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## **Research Article**



## Growth Performance, Immunity, and General Health Status of Newborn Friesian Calves Fed Milk Supplemented with Propolis, Thyme, or their Combination, as Antioxidants, During the Suckling Period

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**Abstract** | Aim of the resent study was to determine impacts of feeding milk supplemented with propolis thyme, or both, as antioxidants, on growth efficiency, liver function, kidney function, total antioxidant capacity, and immune response of Friesian calves from 7 to 105 days of age as an experimental period. Newborn male calves (n=20) weighing 37.92±0.63 kg were divided into 4 groups, five calves/group. The first group (G1, control) included animals without treatment. During the whole suckling period each animal in G2, G3, and G4 daily received milk supplemented with propolis (5 g), thyme oil (2 ml), or 5 g propolis plus 2 ml thyme oil, respectively. Results showed that animals in fed both propolis and thyme combination showed the highest LBW, total gain, counts and percentages of blood cells, hemoglobin, total proteins and their fractions, total lipids, total cholesterol, and glucose, and the lowest urea and creatinine levels (P<0.05). Plasma immunoglobulins (IgG, IgA, and IgM) and total antioxidant capacity increased to the maximal values, while AST and ALT activities decreased to the minimum values in G4. Feeding Friesian calves on milk supplied with propolis (5 g) plus thyme oil (2 ml) per calf during the suckling period can improve growth performance, general health status, immune response, and total antioxidant capacity. From an economical point of view, this treatment could increase profits by reducing morbidity, diarrhea cases, and mortality, and improving the performance of breeding male calves during suckling and the early post-weaning period.

Keywords | Friesian-calves, Propolis, Thyme, Immunoglobulins, Hematology, Blood constituents

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## **INTRODUCTION**

Newborn calves are the foundation of replacement animals that can be an important asset for the farmer

to generate income for dairy farms (Anjum et al., 2013). During the first fourteen days of newborn calf's life, they are exposed to several stressors because they suffer from the change in environmental conditions including

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diet, handling stress, and low immunity which increases diarrhea infection (Mendoza et al., 2011), which may increase morbidity and mortality rates (Ribeiro et al., 2009). Also, during this period calves are under different health and welfare problems, leading to increased mortality rate (Raboisson et al., 2013). Weaning of calves at an early age is practiced to reduce the cost and labor of feeding (Quigley et al., 1991). Several researchers (Soberon et al., 2012; Soberon and Van Amburgh, 2013) indicated a relationship between pre-weaning daily gain and productive and reproductive performances of dairy calves. In the cattle industry, increasing calf mortality is one of the most managerial problems (Karsll and Evci, 2018).

Many types of natural antioxidants are used to achieve good performance and health status in raising calves such as propolis and thyme. Propolis is a sticky and semi-solid organic substance. It is a mixture of some bees wax and biochemically changing resinous substances (Kara et al., 2014). It has an activity against oxidation, microbes, and toxicity, and acts as immunomodulatory agent for containing flavonoids, phenolic acids, and terpenoid contents (Kadhim et al., 2018) and also has a positive impact, as a flavor enhancer (Denli et al., 2005). Moreover, Zafarnejad et al. (2017) and Prakatur et al. (2019) reported that propolis is a feed additive, to promote the performance, the activity of the digestive enzymes and microbiota, and immunity. In sheep, raw Chinese propolis improved the performance and milk production of ewes, and the immunity of lambs (Shedeed et al., 2019). Adding 150 µL of propolis per kg LBW to milk showed a positive effect on the performance, antimicrobial, antioxidant, and immune parameters of suckling lambs (Cecere et al., 2021). Fecal score and the duration and veterinary expenses for diarrhea in calves were improved (Slanzon et al., 2019).

Plant containing essential oils (EO) is considered as drug source and acts against microbes, inflammation, and oxidation. Thus, it is used to improve animal productivity (Simitzis and Deligeorgis, 2011). *Thymus vulgaris* (THV) has biological compounds such as pinene, thymol, and caryophyllene (Boskabady et al., 2006). Growth performance parameters and immune response of calves was affected by THV (Seifzadeh et al., 2016). Addition of THV in the diet improved cell-mediated, humoral immune responses, and health status of growing calves (Wafa et al.,

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2021, 2022). Thymol (476 g/kg), gamma-Terpinene (309 g/kg), and para-Cymene (84 g/kg) are the main constituents of THV essential oil (Boruga et al., 2014). Studies on the effect of propolis in comparing with THV or their combination on ruminants, particularly calves during the suckling period, are limited. In our study, we proposed that propolis, thyme, or their combination may be effective, as antioxidants to substitute antibiotics.

