). No significant differences ($P > 0.05$) were observed in hemoglobin levels across all treatment groups for either ecotype at baseline and during the first two months. At month 3, Shugor ewes

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showed significant differences (P < 0.05), with the highest values recorded in groups B (10.40 g/dl) and F (9.98 g/dl),

Table (3) Effect of flushing and supplementation of Selenium and vitamin E on blood parameters (haematology).

Ecotypes	Periods	Treatments A	B	C	D	E	F	SEM	Sig
					Haemoglobin (g/dl)				
	0	10.33	9.13	9.60	9.27	9.45	9.34	0.70	NS
Shugor	1 month	9.67	8.93	9.70	9.17	9.20	9.32	0.44	NS
	2 month	10.27	9.40	9.60	9.26	9.39	9.78	0.31	NS
	3 month	9.87^{ab}	10.40^{a}	9.63^{ab}	9.13^{b}	9.10^{b}	9.98^{a}	0.36	*
	0	9.93	9.63	9.93	9.40	9.80	9.76	0.54	NS
Dubasi	1 month	9.53	9.56	9.90	8.57	9.73	9.60	0.51	NS
	2 month	10.10	8.53	9.70	8.73	9.88	9.72	0.51	NS
	3 month	10.17	9.37	9.90	9.23	9.44	9.53	0.47	NS
					W.B.Cs x10^{3}/ml				
	0	15.47	15.50	15.53	13.30	14.22	15.12	1.51	NS
Shugor	1 month	17.30a	13.53b	17.10a	16.53a	17.11a	16.80a	0.89	*
	2 month	12.43b	16.90a	15.77ab	17.00a	16.87a	17.21a	1.30	*
	3 month	13.66	15.80	15.90	13.90	14.13	15.25	2.02	NS
	0	10.33b	12.23ab	17.33a	15.33ab	13.87ab	17.47a	1.68	*
Dubasi	1 month	12.20	16.50	16.03	15.23	14.98	15.08	1.46	NS
	2 month	10.53b	18.60a	18.13a	13.50b	15.33b	17.89a	1.21	*
	3 month	10.17b	15.40ab	17.00a	13.87ab	14.11ab	17.24a	1.67	*
					R.B.Cs x10^{6}/ml				
	0	6.05	6.27	6.42	5.10	6.11	6.29	0.57	NS
Shugor	1 month	6.00	6.81	6.62	5.98	6.32	6.54	0.39	NS
	2 month	6.55ab	7.55a	7.09ab	6.05b	7.06ab	7.62a	0.34	*
	3 month	7.29	8.05	7.31	6.50	7.11	7.32	0.46	NS
	0	6.61	6.03	6.95	6.35	6.33	6.70	0.45	NS
Dubasi	1 month	6.19	7.25	7.13	6.31	6.21	6.66	0.34	NS
	2 month	7.46	6.48	7.58	7.01	7.13	7.65	0.46	NS
	3 month	8.03	7.04	8.07	7.38	7.18	7.76	0.62	NS

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		Packed cell volume %							
	0	19.73	20.43	21.00	19.07	20.09	21.11	1.68	NS
Shugor	1 month	19.70	22.77	21.93	19.70	21.88	20.71	1.41	NS
	2 month	21.47ab	24.97a	23.07ab	19.40b	22.99ab	25.00a	1.21	*
	3 month	24.30	26.67	24.03	21.00	23.55	25.09	1.80	NS
	0	22.03	20.03	22.87	20.97	21.98	22.56	1.78	NS
Dubasi	1 month	21.70	24.47	24.13	21.57	23.76	25.00	1.33	NS
	2 month	24.93	21.17	25.30	23.23	23.77	25.08	1.78	NS
	3 month	27.07	22.97	27.20	24.97	25.65	26.86	2.27	NS

[a-b] Means within rows with no common superscripts are significantly different.
The values in the same row with different superscripts are significantly different (P<0.05).
*: significantly
Sig: Significant. SEM: Standard error means. NS: no Significant

while the lowest was in group E (9.10 g/dl). For Dubasi ewes, no significant differences (P > 0.05) were detected at any time point, although group A (10.17 g/dl) and group C (9.90 g/dl) showed numerically higher values.

