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Throw light on the causes of appearance of parakeratosis in buffalo calves in Sharkia Governorate with trial of treatment

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ABSTRACT

The present study was designed to investigate the clinical impacts of parakeratosis on health status and hematobiochemical parameters among buffalo calves and evaluate the effect of ration fortification with either mineral mixture with or without addition Nigella sativa seed on clinical health condition.

About thirty five buffalo calves with age of two years and average B. wt (300-350 kg) were used in this study. Two skin scrapings from affected part and fecal samples were collected from parakeratotic calves for parasitological and mycological examination. Fecal samples were examined parasitologically. Post examination skin scrapings and fecal samples, 20 calves free from mites, fungus and parasite were used in this study (5 healthy –15 suffering from parakeratosis) were divided into four equal groups, Gp (1) apparently healthy calves (-ve control), Gp (2) calves suffering from parakeratosis (+ve control), Gp (3) calves suffering from parakeratosis supplemented with 2 kgm mineral mixture/ton ration for 2 month and Gp (4) calves suffering from parakeratosis supplemented with mineral mixture 2 kgm, mineral mixture/ton ration and 0.05% nigella sativa powder for 2 month. All calves were feed with 1kgm ration/50 kgm body weight and opened feeding hay.

Parakeratosis calves (Gp 2) showed alopecia; thickening, hardening, and skin cracking, body temperature exhibited a normal pattern while heart and respiratory rate increased. Three blood samples were collected from all calves at 1st and 10th day post treatment for hematobiochemical testes. Hematological testes implied that Parakeratotic calves in Gp 2 exhibited significant reduction in RBCs, Hb and PCV while WBCs, neutrophils, eosinophil, and monocyte showed non significant difference. Moreover, lymphocytes, basophil, phagocytic index, phagocytic % and killing percentage were recorded a non significant decrease. G3 & G4 showed improvement in RBCs

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count in a non-significant and significant manner respectively with non significant changes in both leukogram and phagocytic index.

Regarding biochemical tests; G2 showed a significant increase in serum total lipids, triglycerides and Malondialdehyde levels. On the other hand, there was a reduction in serum selenium, zinc, copper, iron, total proteins, albumin, globulins, cholesterol, superoxide dismutase, catalase and thyroid hormones (T_3 and T_4) levels. aspartate aminotransferase, alanine aminotransferase and A/G ratio exhibited a non significant increase, while alkaline phosphatase displayed non significant decrease in comparison with (G1).

Supplementation of parakeratic calves with mineral mixture only (G3) or with addition of Nigella sativa (G4) for 2 months showed improvement in skin conditions with complete disappearance of skin lesions, restored skin elasticity, hair growth. Furthermore, the hematobiochemical parameters showed improvement pattern in G & G4. Mineral mixture as well as Nigella sativa enhanced and brought hematobiochemical and oxidative stress biomarkers levels closer to normal.

It could be concluded that trace elements specially zinc deficiency is the main cause of parakeratosis in calves. Most of clinical signs and hematobiochemical alterations had been modulated and improved post supplementation with mineral mixture alone or with 0.05% of Nigella sativa seeds powder.

INTRODUCTION

Buffaloes are originally from Asia and they are distributed in tropical and subtropical country **(Shalash 1984).** Animal health requires good nutritional status and nutrition composition (protein, carbohydrates and macro and micro elements) **(Mohameden, et al. 2023).** Nutritional deficiency diseases were developed when inadequate amounts of essential nutrients provided to animals (**Radostitis, et al. 1995**).

Parakeratosis is a metabolic disorder characterized by deficiency of both macro and micro elements (**Ebrahim 2015).** Trace minerals are playing a role in vital processes in body and growth of animals (**Rucker, et al. 2008**). Deficiency of vitamines, macro and trace elements induce many metabolic diseases beside induce adverse effect on skin and hair (**Radostits, et al. 2000**). Parakeratosis and hyperkeratosis with nuclei present in keratinized skin **(Kaneko, 1989).** Trace elements are required by animal body for maintaining its normal vital metabolic processes because its act as cofactors as activators of enzymes (**Underwood, 1982**). Zinc deficiency induces parakeratosis, dermatophilosis and dermatophytosis (**Smith, 2002**). Parakeratosis is characterized by in appetance, emaciation, focal and diffuse thickening & skin scales, alopecia and fissures (**Boland, 2003**).