To reduce mortality and improve Friesian calf performance, we proposed that supplementation of the suckled milk with propolis, thyme, or their combination, as natural antioxidants, may have positive impacts on growth performance, liver and kidney function, total antioxidant capacity, and immune response from birth to weaning.

#### MATERIALS AND METHODS

This study was conducted in frame of a corporation between Animal Production Department, Faculty of Agriculture, Tanta University and Animal Production Research Institute (APRI), Egypt. According to the ethical standards in dealing with animals for scientific purposes were approved during the academic year 2019 – 2020 by the ethical committee of Faculty of Agriculture, Tanta University (Session 6: 13/1/2020), and were updated by the Supreme Council of Egyptian Universities (No 2020.03.21). All ethical standards (AY<sub>2019-2020</sub>/ Session 6/ 2020.01.13) are in line with international standards in dealing with animals for scientific purposes.

#### ANIMALS AND EXPERIMENTAL DESIGN

A total of 20 newborn male Friesian calves were used in the present study. Within 30-60 minutes after parturition, newborn calves were suckled colostrum from their dams (1-2 kg) using feeding bottles up to three days of age, then calves were artificially suckled milk from their dams at morning and evening based on 10% of calf weight for six weeks. Avery week from birth up to weaning, 1% reduction in suckled milk (based on LBW) was achieved to calves in all groups. Starting at three weeks of age, starter, berseem hay, and rice straw were offered to calves *ad libitum*. Table 1 shows the chemical composition of feeds offered to calves during the experimental period. After one week of birth, the average initial live body weight was 37.92±0.63 kg.

Table 1: Chemical analysis (on DM basis)	of starter, berseem hay, and rice straw	fed to calves.
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Item	Dry matter (%)	Chemical composition (%, on DM basis)					
		Organic matter	Crud protein	Crud fiber	Ether extract	Nitrogen free extract	Ash
Starter	88.98	92.55	19.24	5.63	2.97	64.71	7.45
Berseem hay	87.39	87.19	13.22	27.18	0.67	46.12	12.81
Rice straw	88.25	81.87	2.68	38.72	1.32	39.15	18.13

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All day times, fresh water was available for animals. During suckling period, no mortality cases were recorded, and all animals were without diseases.

After calving, newborn calves at one-week old were allocated into 4 groups (five animals/group). The control group was fed a basal diet without treatment. Daily milk amount fed to calves during the whole suckling period (weaning at 105 d) was supplemented with 5 g crud propolis/calf in G2 (Shedeed et al., 2019), 2 ml thyme oil/ calf in G3 (El-Essawy et al., 2021), and a combination of 5 g propolis and 2 ml thyme oil/calf in G4.

#### **EXPERIMENTAL PROCEDURES**

#### CALVES LIVE BODY WEIGHT

Calf's live body weight was recorded at 7 days of age, weaning (105 days of age), and post-weaning (165 d of age), while body weight gains were calculated as a total or daily gain.

#### **BLOOD SAMPLING**

Before receiving morning milk meal, the samples of blood were collected from the jugular vein puncture at weaning age (105 d) from all calves for analytical assays.

Blood samples were taken into heparinized test tubes. Each sample was divided into two portions, the first was taken as whole blood for determining the hematological parameters, while the second portion was centrifuged (3000 rpm for 20 min) to isolate blood plasma, then plasma samples were preserved (-20°C) up to different analyses.

According to Killingsworth and Savory (1972), plasma immunoglobulin (IgG, IgM, and IgA) concentrations were determined in the blood plasma of calves.

In the whole blood samples, hemoglobin level and hematocrit value were measured (Henry, 2001) using Mission<sup>®</sup> Plus kit (REF C132-3031, USA). Blood cellular counts (red and white blood cells) was determined using a hemocytometer.