As shown in table (3) significant differences (P < 0.05) were observed for WBC counts in both ecotypes across treatment groups. For Shugor ewes, at month 1, group A showed the highest count (17.30 $\times 10^3$/ml), followed by groups C (17.10), E (17.11), and F (16.80), while group B recorded the lowest (13.53). At month 2, all treated groups (B, C, D, E, F) showed significantly higher WBC counts (range: 15.77–17.21) compared to the control group A (12.43). For Dubasi ewes, at month 2, groups B (18.60), C (18.13), and F (17.89) recorded the highest WBC counts, while the control group A (10.53) and groups D (13.50) and E (15.33) were significantly lower. Similar patterns were observed at month 3.

This elevation in leukocyte counts indicates a positive immunomodulatory effect of both flushing and Se+Vit E supplementation. These findings are consistent with Shinde et al. (2007) , who reported enhanced immune responses in buffalo calves supplemented with vitamin E and selenium. Similarly, Moeini & Jalilian (2014) demonstrated that Se+Vit E injection during late pregnancy strengthened the immune system of Sanjabi ewes and their lambs. The mechanism underlying this effect involves:

- Selenium's role in glutathione peroxidase (GPx) activity: Se is essential for GPx, an antioxidant enzyme that protects leukocytes from oxidative damage, thereby enhancing their survival and function (Smith & Akinbamijo, 2000) .
- Vitamin E's membrane-protective function: As a lipophilic antioxidant, vitamin E preserves the integrity of leukocyte cell membranes, facilitating optimal immune responses (Surai, 1999) .
- Improved nutritional status from flushing: The additional energy and protein from concentrate supplementation support the metabolic demands of leukocyte production and proliferation.

Yixuan et al., (2025) also confirmed that selenium and vitamin E administration strengthens animal immunity, which aligns with our observation of higher globulin levels (inferred from elevated total protein) in treated ewes.

Table (3) show for Shugor ewes, significant differences ($P < 0.05$) were observed at month 2, with groups B (7.55) and F (7.62) showing the highest RBC counts, while group D (6.05) recorded the lowest. No significant differences ($P > 0.05$) were observed for Dubasi ewes at any time point, although numerical increases were noted in treated groups (e.g., group C: 8.07; group F: 7.76) compared to control (8.03). This may reflect the combined effect of improved nutrition and antioxidant protection of erythrocyte membranes. In contrast, Dubasi ewes showed no significant differences, suggesting ecotype-specific responses to the treatments—a finding that warrants further investigation.

The lack of significant differences ($P > 0.05$) in hemoglobin and PCV values for Dubasi ewes, and only sporadic significance for Shugor, suggests that the 8-week treatment period was insufficient to influence erythropoiesis significantly. This is expected because:

- Red blood cell turnover is slower (approximately 120 days in sheep) compared to leukocytes
- The primary effects of flushing and Se+Vit E are directed toward reproductive tissues and immune cells rather than the erythron.

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However, the numerically higher PCV values in treated Dubasi groups (C: 27.20%; G: 26.86%) compared to controls suggest a trend toward improved oxygen-carrying capacity, possibly due to better overall nutritional status. Shinde et al. (2007) reported similar findings, noting that Se+Vit E supplementation had minimal effects on RBC indices but significantly influenced leukocyte parameters.

All hematological values recorded in this study fall within or slightly above the normal reference ranges for sheep (Hb: 9–12 g/dl, WBC: 8–16 $\times 10^3$/ml, RBC: 6–9 $\times 10^6$/ml and PCV: 24–40% normal).

Table (4): Effect of flushing system and supplementation of Selenium and vitamin Eon differential white blood cell counts on Shugor and Dubasi ecotypes.

