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Effect of Probax, Thyme Extract, and their Combination on Growth Performance, Rumen Function, and Blood Parameters of Friesian Calves

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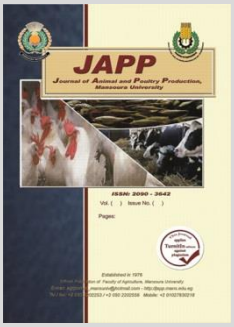

ABSTRACT

This study aimed to examine the effect of Probax (*Lactobacillus acidophilus*), thyme *(Thymus vulgaris*) extract (THM), and their combination on productive performance, rumen function, hematological and blood biochemical components, and immunity of newborn Friesian calves from calving to weaning (105 d) and post-weaning (150 d of age). Total of 20 male Friesian calves at three days of age and 35.07±0.56 kg body weight were allotted into four groups (5 calves/group). In the 1st group (G1), calves were fed whole cow milk and the starter plus berseem hay after 7 days of age (control). Calves were fed the same control diet supplemented with 50 mg Probax /kg BW (G2), 40 mg of THM/kg BW (G3), and 50 mg of Probax and 40 mg of THM (G4). Growth performance parameters, ruminal VFAs concentration, protozoal count, RBCs, Hb, PCV, and concentration of total proteins, albumin, globulin, glucose, urea, total cholesterol, triglycerides, IgG, IgM, and IgA in blood serum were the highest (P<0.05), while creatinine concentration, and AST and ALT activities were the lowest in G4. Dietary supplementation of Probax+THM (50 and 40 mg/kg BW) improved growth performance, rumen function, hematology, blood biochemicals, and immunity of Friesian calves at weaning, and at 150 days of age.

***Keywords*:** Calves, weight gain, immunoglobulins, rumen

INTRODUCTION

In the agricultural production system of developing countries, livestock is playing an essential role in the national economy (Yeshiwas and Fentahun, 2017). Egypt is one of the largest country's that imports live cattle and beef. Increasing per capita animal protein consumption by 4 g/day by the year 2030 is one of the main objectives of developing animal production in Egypt (USFAS, 2017).

From the most critical interval in life of newborn calves is the 1st few weeks of age. During this interval, they are exposed to several health problems which may lead to an increase in the mortality rate to about 10% (Raboisson *et al.*, 2013). According to Namur *et al.* (1988), feed additives can be classified into two categories, the first one contains the additives which are essential for animal biological function such as vitamins and trace elements. The second category comprises the additives which are not essential for animal biological function, but they had a positive effect on animal performance that includes metabolic modifiers, growth promoters and probiotics.

The probiotics or direct fed microbes (DFM) are defined as a live microbial feed supplement, which affects beneficially animal health by improving its intestinal microflora. Lactobacilli is one of the most common probiotics used in animal feeding. The mode of action of probiotics or DFM depends on the rumen microbial population. *Lactobacillus acidophilus* strain is one of the dominant lactobacilli in the human intestine (Oh *et al.*, 2000), that making it widely used as a feed additive in livestock. Studies indicated that probiotic supplementation can increase levels of animal blood immunoglobulin (Hosono *et al.*, 2003; Heinrichs *et al.*, 2009).

The plant is one of the drug sources which prompted researchers to indicate that it acts as antimicrobial, anti-inflammatory and antioxidant agent, due to their content of essential oils (EOs) and used for improving the growth and production of animal (Simitzis and Deligeorgis, 2011). *Thymus vulgaris* (THM), as an annual plant growing in different world parts, is commonly used in folk medicine. It had various medical abilities (expectorant, antitussive, antispasmodic, anthelminthic, carminative and diuretic). THM oil contains many chemical compounds with biological activity (pinene, thymol and caryophyllene) which are used in various diseases (Lee *et al.*, 2005; Boskabady *et al.*, 2006) with minimal side effects (Al-Asmari *et al.*, 2017). In animal nutrition, different authors tested the effect of THM plant on dry matter intake, gain performance and immune parameters of calves (Seifzadeh *et al.*, 2016). In growing calves, dietary supplementation of THM increased cell-mediated and humoral immune responses with beneficial effects on their health status (Wafa *et al.*, 2021).