Plant products are known to be rich in flavonoids, phenolic compounds, coumarins and terpenoids and other antioxidant constituents which improve body performance (**Aggarwal et al. 2008**). Nigella sativa is a very important herb which used in folkloric medicine belongs to botanical family of Ranunculaceae (**El-Kholy, et al. 2007**). Nigella Sativa contains many trace elements such as iron, zinc, calcium and copper (**Gilani et al. 2004**). Nigella sativa are used as a feed additive (**Salem, 2005**). Two thousand years ago; Nigella sativa has been traditionally used by human to treat many diseases (**Khare, 2004**).

The present study was carried out to evaluate some adverse effects of parakeratosis on haemato-biochemical, oxidative stress parameters and study the impact of mineral mixture alone or with Nigella sativa seeds on improvement of parakeratosis in buffalo calves

MATERIALS AND METHODS

Animals and Experimental design

About thirty five buffalo calves aged two years, average B. wt (300-350 kg) were used in this study. Two skin scrapings and faecal sam-

ples were collected from each calves for parasitological and mycological examination 1st skin sample mixed with 10% potassium hydroxide for detection of mites **(Kelly, 1984)**,

the second skin sample was cultured on sabauruds agar containing 100 ug/ ml of gentamycin sulfate and incubated at 37°C to isolate ringworm fungus **(Al-Doory, 1980)** and fecal samples were examined detection internal parasite (**Soulsby, 1986**). Post parasitological and mycological examination, twenty calves out thirty five free from mites, internal parasite and fungus infection, subsequentally the free calves were subjected for this study and divided for four groups Gp (1) healthy calves (-ve control), Gp (2) calves suffering from parakeratotosis (+ve control), Gp (3) calves suffering from parakeratotosis treated by 2 kgm mineral mixture/ton ration for 2 month and Gp (4) calves suffering from parakeratotosis treated by 2 kgm mineral mixture/ton ration and 0.05 % cursed nigella sativa for 2 month. All calves were received 1kgm ration/50 kgm body weight and opened feeding hay.

Three blood samples were taken from each calve at 1st and 10th day post treatment

1st sample was taken on tube contain EDTA, for estimation blood picture (**Jain 2000**), 2nd**sample** was taken in heparinzed tube for estimation phagocytic% and killing % (**Rouse, et al. 1980), Woldehiwet and Rowan (1990).**

3rd**sample** was taken for obtain serum for mea-suring selenium (**Fernadez and Kahr1971),** cupper (**Zak 1958**) iron (**Drsuxc 1977**), zinc **(Versieck et al. 1974),** aspartate aminotransferase (AST), alanine aminotransferase (ALT) (**Reitman and Frankel 1957**) alkaline phosphatase (ALP) (**Kind** and **King 1954**),T. protein (**Doumas et al. 1981**), albumin (**Bauer 1982**), Globulin was determined by substraction of obtained serum albumin from total protein **(Doumas& Biggs 1972),** T. lipids (**Knight et al. 1972**) cholesterol **(White et al. 1970)** triglyceride (**Wahlefeld and bergmeyer 1974**), Malanodialdhyde (MDA) (**Nielsen et al. 1997**), catalase (CAT) (**Sinha 1972**), Superoxide dismutase (SOD) (**Nishikimi et al. 1972**), triiodothyronine (T3), thyroxine (T4) (**Abraham 1981**) by RIA Kits

Additives

A) Kemeta mineral mixture: It is a trade name of mineral mixture produced by Kemeta Comp for Veterinary Pharmaceutical preparation, Egypt. Each 1 kgm contains, Zinc 50000 mg, Maganese 60000 mg, iron 30000 mg, Copper 4000 mg, Iodine 300 mg, Cobalt 100 mg, Selenium100 mg, Calcium carbonate up to 1 kgm.