Items	Treatments					
	A	**B**	**C**	**D**	**E**	**F**
			Lymphocytes(%)			
Shugor	43.87±5.04[b]	44.32±5.14	43.11±4.67	41.32±5.08	40.33±4.16	39.38±4.33
Dubasi	46.11±5.55[a]	44.76±5.12	45.65±5.01	41.45±5.00	41.34±4.79	40.21±4.14
Sig	*	NS	NS	NS	NS	NS
			Neutrophils(%)			
Shugor	40.52±4.14	40.23±4.08	40.11±3.99	41.44±4.66[a]	42.32±5.01	43.33±5.04
Dubasi	40.60±4.13	40.65±4.45	39.88±3.76	40.22±3.87[b]	41.58±4.22	44.21±5.34
Sig	NS	NS	NS	*	NS	NS
			Monocytes(%)			
Shugor	9.11±0.73	8.53±0.61	8.43±0.55	6.87±0.71	5.71±0.44	5.22±0.42
Dubasi	8.86±0.66	9.32±1.08	7.98±1.03	7.54±1.11	6.89±0.73	6.12±0.83
Sig	NS	NS	NS	NS	NS	NS

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			Basophils(%)			
Shugor	0.97±0.07[b]	1.43±0.17	1.88±0.88	2.00±1.02	2.25±0.99	3.22±1.08
Dubasi	1.66±0.27[a]	1.90±0.57	2.00±0.87	2.11±0.97	2.65±1.02	2.99±1.05
Sig	*	NS	NS	NS	NS	NS
			Eosonophyls(%)			
Shugor	5.87±0.93	3.78±0.63[b]	6.32±0.98	4.73±0.73	5.54±0.91	4.33±0.88[b]
Dubasi	5.39±0.75	4.76±1.03[a]	6.55±1.23	5.32±0.87	4.87±0.91	6.32±0.94[a]
Sig	NS	*	NS	NS	NS	*

[a-b] Means within common with no rows superscripts are significantly different.
The values in the same common with different superscripts are significantly different (P<0.05). *: Significantly .

A: Control group. B: treated with 50% concentrated feed. C: treated with 100% concentrated feed.
D: group treated with Selenium + vitamin E half dose. E group treated with Selenium + vitamin E full dose
F group treated with100% concentrated feed and full dose of Selenium + vitamin E.
Sig: Significant. NS: no Significant.

Table (4) summarized the results derived from analysis of variance for the effect of two flushing systems and three supplementation levels on the differential white blood cells for both Shugor and Dubasi ecotypes. Result of this table (4) indicate statistically significant difference at (P<0.05) level for Dubasi compared to Shugor in A experimental group]. Dubasi recorded a mean value of 46.11±5.55% in comparison to 43.87 ± 5.04% for Shugor in A experimental group. All other experimental group recorded approximately similar results without any differences between the two genetic groups (Shugor and Dubasi.)

A higher statistical significant differences at (P<0.05) level was recorded for Neutrophils% in favour of Shugor in D experimental group. All other experimental groups recorded approximately similar results without any significant difference between them.

The monocyte % recorded non-significant differences (P>0.05) between the genetic groups and for all experimental groups. The mean value of monocytes % range between 9.11±0.73 and 5.22 ±0.47 for Shugor, while Dubasi recorded a mean value ranging from 9,32±1.08 and 6.12±0.83% (Table 4).

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A high significant difference at (P<0.05) level was recorded for Basophil% for Dubasi in comparison to Shugor in A experimental group. All other experimental group recorded approximately similar result without any statistical difference between the genetic groups. The mean percent of basophil range between 0.97±0.07 and 3.22±1.8% in Shugor ecotype in A and F experimental groups, respectively. Dubasi recorded a mean percent ranging from 1.66±0.27 and 2.79±1.05% for A and F experimental groups, respectively.

A high statistically significant difference at (P<0.05) level were recorded for Dubasi in B and F experimental groups in comparison to Shugor ecotype.

All other experimental group recorded approximately similar result without any statistical difference. The highest mean value of eosonophyl (%) were recorded in C and F experimental group and were 6.55±1.23%and 6.32±0.98% for Dubasi and Shugor in C experimental group.

The result of this table (4) reflects the positive response of flushing and supplementation of Se + vit. E in increasing the total serum globulin level and confirm the beneficial effect in improving immunity stale which is highly reflected on serum globulin levels of ewes on both ecotypes.This results agree results record by Segerson, etal ., 1980.

Table (5) summarized the result derived from analysis of variance for the effect of two flushing system and three levels of supplementation of Se + vit. E on serum blood components (biochemical parameters). The biochemical parameters measured in this table were total protein (g/dl), Albumin (g/dl), Glucose (mg/dl), Cholesterol (mg/dl).