The present study aimed to examine the effect of Probax, as a commercial source of DFM (*Lactobacillus acidophilus*), THM extract, as a probiotic, or their combination on growth performance, rumen and blood parameters, and immune system of Friesian newborn calves.

MATERIALS AND METHODS

The present study was carried out in cooperation between Cattle Breeding Research Department, Animal Production Research Institute, Agricultural Research Center

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Animals

This study included a total of 20 male Friesian calves at three days of age with 35.07±0.56 kg, as average live body weight. Calves were allotted into four similar groups, 5 calves/group. After calving, animals were fed on dam colostrum (about 1.75 kg) for three days using a feeding bottle starting 20-30 minutes after parturition. During the suckling period (3-105 day of age), calves in all groups were given similar amount of the whole milk. Thereafter, up to 150 days of age, all calves were fed same rations. The studying protocol which used in the current study was in accordance with the Directive 2010/63/EU for animal protection (Official Journal of the European Union, 2010).

Feeding system and experimental groups

All calves were in healthy appearance and free of any diseases, kept individually in pens (1.0 × 1.5 m) bedded with rice straw. Calves in the 1st group were fed whole cow milk in two meals at 6.0 a.m. and 6.0 p.m. and the starter were given with good quality berseem hay (BH) after 7 days of age. This group was considered as a control group without any treatment (G1). Calves in G2 were fed the same control diet supplemented with 50 mg/kg BW of Probax® (Dugok-Ri, Sinam-Myeon Co Ltd., Yesan-Gun, Chungcheongnam-Do 340-861, Korea) containing 1.0 x 10^{10} CFU *Lactobacillus* and Dextrose up to 1 kg. Calves in G3 were fed on the control diet supplemented with 40 mg of thyme aqueous extract/kg of body weight. In G4, calves were received 50 mg of Probax® /kg BW in combination with 40 mg of thyme extract.

Feed additive (Probax® and/or thyme extract) was added to milk of calves according to the treatment of groups by mixing in morning milk meals just before feeding. After 2 hours, a starter was allowed to calves with free choice and freshwater was available for free.

Feeding calves on all feed supplements in the whole milk lasted from 4 to 105 days (weaning), then calves in all groups were fed the same amount of concentrate feed mixture (CFM), berseem hay (BH), and rice straw (RS) up to 150 days of age.

The daily feed allowance of calves was adjusted biweekly based on their body weight according to NRC (2001) recommendations.

Representative samples of the whole milk, starter, CFM, BH, and RS were chemically analyzed for DM, CP, EE, CF and ash contents according to the methods of A.O.A.C. (2006), while, the nitrogen-free extract (NFE) was calculated. Chemical analysis of feedstuffs (milk, starter, CFM, BH and RS) during the experimental period is shown in Table 1. The calculated chemical composition of the experimental diets (% on DM basis) is shown in Table 2.

Growth performance parameters

Throughout the experimental period (4-150 days of age), live body weight (LBW) and dry matter intake (milk, starter, CFM, BH and RS) of calves were weekly recorded, then total body gain was calculated.

Table 1. Chemical analysis (on DM basis) of milk, starter, CFM, berseem hay and rice straw fed to calves.

Item	Milk	Starter*	CFM**	Berseem hay (BH)	Rice straw (RS)
DM (%)	12.90	91.17	89.00	91.15	89.15
Chemical composition (%)					
OM	94.70	91.11	88.50	89.44	80.25
CP	25.01	17.70	14.32	14.79	3.35
CF	0.00	6.05	15.10	27.89	35.89
EE	32.12	3.27	4.22	2.98	1.70
NFE	37.57	64.09	45.86	43.78	39.31
ASH	5.30	8.89	11.5	10.56	19.75

***Starter were composed of yellow corn (38%), soybean meal (23%), wheat bran (35%), molasses (2%), premix (1%) and common salt (1%). Every one kg of premix contained Vit. A ($3.3x10^6$ IU); Vit. E (3.3g); Vit. D3 ($3.3x10^6$ IU); Vit. K (0.33g); Vit. B (10.33g); Vit. B2 (1.33g); Vit B5 (6.67g); Vit B6 (0.50g); Vit. B12 (3.3g); Pantothenic acid (3.3g), Folic acid (0.33g); Biotin (16.67mg); Cholin (166.67g); Copper (1g); (Iron 10g); Mn (13.3g); Zn (15g); Iodine (0.1g); Se (0.03g) and Carrier $CACO_3$ up to 1 kg.**

****CFM; concentrate feed mixture was composed of 37% yellow corn, 30% undecorticated cottonseed, 20% wheat bran, 6.5% rice bran, 3% molasses, 2.5% limestone, 1% common salt.**

Table 2. Calculated chemical composition of the experimental rations (% on DM basis).