B) Nigella sativa is an annual flowering plant in the family Ranunculaceae .Nigella sativa grows to 20-30 cm tall, with finely divided, linear (but not thread like) leaves. Thymoquinone is found in herbs and spices. Thymoquinone is a major constituent of seed

Statisticalanalysis was performed by analysis of variance (ANOVA). Duncan's Multiple Range **(Tambane and Dunlop, 2000)**

Table 1. Ingredient composition of experimental concentrate mixture (kg)

Ingredients	%	Chemical analysis of ration	
Yellow corn	41.5	Crude protein %	14.8
Wheat bran	17	Crud fiber %	8.3
Barley	12	Crude fat %	4.86
Soybean meal	10	Ash %	9
Cotton seed meal	13	NFE %	51.04
Vitamin & minerals premix	2	Moisture %	12
Lime stone	2		
Bone meal	2		
Salt	0.5		

RESULTS

Table 2. Clinical signs of diseased buffalo calves affected with parakeratosis

Appetite	Partial loss of appetite
Mucous membranes of eye	Pale
Depigmentation of hair	present
Parakeratosis of skin	present
Alopecia	Partial alopecia
weakness	Partial weakness
Body temperature C°	increase (control 38.7±0.42–diseased 39.02±0.21)
Respiratory rate/mint	increase (control20.26±2.12–diseased 29.13±0.16)
Heart rate/mint	increase (control 71.06±1.03–diseased 82.21±0.43)

Table 3. Effect of parakeratosis on blood picture, phagocytic activity and killing percentage in calves 1st and 10th day post treatment (n= 5).

Parameters	Period& groups		1st day Gp (1)	1st day Gp (2)	1st day Gp(3)	1st day Gp (4)	10th day Gp (1)	10th day Gp (2)	10th day Gp 3)	10th day Gp (4)
Erythro gram	RBCs (106/mm3)		7.68± 0.84a	5.72± 0.82b	6.89± 0.68b	7.59± 0.82a	7.64± 0.70a	5.80± 0.61b	7.03± 0.69b	7.65± 0.91a
	Hb (gm/dl)		11.98± 0.21a	9.34± 0.89b	11.42± 0.55a	11.88± 0.77a	11.78± 0.54a	9.21± 0.44b	11.54± 0.61a	11.93± 0.69a
	PCV %		31.46± 1.24a	26.08± 1.05b	31.05± 1.12a	31.23± 1.30a	31.71± 1.09a	26.21± 1.13b	31.15± 1.04a	31.69± 1.61a
Leu-kogram (10^3/ mm^3)	W.B.Cs		11.86± 0.91a	12.21± 0.83a	11.92± 0.83	11.83± 0.95a	11.94± 0.79a	12.33± 0.83a	11.97± 0.83a	11.99± 0.83a
	dif-ferential	Neutrophils	3.87± 0.55a	4.06± 0.78a	3.85± 0.51a	3.85± 0.47a	3.80± 0.84a	4.15± 0.89a	3.98± 0.37a	3.82± 0.44a
		Lymphocytes	4.23± 0.55a	4.04± 0.93a	4.19± 0.58a	4.24± 0.37a	4.31± 0.60a	4.15± 0.87a	4.28± 0.62a	4.31± 0.59a
		Eosinophils	1.29± 0.21a	1.32± 0.15a	1.27± 0.19a	1.28± 0.21a	1.34± 0.48a	1.41± 0.19a	1.39± 0.22a	1.35± 0.18a
		Basophils	1.21± 0.12	1.09± 0.23	1.19± 0.18	1.22± 0.16	1.25± 0.14	1.09± 0.15	1.23± 0.19	1.26± 0.12
		Monocytes	1.26± 0. 29a	1.50± 0. 17a	1.42± 0. 21a	1.25± 0. 19a	1.24± 0. 16a	1.53± 0. 21a	1.29± 0. 24a	1.25± 0. 21a
Phagocytic		%	67.32± 1.34a	66.93± 1.87a	67.05± 1.13a	67.21± 1.59a	67.71± 1.09a	67.32± 1.66a	67.69± 1.66a	67.72± 1.62a
		index	4.26± 0.98a	3.95± 0.63a	4.15± 0.74a	4.22± 0.48a	4.32± 0.44a	3.98± 0.38a	4.28± 0.63a	4.30± 0.77a
Killing %			46.21± 0.78	45.97± 0.33a	46.14± 0.94a	46.18± 0.53a	46.51± 0.39a	46.43± 0.64a	46.47± 0.38a	46.50± 0.39a