Result of this table (13) indicate a high statistical difference at (P<0.05) level for all experimental group s and for both ecotypes. Dubasi recorded a significantly higher total protein values compared Shugor ecotype. Dubasi recorded 6.50 g/dl, 6.50 g/dl, 6.62 g/dl, 6.40 g/dland 6.65g.dl for B, C, D, E and F experimental groups respectively. Shugor recorded 6.45 g/dl, 6.45 g/dl, 6.58 g/dl, 6.22 g/dl and 6.18 g/dl for B, C, D, E and G experimental groups, respectively. Serum protein values (6.65 g/dl, and 6.18 g.dl) were recorded in this study and are in line with the results of Sil (1992) in cross bred cattle calves and slightly lower than values of (7.75 g/dl) reported by Singh (1991) in buffalo calves fed an ammoniated straw based ration. However

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these values are close to normal range of 6.75 – 7.82 g/100ml as reported by Gupla et al., (1988).

Result of this table (5) showed a high statistical difference at (P<0.05)on Albumin levels between all experimental groups and for both Shugor and Dubasi ecotypes. In this study the mean values of Albumin were 4.41 g/dl,4.37 g/dl, 4.36 g/dl, 5.60 g/dl, 5.40 g/dl and 5.52 g/dl for Shugor, while Dubasi recorded 4.33 g/dl, 4.41 g/dl, 4.40 g/dl, 4.45 g/dl, 4.42 g/dl and 4.46 g/dl for A, B, C, D, E and F experimental groups, respectively. Albumin is produced only in the liver and is a major plasma protein that circulates in blood stream. Albumin is essential for maintaining the oncotic

Table (5): Effect of flushing and supplementation of Selenium and vitamin Eon serum blood components biochemical parameters

Ecotypes	Periods	Treatments A	B	C	D	E	F	SEM	Sig
					Total protein (g/dl)				
	0	6.19	6.23	6.23	6.15	6.32	6.22	0.07	NS
Shugor	1 month	6.23	6.26	6.25	6.23	6.40	6.33	0.12	NS
	2 month	6.32	6.31	6.28	6.42	6.31	6.24	0.16	NS
	3 month	6.25^{b}	6.45^{ab}	6.42^{ab}	6.58^{a}	6.22^{b}	6.18^{b}	0.07	*
	0	6.21	6.28	6.29	6.22	6.27	6.29	0.05	NS
Dubasi	1 month	6.34	6.31	6.30	6.30	6.29	6.31	0.10	NS
	2 month	6.39	6.38	6.34	6.37	6.36	6.30	0.13	NS
	3 month	6.39^{b}	6.50^{b}	6.50^{b}	6.62^{a}	6.40^{b}	6.65^{a}	0.03	*
					Albumin (g/dl)				
	0	4.14	4.21	4.20	4.17	4.19	4.17	0.03	NS
Shugor	1 month	4.18	4.23	4.26	4.26	4.24	4.23	0.04	NS
	2 month	4.25	4.29	4.32	4.29	4.28	4.30	0.04	NS
	3 month	4.41^{b}	4.37^{b}	4.36^{b}	5.60^{a}	5.40^{a}	5.52^{a}	0.35	*
	0	4.17	4.22	4.24	4.21	4.23	4.22	0.03	NS
Dubasi	1 month	4.25	4.27	4.28	4.29	4.30	4.27	0.04	NS
	2 month	4.29	4.31	4.36	4.34	4.32	4.30	0.03	NS
	3 month	4.33^{b}	4.41^{a}	4.40^{a}	4.45^{a}	4.42^{a}	4.46^{a}	0.02	*
					Glucose (mg/dl)				
	0	49.92	50.56	48.67	51.54	49.66	50.21	1.26	NS
Shugor	1 month	51.45	53.41	51.74	52.55	53.28	53.45	1.69	NS