Item	Suckling period	Post-weaning period
DM	24.24	89.65
OM	94.11	87.19
CP	23.69	12.36
CF	27.89	22.79
EE	2.03	3.38
NFE	40.50	48.66
ASH	5.89	12.81

Ruminal parameters

Rumen liquor samples were collected from three calves in each group at the end of the experiment by stomach tube, 3 hours after feeding, for three consecutive days. The ruminal pH value was measured immediately after collection using a digital pH meter (Sophisticated microprocessor, pH meter).

The rumen fluid was strained through four layers of cheesecloth into plastic containers and kept frozen for later analyses. The concentration of volatile fatty acids (VFAs) was determined by acidification of the first portion of the ruminal sample with concentrated ortho-phosphoric acid and 0.1M hydrochloric acid, while the concentration of ammonia-N was determined by alkalization of the second portion of the sample with 0.1M NaOH. The protozoal count in ruminal liquor samples (fixed with 10% formalin solution in sterilized 0.9% normal saline; 1:9, v: v) was estimated directly by microscopic examination according to Collins and Lyne (2004).

Blood Sampling

After three hours of morning feeding, two blood samples were taken from the jugular vein of each calf in different experimental groups. In the 1st sample, blood was taken in a tube containing EDTA for hematological parameters, while the 2nd blood sample was taken in tubes without anticoagulant to allow blood clot, then centrifuged (3000 rpm for 20 min), and blood serum was isolated and stored untill analysis (-20°C). In the whole blood, red (RBCs) and white (WBCs) blood cells, haemoglobin concentration (Hb), and packed cell volume (PCV) were determined immediately.

Blood serum samples were collected at weaning (105 d of age) and end of the experiment (final at 150 d of age) for analyses of biochemical including total proteins (Henry, 1974) and albumin (AL, Doumas *et al.*, 1971) concentrations.

Concentration of globulin (GL) was obtained by subtraction AL concentration from total proteins concentration. Concentration of serum glucose, creatinine, urea, total cholesterol, and triglycerides were determined according to Trinder (1969), Bartles *et al.* (1972), Bull *et al.* (1991), Richmond (1973), and McGowan *et al.* (1983), respectively. Enzyme activity of transaminases in blood serum including AST and ALT were also measured (Reitman and Frankel, 1957).

Concentrations of immunoglobulins types (G, M and A in serum of blood collected on the day from calves at calving (0 day) and on day 90 of age were estimated quantitatively by ELISA (Bovine IgG, IgM, and IgA ELISA Quantitative kit, Bethyl laboratories, UK) (Killingsworth and Savory, 1972).

Statistical Analysis

The obtained data were statistically analyzed by SPSS analysis program (IBM SPSS, 2017) using one way-ANOVA. Duncan Multiple Range Test (Duncan, 1955) was used to separate the significant differences at P≤0.05 among groups.The following model was used for data analyzing:

$$Y_{ij}=\mu + T_i + e_{ij}$$

where: Y_{ij} = the observation, μ = overall mean, T_i = Effect of the treatment, and e_{ij} = Random error component assumed to be normally distributed.

RESULTS AND DISCUSSION

Results

Feed intake, live body weight, and total weight gain

Results in Table 3 show insignificant differences in LBW of calves at 4 days of age. Live body weight of calves increased (P<0.05) in G3 and G4 at 105 days, and in all treatment groups (G2-G3) at 150 days as compared to G1 (control). Total body gain was increased (P<0.05) in G3 and G4 in comparison with G1 at all intervals, while increased (P<0.05) in G2 in comparison with G1 at 106-150 and 4-150 days of age (Table 3).