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 4. Effect of parakeratosis on Serum trace elements in buffaloe calves 1st and 10th day post treatment (n=5).

Groups	Period	1st day Gp (1)	1st day Gp (2)	1st day Gp 3)	1st day Gp (4)	10th day Gp (1)	10th day Gp (2)	10th day Gp (3)	10th day Gp (4)
Copper (µg/dl)		32.58± 1.72a	27.12± 1.81b	32.43± 1.96a	33.02± 1.62a	32.21± 1.48a	27.83± 1.44b	31.55± 1.83a	32.15± 1.91a
Selenium (µg/dl)		49.21± 1.44a	42.71± 1.82b	48.65± 1.38a	48.98± 1.58a	48.52± 1.56a	41.67± 1.37b	48.98± 1.82a	48.99± 1.44a
Zinc (µg/dl)		42.22± 1.95a	33.22± 1.61b	42.83± 1.22a	43.18± 1.66a	41.85± 1.55a	33.86± 1.28b	42.90± 1.41a	43.05± 1.83a
Iron (µg/dl)		120.6± 1.44a	115.1± 1.33b	120.3± 1.17a	121.08± 1.87a	120.18± 1.82a	114.99± 1.22b	121.08± 1.49a	120.32± 1.57a

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 5. Effect of parakeratosis on liver function in calves at1st and 10th day post treatment (n= 5).

Period & groups Parameters		1st day				10th day			
		Gp (1)	Gp (2)	Gp 3)	Gp (4)	Gp (1)	Gp (2)	Gp 3)	Gp (4)
Liver Ezymes (IU/L)	AST	67.55± 1.49a	69.07± 1.26a	68.08± 1.33a	67.69± 1.59a	67.43± 1.73a	68.08± 1.69a	67.75± 1.58a	67.98± 1.48a
	ALT	37.88± 1.50a	39.09± 1.49a	38.47± 1.59a	37.50± 1.09a	37.95± 1.39a	39.58± 1.33a	38.36± 1.69a	37.89± 1.43a
	ALP	69.89± 1.49a	69.12± 1.37a	69.65± 1.27a	69.83± 1.08a	69.80± 1.81a	69.02± 1.51a	69.86± 1.66a	69.87± 1.15a
Protein profile (g/L)	T. protein	7.99± 0.65a	5.74± 0.33b	7.69± 0.53a	7.80± 0.53a	7.89± 0.38a	5.60± 0.25b	7.73± 0.66a	7.88± 0.52a
	Albumin	4.14± 0.63a	3.08± 0.52b	3.90± 0.71a	3.97± 0.59a	4.14± 0.71a	3.06± 0.44b	3.89± 0.60a	3.99± 0.72a
	Globulin	3.85± 0.27a	2.66± 0.17b	3.79± 0.55a	3.83± 0.63a	3.85± 0.49a	2.54± 0.32b	3.86± 0.63a	3.89± 0.55a
	A/G ratio	1.08± 0.12a	1.16± 0.23a	1.03± 0.19a	1.04± 0.18a	1.08± 0.22a	1.20± 0.24a	1.01± 0.15a	1.03± 0.21a

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 6. Effect of parakeratosis on Serum some biochemical parameters in calves at 1st and 10th day post treatment (n=5).