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The concentration of total proteins, albumin, glucose, total lipids, and total cholesterol in blood plasma samples were also assayed according to Henry (1964), Doumas et al. (1971), Trinder (1969), Zöllner and Kirsch (1962), and Richmond (1973), respectively. The activity of liver enzymes, aspartate transaminase (AST) and alanine transaminase (ALT) were determined in blood plasma according to Reitman and Frankel (1957). Plasma creatinine level (Henry, 1974) and urea (Patton and Crouch, 1977) were determined as kidney function markers. However, the concentration of globulin was determined by the difference between total proteins and albumin concentrations. Total antioxidant capacity was determined as the methods described by Koracevic et al. (2001).

#### **STATISTICAL ANALYSIS**

SPSS Program (2010) version 15 was used to the statistical analysis of the obtained data. One-way analysis of variance was used to explore the significant effects of treatment. The significant differences were separated at a level of P<0.05 by Duncan Multiple Range test (Duncan, 1955).

#### **RESULTS AND DISCUSSION**

#### LIVE BODY WEIGHT AND GROWTH RATE

Results in Table 2 showed that live body weight (LBW) and weight gain (total and daily) of calves at weaning (105 d of age) and post weaning (165 d of age) significantly increased in all treatment groups than in control, being with maximum values (P<0.05) in calves fed the combination.

#### **I**MMUNITY STATUS

Table 3 revealed that the concentration of plasma immunoglobulins of newborn calves at the end of the experiment showed a significant (P<0.05) increase in all treatment groups in comparison with control one, but a more significant (P<0.05) increase was recorded in G4 compared with G2 and G3.

<b>Table 2:</b> Effect of treatments on live body weight and growth rate of Friesian calves at different	stages.
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Item	Experimental group					
	G1 (Control)	G2 (Propolis)	G3 (Thyme)	G4 (Combination)		
Live body weight of calves (kg)						
Initial	37.96±1.90	37.94±1.12	37.84±1.50	37.94±0.83		
At weaning (105-d old)	78.80±1.52°	$96.32 \pm 1.30^{b}$	95.12±1.27 <sup>b</sup>	119.94±2.11ª		
Post-weaning (165-d old)	98.17±1.51°	$129.92 \pm 1.27^{b}$	$127.98 \pm 1.30^{b}$	178.44±2.12ª		
Total body weight gain (kg)						
Initial to weaning	40.84±2.19°	58.38±1.73 <sup>b</sup>	57.28±2.07 <sup>b</sup>	82.00±1.33ª		
Initial to 165 d old	60.21±2.20 <sup>c</sup>	$91.98 \pm 1.72^{b}$	$90.14 \pm 2.06^{b}$	140.50±1.34ª		
Average daily gain (kg/h/d)						
Initial to weaning	$0.38 \pm 0.02^{\circ}$	$0.56 \pm 0.01^{b}$	$0.54 \pm 0.02^{b}$	0.78±0.01ª		
Initial to 165 d old	$0.36 \pm 0.01^{\circ}$	$0.55 \pm 0.01^{b}$	$0.55 \pm 0.02^{b}$	0.85±0.01ª		
a-b: Means in the same column with different superscripts differ significantly (P<0.05).						

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**Table 3:** Effect of treatment on immunoglobulins (IgG, IgM, and IgA) concentrations in the blood plasma of Friesian calves in experimental groups at 165-days of age.

Item	Experimental group				
	G1 (Control)	G2 (Propolis)	G3 (Thyme)	G4 (Combination)	
IgG (g/L)	11.55±0.14°	13.44±0.12 <sup>b</sup>	13.36±0.15 <sup>b</sup>	15.05±0.16ª	
IgM (g/L)	1.69±0.05°	$2.41 \pm 0.07^{b}$	$2.37 \pm 0.08^{b}$	3.06±0.06 <sup>a</sup>	
IgA (g/L)	$0.52 \pm 0.04^{\circ}$	$0.68 \pm 0.03^{b}$	$0.66 \pm 0.02^{b}$	0.89±0.03ª	

a, b, c: Means in the same column with different superscripts differ significantly (P<0.05).

Table 4: Hematological parameters in the blood of Friesian calves in the experimental groups at the end of the experiment.