Dry matter intake of calves increased (P<0.05) in all treatment groups as compared to controls during the suckling interval (4-105 d). During the post-weaning interval (106-150 d) or the entire length of the experimental period (4-150 d), dry matter intake of calves was increased (P<0.05) in G3 and G4 compared with G1 (Table 3).

Rumen function

Results in Table 4 reveal that the concentration of VFAs in rumen liquor of calves during the post-weaning period was increased (P<0.05) in G3 and G4, while protozoal count increased (P<0.05) in G4. The differences in ammonia-N concentration and pH values were not significant.

Table 3. Effect of Probax, thyme and their combination on growth performance parameters of calves during the experimental period.

Parameter	Experimental group				SEM	P-value
	G1 (control)	G2 (Probax)	G3 (THM)	G4 (Probax+THM)		
			Live body weight (kg)			
4 d	38.02	39.00	38.00	38.60	0.56	0.824
105 d	66.60^{b}	70.60^{ab}	75.80^{a}	77.00^{a}	1.38	0.033
150 d	79.40^{c}	91.40^{b}	99.40^{a}	102.40^{a}	2.28	0.000
			Bodyweight gain (kg)			
4-105 d	28.40^{b}	31.60^{b}	37.80^{a}	38.40^{a}	1.33	0.006
106-150 d	12.80^{c}	20.80^{b}	23.60^{ab}	25.40^{a}	1.19	0.000
4-150 d	41.20^{c}	52.40^{b}	61.40^{a}	63.80^{a}	2.29	0.001
			Dry matter intake (kg/h/d)			
4-105 d	1.00^{c}	1.19^{b}	1.25^{a}	1.26^{a}	0.001	0.041
106-150 d	2.53^{c}	2.57^{bc}	2.77^{b}	2.97^{a}	0.042	0.028
4-150 d	1.77^{c}	1.88^{bc}	2.01^{ab}	2.12^{a}	0.061	0.045

In the same row, ^{a,b,c} mean different significant differences between groups at P< 0.05.

Table 4. Effect of Probax, thyme and their combination on ruminal liquor parameters of calves post-weaning.

Item	Experimental group				SEM	P-value
	G1 (control)	G2 (Probax)	G3 (THM)	G4 (Probax+THM)		
pH value	5.53	5.74	5.81	5.82	0.14	0.125
NH_3-N (mg/100 ml)	22.50	22.67	20.92	19.07	1.25	0.899
TVFAs (meq/100 ml)	11.90^{b}	12.83^{b}	13.55^{a}	13.90^{a}	1.58	0.038
Protozoal count (10^6/mm^3)	1.034^{b}	1.109^{b}	1.277^{ab}	1.385^{a}	0.051	0.031

In the same row, ^{a,b} mean different significant differences between groups at P< 0.05.

Blood parameters

Hematological parameters

Results in Table 5 revealed that calves in G4 showed higher (P<0.05) count of RBCs, and Hb concentration at weaning and final stages as well as higher (P<0.05) PCV value at final stage than in G1. However, WBCs count was not affected by treatment.

Table 5. Effect of Probax, thyme and their combination on hematological parameters of calves during the experimental period.

Hematological parameter	Stage	Experimental group				SEM	P-value
		G1 (control)	G2 (Probax)	G3 (THM)	G4 (Probax+THM)		
RBCs (10^6/mm^3)	Weaning	8.38^{b}	9.04^{b}	10.08^{ab}	11.80^{a}	0.41	0.010
	Final	8.92^{b}	9.70^{b}	10.84^{ab}	12.30^{a}	0.44	0.019
WBCs (10^3/mm^3)	Weaning	9.12	9.32	9.66	10.52	0.46	0.741
	Final	8.88	9.36	9.78	10.18	0.43	0.785
Hemoglobin (g/dL)	Weaning	8.78^{b}	8.87^{b}	10.18^{ab}	11.77^{a}	0.45	0.041
	Final	7.66^{b}	8.12^{b}	9.70^{ab}	11.43^{a}	0.51	0.021
PCV (%)	Weaning	25.90	25.55	29.37	31.69	1.18	0.205
	Final	22.19^{b}	23.60^{ab}	28.52^{ab}	30.68^{a}	1.40	0.017

In the same row, ^{a,b} mean different significant differences between groups at P< 0.05.