Parameters	Period & groups	1stday				10thday			
		Gp (1)	Gp (2)	Gp 3)	Gp (4)	Gp (1)	Gp (2)	Gp (3)	Gp (4)
Lipid pro-phile (mg/dl)	T lipids (mg/dl)	378.17± 1.32b	385.21 ±1.63a	379.10 ±1.32b	378.07± 1.35b	378.26± 1.47b	384.67± 1.98a	379.90± 1.89b	378.74± 1.75b
	Triglycerides (mg/dl)	32.56± 1.51a	38.07± 1.21b	33.68± 1.17a	32.84± 1.44a	32.38± 1.19a	37.94± 1.32b	32.69± 1.31a	32.56± 1.38a
	Cholesterol (mg/dl)	92.17± 1.28a	88.23± 1.16b	91.88± 1.06a	92.09± 1.62a	92.53± 1.19a	88.05± 1.32b	92.14± 1.18a	92.23± 1.33a
Oxida-tive stress bi-omarkers	CAT (U/L)	14.34± 1.89aa	11.21± 1.81b	14.25± 1.73a	14.27± 1.75a	14.61± 1.76a	11.52± 1.59b	14.59± 1.96a	14.68± 1.49a
	SOD (U/L)	83.33± 1.89a	79.59± 1.93b	83.21± 1.97a	83.40± 1.52a	83.28± 1.97a	80.05± 1.85b	83.30± 1.55a	83.32± 1.44a
MDA (nml/ml)		14.43± 0.89b	19.28± 0.87a	14.98± 0.67b	14.73± 0.82b	14.58± 0.57b	19.08± 0.49a	14.70± 0.33b	14.69± 0.56b
T3 (ng/dl)		130.20± 2.68a	124.17 ±1.33b	129.08 ±1.05a	129.89± 1.99a	130.13± 1.98a	124.81± 1.71b	130.02± 174a	131.08± 1.17a
T4 (ng/dl)		3.93± 0.65a	3.29± 0.26a	3.58± 0.46a	3.75± 0.71a	3.80. ± 0.63a	2.73± 0.51a	3.79± 0.82a	3.79± 0.89a

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

DISCUSSION

The impact of parakeratosis and ration fortification with either minerals mixture alone or with addition of Nigella sativa on body condition and clinical signs in buffalo calves are presented in table (2). Parakeratotic calves (G2) showed alopecia, thickening, hardening and skin cracks (table 2). Feeding of parakeratic buffalo calves on a ration fortified with either 2 kg mineral mixture/ton alone (G3) or plus 0.05% nigella sativa powder (G4) showed improvement in skin conditions appeared in skin lesions healing, skin elasticity restore and hair growth at the end of the trial.

More or less similar observations were reported previously.Calves suffering from trace elements deficiency showed different skin lesion as hair loss, scales, deep fissures around the hooves, hard dehydrated skin, dermatitis, alopecia, and low weight gain (**Sadiek, et al.1994, Miller and Miller 2000).** Zinc deficiency induce parackeratosis, thickening, hardening and cracking of skin of all animal species **(Berger 2002).**Copper and zinc deficiency induced alopecia, fissure and parakeratosis (**Hosned, et al. 2007; Mamdouh and Ahmed (2021)**. Hypozincemia caused thickening and hardening **(Al-Saad, et al. 2010)** decreased feed intake (**Saurabh and Promila, 2018)** anorexia, dermatitis. Parakeratotic buffalo calves received mineral mixture containing adequate amounts of zinc and copper for 30 days revealed improvement of healthy status (**Sadiek, et al. 1994)** and serum biochemical parameters (**Pankaj, et al. 2014**) and stunted growth (**Yanuartono, et al. 2024).** Trace elements improved skin status in farm animals (**Radostites, et al. 2000**)

Data concerning the hematological parameters are illustrated in table (3). It was clear that parakeratotic calves (G2) exhibited significant reduction in RBCs, Hb, and PCV% and non significant decrease in lymphocytes, basophils, phagocytic index and killing percentage. Meanwhile, WBCs, neutrophils, eosinophil and monocytes demonstrated non significant increasein comparison with control calves (G1). Minerals mixture supplementation alone (G3) or with addition of Nigella sativa powder (G4) improved RBCs count in a non-significant and significant manner respectively. G3 and G4 showed non significant changes in both leukogram and phagocytic index.