Item	Experimental group				
	G1 (Control) G2 (Propolis)		G3 (Thyme)	G4 (Combination)	
RBCs (x10 <sup>6</sup> /mm <sup>3</sup> )	$8.32 \pm 0.17^{b}$	8.89±0.21 <sup>ab</sup>	$8.83 \pm 0.22^{b}$	9.45±0.20 <sup>a</sup>	
WBCs (x10 <sup>3</sup> /mm <sup>3</sup> )	9.21±0.16 <sup>c</sup>	$10.31 \pm 0.17^{b}$	$10.18 \pm 0.16^{b}$	11.02±0.18 <sup>a</sup>	
Hemoglobin (g/dl)	$10.10 \pm 0.13^{b}$	10.34±0.25 <sup>b</sup>	$10.18 \pm 0.19^{b}$	11.60±0.18ª	
PCV (%)	28.97±0.27°	$33.86 \pm 0.33^{b}$	$33.00 \pm 0.36^{b}$	37.19±0.44 <sup>a</sup>	

a, b, c: Significant differences among groups at P<0.05.

**Table 5:** The concentration of blood biochemicals in the plasma of Friesian calves in experimental groups at the end of the experiment.

Item	Experimental group			
	G1 (Control)	G2 (Propolis)	G3 (Thyme)	G4 (Combination)
Protein metabolites				
Total proteins (mg/dl)	5.64±0.10 <sup>c</sup>	$6.59 \pm 0.15^{b}$	$6.47 \pm 0.14^{b}$	7.53±0.19ª
Albumin (mg/dl)	2.95±0.09°	$3.39 \pm 0.10^{b}$	3.33±0.09 <sup>b</sup>	3.83±0.13ª
Globulin (mg/dl)	2.68±0.11°	$3.20\pm0.13^{b}$	$3.13 \pm 0.14^{b}$	3.70±0.14ª
Albumin: globulin ratio	1.10	1.05	1.06	1.04
Carbohydrate metabolites				
Glucose (mg/dl)	43.34±1.14°	$48.08 \pm 0.81^{b}$	$47.03 \pm 0.86^{b}$	53.11±1.07ª
Lipid profile				
Total lipids (mg/dl)	487.16±5.48°	$527.18 \pm 5.97^{b}$	$525.01 \pm 5.58^{b}$	568.64±7.06ª
Cholesterol (mg/dl)	83.10±1.16 <sup>c</sup>	$89.63 \pm 1.10^{b}$	$87.70 \pm 1.04^{b}$	95.86±1.15ª
Cholesterol (mg/dl)	83.10±1.10°	89.03±1.10"	87.70±1.04°	95.80±1.15"

a, b, c: Significant differences among groups at P<0.05.

#### HEMATOLOGICAL PARAMETERS

Count of WBCs and PCV percent in the whole blood of calves at the end of the experimental period were significantly (P<0.05) improved by propolis (G2), thyme (G3), and their combination (G4) treatments in comparing with the control. However, only combination treatment significantly (P<0.05) improved all hematological parameters (Table 4).

#### THE CONCENTRATION OF PLASMA METABOLITES

Plasma total proteins, albumin, and globulin concentrations increased (P<0.05) in treatments as compared to control group with nearly similar albumin: globulin ratio in all experimental groups. Also, glucose concentration, as a carbohydrate metabolite as well as total lipids and cholesterol levels, as lipid profile, significantly (P<0.05) increased in all treatment groups as compared to the control group. Calves in G4 showed significantly (P<0.05)

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the highest values of plasma metabolites as compared to those in G1, G2, and G3 (Table 5).

#### MARKERS OF LIVER AND KIDNEY FUNCTIONS

As shown in Table 6, transaminases (AST and ALT) activities and kidney function in terms of plasma urea and creatinine in the blood plasma of calves significantly reduced by in G2, G3, and G4 in comparison with G1. Animals in G4 exhibited significantly (P<0.05) the best liver and kidney function markers in comparison with other treatment groups and control.

#### TOTAL ANTIOXIDANTS CAPACITY

Level of total antioxidant capacity (TAC), as an antioxidant bio-marker in the blood plasma of calves at the end of the experimental period, was higher (P<0.05) in all treatment groups than in the control one, but G4 showed the highest (P<0.05) values (Figure 1).

**Table 6:** Enzyme activity (AST and ALT) and total antioxidant capacity in the blood plasma of Friesian calves in experimental groups at the end of the experiment.