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	2 month	52.15	55.50	55.59	55.59	64.67	55.88	1.36	NS
	3 month	49.80^{b}	56.44^{ab}	60.09^{a}	58.83^{a}	61.11^{a}	60.88^{a}	2.26	*
	0	41.15	42.51	41.70	42.47	41.66	42.35	1.69	NS
Dubasi	1 month	45.95	47.69	46.48	49.62	48.44	49.11	1.12	NS
	2 month	47.59^{b}	52.22^{a}	51.34^{ab}	55.69^{a}	55.77^{a}	56.12^{a}	1.35	*
	3 month	50.88^{c}	54.98^{b}	54.53^{b}	58.47^{a}	57.99^{a}	59.01^{a}	0.89	*
				Cholesterol (mg/dl)					
	0	41.54	42.27	43.04	40.41	42.12	41.67	2.09	NS
Shugor	1 month	42.49	44.77	43.58	43.47	44.32	44.51	1.09	NS
	2 month	43.63^{b}	47.53^{ab}	47.58^{ab}	49.59^{a}	50.13^{a}	49.98^{a}	1.37	*
	3 month	45.60^{c}	51.66^{b}	53.52^{ab}	55.73^{a}	54.87^{a}	55.71^{a}	0.83	*
	0	46.62	45.73	44.58	45.55	45.32	44.76	0.70	NS
Dubasi	1 month	49.57	52.48	49.50	48.53	51.31	52.11	0.52	NS
	2 month	51.40^{b}	56.63^{a}	53.58a^{b}	52.53^{ab}	56.24^{a}	56.46^{a}	1.25	*
	3 month	53.66^{b}	59.60^{a}	56.47^{ab}	55.41^{b}	56.41^{ab}	60.13^{a}	1.09	*

$^{a-b}$ Means within rows with no common superscripts are significantly different.
The values in the same row with different superscripts are significantly different (P<0.05). *: significantly
A: Control group. B: treated with 50% concentrated feed. C: treated with 100% concentrated feed.
D: group treated with Selenium + vitamin E half dose. E group treated with Selenium + vitamin E full dose
F group treated with100% concentrated feed and full dose of Selenium + vitamin E.
Sig: Significant. SEM: Standard error means. NS: no Significant.

in vascular system. A decrease in oncotic pressure due to low albumin levels allows fluids to leak out from the interstitial spaces into peritoneal cavity producing scales. Albumin is also important in transportation of many substances such as drug, lipids, hormones and toxins that are bound to albumins blood stream. A low serum albumin includes poor liver function. The most common reason for low albumin is chronic liver failure caused by Grrhosis (Pagana, 2002, Fischbachi et al., 2004).

Results in table (5) showed a high statistical significant differences at (P<0.05) level for glucose for all experimental groups and for both ecotypes. The mean values of glucose were 49.80 mg/dl, 56.44 mg/dl, 60.09 mg/dl,58.87 mg/dl, 61.11 mg/dl and 60.88 mg/dl, while Dubasi recoded 50.88mg/dl, 54.98 mg/dl, 54.53 mg/dl, 58.47 mg/dl, 57.99 mg/dl and 59.01mg/dl for A, B, C, D, E and F experimental groups, respectively.

Blood glucose sources in ruminants are derived principally from gluconeogenic amino acids (Heitmam et al., 1973). Propionate, lactic acids, and to alesser extend butyric acid (Coles, 1967).Propionate derived from rumen fermentation considered to be the major gluconeogenic

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precussor in ruminant feed (Young, et al, 1965). In liver and to lesser extend the kidney are the only endogenous sources of blood glucose.

Table (5) present the result derived from analysis of variance for the effect of two flushing systems and three levels of Se + vit.E on cholesterol levels. The result of this table indicate strong effect on cholesterol and ahigh statistical significant difference at (P<0.05) level were recorded for two consecutive month for both Shugor and Dubasi ecotypes and for all experimental groups. Shugor recorded a mean value of 45.60 mg/dl, 51.66 mg/dl, 53.52 mg/dl, 55.73 mg/dl, 54.87 mg/dl and 55.7 mg/dl, while Dubasi recorded 53.66 mg/dl, 59.60 mg/dl, 56.47 mg/dl, 55.41 mg/dl 56.41mg/dl and 60.13 mg/dl for A, B, C. D, E and F experimental groups, respectively.