The hematological changes observed in G2 might be attributed to one or more of the following possibilities; firstly, trace elements deficiency causes reduction in haematopioesis **(Mullally, et al. 2004**) as zinc deficiency induces protein synthesis reduction and decline in blood cells generation consuently (**Payne,1989**). Secondly, Copper deficiency leads to reduction of iron absorption and reduction in Hb formation accordingly (**Abdou 2005).** Accordingly; post feeding of parakeratotic buffalo calves on a ration fortified mineral mixture alone (G3) or plus 0.o5% nigella sativa powder (G4) improved hematological parameters

These observations corroborate the findings of others. Parakeratotic calves showed significant reduction in RBCs, Hb, PCV% and non significant increase in WBCs, neutrophils, eosionphils and monocytes (**Alam, et al. 2010).** Zinc deficiency in calves showed the same records **(Tamadhir (2015), Hegab and Mohamaden (2023**). Calves suffering from alopecia and skin lesions showed reduction in RBCs, Hb, PCV% and phagocytic % (**Mamdouh and Ahmed 2021).**

Parakeratotic buffalo calves received mineral mixture containing adequate amounts of zinc and copper for 30 day revealed improvement health status (**Sadiek, et al. (1994).**

Nigella sativa improved RBCs **(Al-Jishi 2000)** and its oil improved counts of leukocytes, haemoglobin and PCV levels. These improvement in both clinical signs and hematological parameters in parakeratotic calves received nigella sativa may be due to presence large amount of trace element (iron, zinc, manganese and copper) in nigella sativa **(Gilani et al. 2004).**

Results showing the impact of parakeratosis on trace elements serum levels are presented in table (4). Calves in G2 showed significant reduction in serum levels of selenium,

zinc, copper and iron. Meanwhile, G3 and G4 exhibited non significant changes among trace elements serum concentrations compared with healthy calves (G1). They enhanced and brought serum levels closer to normal. The reduced trace elements serum levels might be due to insufficient dietary intake. The declined trace elements levels come in accordance with findings of other authors (**Sadiek, et al. (1994; Abdeen and Mona 2007**; **Al-Saad, et al. 2010; Alam, et al. 2010)**. Nigella sativa contains large amount of trace elements (iron, zinc, manganese and copper) **(Gilani et al. 2004),** thus supplementation on a ration fortified with it improved trace elements serum levels.

Hepatic function indicators either liver enzymes or serum proteins levels in buffalo calves are presented in table (5). AST, ALT & ALP exhibited non significant increase in all experimental groups. G2 showed a little pit increase but still non significant of both AST & ALT. G3 and G4 showed non significant changes in hepatic enzymes compared with healthy calves (G1). They enhanced and brought serum levels closer to normal.

The little pit increase in (AST) and (ALT) levels in G2 could be attributed to zinc deficiency. Zinc is essential for protein metabolism and enzyme function, thus hepatic protein and hepatic enzymes synthesis might be impaired leading to an increase in AST and ALT production as a consequence of zinc deficiency (**Kaneko, 1989)**.

Regarding serum proteins; parakeratotic calves (G2) demonstrated a decline in total proteins, albumin and globulins concentrations compared to all groups. While there was no significant difference in calves fed on a ration fortified with either mineral mixture alone (G3) or plus nigella sativa powder (G4) (table 5). Zinc plays a crucial role in protein synthesis directly or indirectly. Zinc help in albumin production from the liver, thus low serum albumin might be due to zinc deficiency via impaired liver function and overall protein metabolism **(Radostitis, et al. 1995).** Zinc deficiency may impair amino acid utilization and protein turnover. Zinc is essential for immune function, and its deficiency can lead to hypo-gammaglobulinemia (low globulin levels), thus parakeratotic calves often have reduced globulin levels, leading to weakened immune responses and higher susceptibility to infections **(Kincaid et al. 1986).** Due to decreased albumin and globulin levels; total proteins concentrations tend to decline accordingly in parakeratotic calves **(Underwood & Suttle, 2001).**