Item	Experimental group			
	G1 (Control)	G2 (Propolis)	G3 (Thyme)	G4 (Combination)
Liver function				
AST (IU/1)	45.11±0.95ª	$39.97 \pm 0.76^{b}$	$39.80{\pm}0.89^{\rm b}$	32.63±0.98°
ALT (IU/I)	21.20±0.49ª	$16.74 \pm 0.52^{b}$	$17.00 \pm 0.55^{b}$	12.68±0.58°
AST: ALT ratio	2.13	2.39	2.34	2.57
Kidney function				
Urea (mg/dl)	3.32±0.08ª	$2.89 \pm 0.09^{b}$	$2.91\pm0.10^{\mathrm{b}}$	2.52±0.10°
Creatinine (mg/dl)	$1.68 \pm 0.07^{a}$	$1.26 \pm 0.06^{b}$	$1.32 \pm 0.05^{b}$	0.88±0.07°

a, b, c: Significant differences among groups at P<0.05.



Figure 1: Total antioxidant capacity level in blood plasma.

Many medicinal herbs and plants are used as feed additives in animal feeding (Allam et al., 1999). In our study, each propolis or thyme treatment had beneficial effects on the growth performance during all age stages of calves, but the most positive impacts were obtained on calves treated with their combination. Total gain of Friesian calves increased (P<0.05) by 44.94, 24.4, and 23.2% in calves fed milk supplemented with a combination of propolis + thyme, propolis, and thyme, in comparison with those free-milk (control), respectively (unshown data). Similar results were reported by Wafa et al. (2022), who found that dietary supplementation with thyme or its combination with Probax significantly increased body weight and gains of weaned Friesian calves with superiority of calves treated with thyme and Probax combination. Seifzadeh et al. (2016) recorded higher final body weight during the suckling period in Holstein's calves fed a diet with thyme than in controls. Ozkaya et al. (2017) and Froehlich (2016) found that final body weight was significantly improved by dietary thyme supplementation of Holstein and Holstein-Friesian calves during the suckling period. In general, dietary herbal extract supplementation had positive impacts on ruminal fermentation and animal productivity (Beauchemin et al., 2007; Benchaar et al., 2008). Regarding the beneficial effects of propolis treatment, Kabiloğlu et

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al. (2022) found that propolis treatment improved the live body weight, gain, and feed conversion ratio of dairy calves. Also, positive effects on the body parameters of calves treated with ethanolic extract of propolis (EEP) were observed by many reports (Tolon et al., 2002; Yaghoubi et al., 2008; Yücel et al., 2015), or when EEP was added to milk replacer feeds (Kupczyński et al., 2012) because propolis had a positive impact on gut health, diarrhea incidence, and growth of newborn calves due to presence of flavonoids (Yaghoubi et al., 2008). The improvement in the growth performance of calves in all treatment groups may be due to the positive effects of thyme oil (Wafa et al., 2021), propolis (Benchaar et al., 2008), or the synergic effect of their combination on nutrient digestibility. In this respect, supplementation of thyme EO in diets of calves significantly improved digestion of DM and OM (Ebrahimi et al., 2018). At post-weaning stage, an increase in volatile fatty acids level and protozoal count in the ruminal fluids as affected by thyme or thyme and Probax treatments of calves (Wafa et al., 2022). On the other hand, Vakili et al. (2013) and Darabighane et al. (2016) observed non-significant differences in growth parameters in calves treated with thyme.

We investigated plasma concentrations of immunoglobulins as markers of immunity in different groups. We found beneficial effects of both propolis or thyme treatment on the immune response of calves, but the most positive impact was more pronounced by their combination in G4. Qiao et al. (2013) concluded that herbs treatment can support the immune response by improving the level of all blood immunoglobulin type. In this respect, Lakhani et al. (2019) stated that the humoral immune response of buffalo calves was enhanced by plant phytogenic. Wafa et al. (2021) indicated an improvement of blood IgG, IgM, and IgA levels in calves fed a diet containing thyme as compared to control. Froehlich (2016) and Ozkaya et al. (2017) indicated that the concentration of IgG, IgM, and IgA were improved by thyme treatment as compared to controls. In calves, thyme extract treatment had beneficial impacts on the