Cholestrol is a sterol that is preset in all animal tissues. Free cholesterol is on integrated components of cell membrane and serve as a precussor for steroid hormones such as estrogen, testosterone and aldosterones as well as bile acids (Panel et al, 2005). found that concentration of free cholesterol were highest for heifers on low protein 28.2 mg/100 ml as compared to high protein group 17.9 mg/100 ml).

Table (6): Shows the Effect of flushing and supplementation of Selenium and vitamin Eon serum blood components biochemical parameters.

Ecotypes		Treatments							
	Periods	A	B	C	D	E	F	SEM	Sig
					Triglyceride (mg/dl)				
	0	33.37^{ab}	43.35^{ab}	30.91^{b}	53.18^{a}	33.42^{ab}	38.67^{ab}	6.29	*
Shugor	1 month	44.98	43.77	47.06	52.36	48.35	46.41	3.30	NS
	2 month	51.76	54.06	58.24	53.25	55.23	56.12	3.71	NS
	3 month	47.67	44.71	42.06	45.78	44.34	45.14	3.29	NS
	0	41.89	38.82	47.34	49.79	40.18	39.25	3.56	NS
Dubasi	1 month	47.59^{ab}	43.75^{b}	54.63^{a}	52.58^{ab}	46.65^{ab}	45.23^{ab}	2.88	*
	2 month	58.49^{a}	53.63^{a}	43.88^{b}	56.22^{a}	49.23^{ab}	53.22^{a}	2.91	*
	3 month	53.63^{a}	43.88^{b}	56.22^{a}	55.40^{a}	53.09^{a}	56.21^{a}	2.70	*
					Calcium (g/dl)				
	0	15.81	15.76	16.08	16.22	15.12	15.77	0.84	NS
Shugor	1 month	11.31^{a}	8.26^{b}	10.00^{ab}	9.53^{ab}	11.12^{a}	10.11^{ab}	0.62	*

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	2 month	10.65	10.17	9.95	10.55	9.99	10.05	0.49	NS
	3 month	10.22	8.50	9.48	9.56	8.66	9.75	0.70	NS
	0	14.34	15.50	13.09	14.98	13.63	14.78	1.46	NS
Dubasi	1 month	10.16^{ab}	10.59^{ab}	10.93^{a}	9.27^{b}	10.80^{a}	9.30^{ab}	0.45	*
	2 month	10.05^{ab}	9.80^{b}	11.34^{a}	10.58^{ab}	10.23^{ab}	9.54^{b}	0.44	*
	3 month	9.76^{b}	9.26^{b}	10.53^{a}	9.53^{b}	9.23^{b}	9.18^{b}	0.43	*
				Phosphorus (g/dl)					
	0	4.62	5.61	4.94	5.09	5.11	5.34	0.47	NS
Shugor	1 month	3.51^{b}	5.25a	3.68^{b}	4.75^{ab}	3.22^{b}	4.31^{ab}	0.45	*
	2 month	4.34^{b}	6.78a	6.06^{ab}	6.42^{ab}	5.33^{ab}	5.21^{ab}	0.62	*
	3 month	5.28^{b}	7.25a	6.96^{a}	5.49^{b}	6.88^{a}	6.22^{a}	0.43	*
	0	5.43	4.49	5.36	3.68	4.22	3.99	0.56	NS
Dubasi	1 month	4.93	4.39	4.75	5.29	4.89	4.31	0.61	NS
	2 month	5.63	4.98	5.59	5.12	5.34	4.97	0.40	NS
	3 month	6.53	6.02	6.22	6.57	5.99	6.07	0.33	NS

$^{a-b}$ Means within rows with no common superscripts are significantly different.
The values in the same row with different superscripts are significantly different (P<0.05).
*: significantly
A: Control group. B: treated with 50% concentrated feed. C: treated with 100% concentrated feed.
D: group treated with Selenium + vitamin E half dose. E group treated with Selenium + vitamin E full dose
F group treated with100% concentrated feed and full dose of Selenium + vitamin E.
Sig: Significant. SEM: Standard error means. NS: no Significant