The above mentioned findings were supported by previous studies. Calves received mineral mixture in ration improved liver function as they reduced enzymes activities and elevated protein profile due to trace element protected hepatocytes and prevented damage and improved albumin synthesis (**Humer, et al. 2019**). Crossbred improved liver function represented by elevation in protein profile and reduction in liver enzymes **(Anil et al. 2020)**. Selenium induced increase in absorption of globulin leading to increase globulin and total protein (**Abbas 2002)**. **Kamalakar, et al. (2015); Amaravathi et al. (2016); Mamdouh and Ahmed (2021)** found that parakeratic buffaloes showed reduction in total proteins, albumin, globulin with elevation in AST and ALT.

Results showing the lipid profile changes are illustrated in table (6).G2 exhibited a significant increase in total lipids & triglycerides, while cholesterol levels reduced significantly compared with G1, G3 and G4.

Decrease in cholesterol levels may be due to copper deficiency or liver insufficiency and hepatic fat metabolism disturbance consequently (**King and Cousins 2006**). Increase in total lipids and triglycerides in calves suffering from parakeratosis may be due to adecrease in food intake stimulating lipolysis leading to release long chain fatty acids as fuel source compensation (**Alam, et al. 2010)**. Nigella sativa induced marked improvement in lipid profiles **(Salem 2005)**.Nigella sativa induced improvement in cholesterol level due to depletion of cholesterol in steroid-genesis in goats (**Habeeb and El Tarabany, 2012**).

Regarding oxidative stress biomarkers;

parakeratotic calves (G2) showed significant decrease in CAT & SOD, while MDA increased significantly. On the other hand, G3 & G4 exhibited no changes compared with control group. The changes in G2 might be mediated through zinc deficiency. Zinc acts as a cofactor for SOD and CAT synthesis and activity, thus its deficiency causes decrease levels of SOD & CAT**.** Reducing such antioxidant enzymes impairs the cell's antioxidant defense system, causing an accumulation of ROS result in lipid and the production of MDA as a marker of oxidative stress (**Prasad, 2009).**

Mineral play a role in scavenging free radicals and reduced MDA and improved antioxidant enzymes (CAT and SOD) (**Pathak et al. 2004)**. Our results are comes in agreement with **Manimaran et al. (2022)** who reported that zinc has powerful antioxidant actions leading to reduction in MDA with increase in CAT and SOD in parakeratotic calves. Zinc improved antioxidant enzymes and reduced MDA (**Suzuki et al. 2011); Al-Ghamdi (2003)** reported that black seed was improved blood picture and antioxidant enzymes.

Data concerning the influence of parakeratosis on thyroid hormones is shown in table (6). Calves of G2 exhibited significant decrease in T3 and non significant decrease of T4. On the other hand, there was no change among G & G4.

Parakeratosis can impact thyroid hormone levels through several mechanisms, primarily due to inflammation and altered lipid metabolism. Inflammation associated with parakeratosis may increase cytokines like IL-6, which can disrupt thyroid function by affecting the conversion of T4 (thyroxine) to inactive reverse T3 (rT3), lowering active T3 levels. Additionally, changes in lipid metabolism and skin cell turnover can interfere with thyroid hormone transport, potentially altering thyroid hormone binding proteins like thyroxine-binding globulin (TBG). This combination of factors can lead to fluctuations in thyroid hormone levels and function **(Wajner et al. 2013; Chatterjee and Das 2016; Bourguignon 2017). Christy and Stella (2007)** recorded that zinc improved thyroid gland function and increase both T3 and T4.

CONCLUSION

It could be concluded that trace elements deficiency is the main causes of parakeratosis in calves. Parakeratosis induces skin lesion and alopecia as well as alterations in hemato-biochemical parameters. Feeding of parakeratic buffalo calves on a ration fortified with either mineral mixture alone or plus 0.05% Nigella sativa seeds powder showed improvement in skin conditions appeared in skin lesions healing, skin elasticity restore and hair growth. Moreover, they improved hemato -biochemical parameters, enhanced and brought them closer to normal.

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