total health scores (Wafa et al., 2021), resisting infections, and immune-related diseases (Amirghofran et al., 2012). In Holstein calves Incidence of diarrhea was reduced and animal health was improved by thyme oil (Darabighane et al., 2016). In accordance with our results on propolis treatment, Emtnan et al. (2005) found that concentrations of immunoglobulins were improved by supplementation of propolis to Baladi goats. However, Shedeed et al. (2019) observed a significant increase in IgA, a tendency to increase in IgM, and an insignificant change in IgG in ewes and lambs of Barki sheep treated with propolis as compared to controls. Increasing immunoglobulin levels in treatment groups may be related to the role of the antioxidant nutrients (natural additive) which can modulate and regulate the early activation steps in the acquired immune response (Ahmed et al., 2009). Also, this increase could be credited to B-lymphocyte stimulation, consequently, the immunoglobulins will elevate initiating and immune response through helper T-cells (Zadik et al., 1994), which may lead to perfect immune competent calves. These impacts were due to presence of compounds such as phenol compounds and flavonoids, which have biological activity in propolis and have positive impact on immunity of calves (Yaghoubi et al., 2008).

Along with the positive impact of treatments on growth and immunity, the obtained hematological parameters were also enhanced, in terms of increasing WBCs count and PCV value by propolis or thyme, but their combination improved RBCs and WBCs, hemoglobin, and hematocrit values of calves. Similarly, Wafa et al. (2021) found that feeding diets with thyme increased hemoglobin concentration, count of RBCs, and PCV of weaned Friesian calves. In newborn calves administrated with thyme oil, Ebrahimi et al. (2018) showed a marked improvement in the count of WBCs. It was proven that WBCs have important role to protect Holstein calves from the infection of bacteria, fungi, and viruses (Cho et al., 2000). In general, Lakhani et al. (2019) indicated that thyme extract can enhance the hematological variables and health status of calves. Also, natural additive supplementation resulted in significant improvement blood cellular counts and hematocrit values (Ahmed et al., 2009). In agreement with the present results concerning the positive effects of propolis on hematological parameters, Shedeed et al. (2019) observed that propolis increased the count of WBCs and RBCs, but the differences in Hb concentration and PCV value were not significant at late pregnancy and after lambing in ewes. Also, Morsy et al. (2016) reported that the count of total leukocytes increased, but Hb and PCV showed an unchanged trend by Brazilian propolis extract for 21 days pre-lambing in ewes. In NZW buck rabbits fed on 150 mg propolis per kg weight, propolis treatment increased the total count of WBCs and RBCs (Elshama et al., 2015). On

the other hand, the dietary supplementation of propolis powder slightly increased WBCs in Hanwoo calves (Sarker and Yang, 2010). The increase of WBCs count in our study was attributed to flavonoid compounds in propolis for the upregulation of expression of toll-like receptors like TLR-2 and TLR-4, and to enhancement of cytokines production such as interleukin-1, -6, and -10 (Orsatti et al., 2010).

The concentration of blood total protein is an indicator of the nutritional status in ruminants (Kumar et al., 1981). Serum albumin is considered as marker for dietary protein level (Agenas et al., 2006). Protein deficiency led to a decrease in humoral and cell-mediated immunity which exposed animals to infection by several diseases (Titgemeyer and Loest, 2001). Our results indicated a positive impact of all treatments on protein metabolism, by increasing concentration of TP, albumin (AL), and globulin (GL); carbohydrate metabolism, by increasing glucose (GLU) level; and lipid metabolism, by improving total lipids (TL) and cholesterol (CHO). Similar results were reported by Wafa et al. (2022) in calves fed diets containing thyme and Probax combination, in terms of increasing plasma TP, AL, and GL. Also, Seifzadeh et al. (2016) reported that dietary THV treatment increased blood total protein of Holstein calves. Feeding diet with thyme EO increased blood serum proteins of dairy calves (Froehlich, 2016). Several authors (Kumar and Dass, 2006; Belibasakis and Tsirgogianni, 1996) reported results on concentration of albumin and globulin in buffalo and dairy calves, respectively, similar to our results. The impact of treatments positively on TP or their fractions may indicate improving the protein anabolism by increasing CP digestibility (Khattab et al., 2011). In accordance with increased plasma glucose concentration in calves to the maximal level by feeding diet supplemented with a combination of propolis and thyme. Similarly, feeding on thyme, Probax, or their combination increased albumin and globulin concentrations in Friesian calves (Wafa et al., 2022), in Holstein calves (Seifzadeh et al., 2016), or feeding Brown Swiss calves on thyme EO (Ebrahimi et al., 2018). On the other hand, Vakili et al. (2013) found that feeding diet supplemented with thyme slightly elevated blood glucose concentration and did not affect blood cholesterol level of Holstein calves. Alleviation in CHO and TL in calves of treatment groups is in association with a similar rise in lipid profile by feeding calves on a diet containing thyme (Wafa et al., 2021). Also, an increase in the blood CHO level was recorded by feeding newborn dairy calves on diet containing thyme (Seifzadeh et al., 2016).