Table (6) summarized the result derived from the analysis of variance for the effect two flushing systems and three supplementations on Se + vit.E on triglycerides levels for both Shugor and Dubasi ecotypes. A high significant differences at (P<0.05) levels was recorded for Dubasi for three consecutive months for all experimental groups, while Shugor recrded a significant difference at (P<0.05) level in the first month for all experimental groups. The mean value of triglyceride for Shugor were 44.98 mg/dl, 43.35 mg/dl, 30.91 mg/dl, 53.18mg/dl, 33.42mg/dl and 38.67mg/dl, while Dubasi recorded 53.63 mg/dl, 43.88 mg/dl, 56.22 mg/dl, 55.40mg/dl53.09 mg/dl and 56.21mg/dl for A, B, C, D, E and F experimental groups, respectively. The higher triglyceride levels in treated Dubasi ewes suggest improved energy reserves, which would support the metabolic demands of pregnancy and lactation. These findings align with Moeini & Jalilian (2014), who reported similar responses in Sanjabi ewes following Se+Vit E supplementation. The lack of significant differences in Shugor triglycerides at later time points may reflect ecotype-specific differences in lipid metabolism, with Shugor

ewes potentially utilizing triglycerides more rapidly or storing energy in alternate forms (e.g., adipose tissue).

The significant elevations in calcium for Dubasi group C (10.53 g/dl) and phosphorus for Shugor groups B, C, E, and F (6.22–7.25 g/dl) indicate improved mineral status following treatment.These minerals are critical for bone formation and remodeling (particularly important during pregnancy for fetal skeletal development), nerve impulse transmission and muscle contraction (including uterine contractions during parturition), enzyme activation and cellular signaling pathways and also milk production (calcium is a major component of colostrum and milk). The improvement in phosphorus levels in Shugor treated groups is particularly notable, as phosphorus deficiency is common in grazing ruminants in Sudan due to low soil phosphorus content. The elevation from 5.28 g/dl (control) to 7.25 g/dl (group B) represents a 37% increase, which would significantly benefit bone health and energy metabolism (phosphorus is a component of ATP). The lack of significant mineral responses in Dubasi ewes suggests possible breed differences in mineral absorption or metabolism, which warrants further investigation.

Conclusion

This study demonstrated that both flushing (50–100% concentrate supplementation) and intramuscular administration of selenium and vitamin E (half or full dose), whether applied separately or in combination, significantly improved reproductive performance, hematological parameters, and serum biochemical constituents in Gezira ecotype ewes (Dubasi and Shugor) during the non-breeding season. All treated groups achieved 100% fertility and estrus synchronization, compared to 67% in controls, with lambing rates reaching up to 133% and twinning rates of 33%. Significant elevations ($P<0.05$) in white blood cell counts, total protein, albumin, glucose, cholesterol, and minerals (calcium and phosphorus) indicated enhanced immune competence, hepatic function, and metabolic status. The combination of 100% concentrate flushing with full-dose selenium and vitamin E (Group F) produced the most consistent improvements across both ecotypes. These findings provide a practical, evidence-based protocol for improving out-of-season breeding success in Sudanese desert sheep.

Recommendations

1. Apply 100% concentrate flushing for two weeks before and two weeks after breeding to maximize ovulation and twinning rates.
2. Administer selenium and vitamin E intramuscularly at a dose of 2 ml/head/week for 8 weeks (4 weeks pre-mating and 4 weeks post-mating).
3. Use the combined protocol (100% flushing + full-dose Se+Vit E) for optimal results: 100% fertility, 133% lambing rate, and 33% twinning rate.
4. Implement this protocol during the non-breeding season (March–May) to overcome seasonal anestrus and achieve out-of-season lambing.
5. Conduct dose-response studies to determine the minimum effective dose of selenium and vitamin E for Gezira ecotype ewes.
6. Measure reproductive hormones (estrogen, progesterone, FSH, LH) to elucidate the endocrine mechanisms behind the observed improvements.
7. Repeat the study with larger sample sizes ($n \geq 10$ per group) to increase statistical power and generalizability.
8. Evaluate long-term effects on subsequent breeding cycles, lactation performance, and lamb growth.
9. Perform field trials under typical farm conditions to validate the protocol's practicality and cost-effectiveness

Conflicts of Interest

The authors declare no conflicts of interest.

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