Our results indicated improvement in kidney and liver functions in all treatment groups, being the best in G4 with normal values of urea, creatinine, AST, and ALT in plasma of Friesian calves (Wafa et al., 2021). Improving

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kidney and liver function by treatments was also proved in the current study as much as in growth performance, immunity, and metabolism of calves. These improvements included a marked decrease in urea and creatinine levels in plasma, as kidney function markers, and decreasing marker of liver function (plasma AST and ALT) as compared to controls. Similar results were reported by Ebrahimi et al. (2018), who found that thyme alone or propolis and thyme combination treatment improved kidney function in Friesian calves, while only propolis and thyme combination showed a marked decrease in plasma activity of AST and ALT, as a good marker for liver function. Similar results were obtained by dietary thyme oil in newborn calves (but other reports concluded that kidney function in calves was not affected by thyme treatment (Vakili et al., 2013; Biricik et al., 2016). Propolis may have hepatoprotective effects or play a role in the prevention of liver injury (Abd-Allah and Daghash, 2019).

When the antioxidant status of calves was examined at the end of the experimental period, all treatments significantly improved TAC in blood plasma. The EO extracted from thyme have potent antioxidant activity (Mimica-Dukić et al., 2016). Dietary supplementation of the EO of thyme containing at least 13.5% thymol increased antioxidant capacity in blood plasma (Giller et al., 2020) and thyme had numerous beneficial effects as antimicrobial, antioxidative, carminative, and antiseptic actions (Baranauskiene et al., 2003). Also, the addition of phenolic thyme EO of propolis reserved significant increase in the antioxidant capacity of cow's milk (Aguiar et al., 2014). Propolis, as an antioxidant, has a positive action on stimulating the antioxidants enzymes which decrease the free radicals, and preservation and enhancement efficiency of immuneorgan functions (Sforcin et al., 2005; Daleprane and Abdalla, 2013). The powerful antioxidant activity of phenolic acids and flavonoids in the propolis (Yaghoubi et al., 2008) had inhibition effects on ROS generation to the preservation of lipid peroxidation (Kurek-Górecka et al., 2014). Propolis treatment significantly reduced MDA level in sheep (Fabris et al., 2013).

## CONCLUSIONS AND RECOMMENDATION

In conclusion, Friesian calves fed milk supplemented with propolis (5 g) plus thyme oil (2 ml) per calf during the suckling period can improve growth performance, general health status, immune response, and total antioxidant capacity. From an economical point of view, this treatment could increase profits by reducing morbidity, diarrhea cases, and mortality, and improving the performance of breeding male calves during suckling and the early postweaning period.

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#### NOVELTY STATEMENT

Several articles studied the effect of treatment with Propolis or Thyme as feed supplementation on cattle new born performance while the novel point in our study is the effect of the combination treatment (Propolis and Thyme) on male Friesian calves performance that can raising as bulls in dairy farms.

#### **AUTHOR'S CONTRIBUTION**

Suggestion, experimental design, and achievement of the experimental work were conducted by all authors. El-Nagar, H.A. and Faraag, M.S. conducted the experimental procedures and data collection. El-Hais A.M., S.E.S. Atia and Badawy, M.I. performed the analytical amd chemical analyses. Wafa, W.M. prepared data to be statistically analyzed and critically writing and revision of the manuscript to publication.

#### **CONFLICT OF INTEREST**

The authors have declared no conflict of interest.

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