Blood serum biochemicals

Data in Table 6 show that weaning and final concentrations of total proteins, albumin, urea, total cholesterol, and triglycerides in blood serum of calves increased (P<0.05), while creatinine concentration was lower (P<0.05) in G3 and G4 than in G1. Serum globulin concentration increased (P<0.05) only in G4 compared with G1. However, at the weaning and final stages, glucose concentrations were higher (P<0.05) in G2, G3, and G4 than in G1, being the highest in G4.

Enzyme activity

Results illustrated in Figures 1 and 2 showed that calves in G4 showed lower activity of AST and ALT at weaning and final stages than those in G1.

Table 6. Effect of Probax, thyme and their combination on biochemical concentrations in blood serum of calves during the experimental period.

Blood biochemical	Stage	Experimental group				SEM	P-value
		G1 (control)	G2 (Probax)	G3 (THM)	G4 (Probax+THM)		
Total proteins (g/dL)	Weaning	6.50^{b}	6.91^{b}	7.37^{a}	7.59^{a}	0.12	0.000
	Final	5.71^{c}	6.07^{b}	6.37^{b}	6.74^{a}	0.09	0.000
Albumin (g/dL)	Weaning	3.83^{c}	4.02^{bc}	4.35^{ab}	4.51^{a}	0.09	0.100
	Final	3.22^{c}	3.49^{bc}	3.69^{ab}	3.99^{a}	0.08	0.004
Globulin (g/dL)	Weaning	2.66^{b}	2.89^{ab}	3.02^{ab}	3.08^{a}	0.07	0.118
	Final	2.49^{b}	2.58^{ab}	2.68^{ab}	2.75^{a}	0.04	0.135
Glucose (mg/dL)	Weaning	87.89^{c}	96.23^{b}	99.02^{b}	109.96^{a}	1.92	0.000
	Final	82.23^{d}	91.37^{c}	96.81^{b}	102.76^{a}	1.83	0.000
Creatinine (mg/dL)	Weaning	1.82^{a}	1.60^{ab}	1.07^{bc}	0.85^{c}	0.14	0.011
	Final	1.99^{a}	1.57^{ab}	1.25^{b}	1.13^{b}	0.11	0.016
Urea (mg/dL)	Weaning	10.79^{b}	11.54^{ab}	12.13^{a}	12.38^{a}	0.19	0.006
	Final	12.32^{b}	12.92^{b}	14.15^{a}	14.26^{a}	0.22	0.000
Total cholesterol (mg/dL)	Weaning	88.98^{b}	92.40^{b}	98.76^{a}	102.86^{a}	1.44	0.000
	Final	85.90^{b}	89.97^{b}	97.28^{a}	99.88^{a}	1.50	0.000
Triglycerides (mg/dL)	Weaning	87.62^{b}	94.56^{ab}	103.52^{a}	105.28^{a}	2.48	0.026
	Final	84.66^{b}	92.84^{ab}	100.86^{a}	102.37^{a}	2.61	0.045

In the same row, ^{a,b,c} mean different significant differences between groups at P< 0.05.

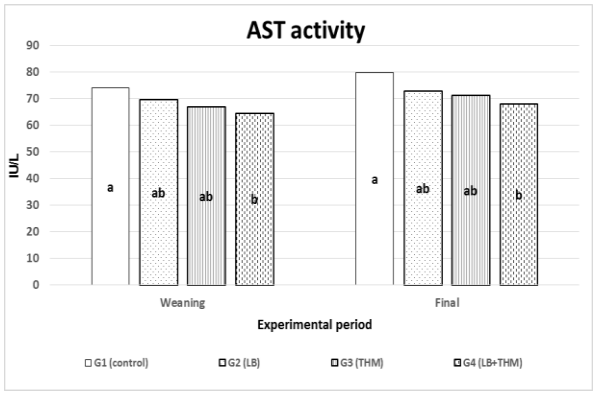

Fig. 1. Effect of Probax, thyme and their combination on AST activity in serum of calves during the experimental period.

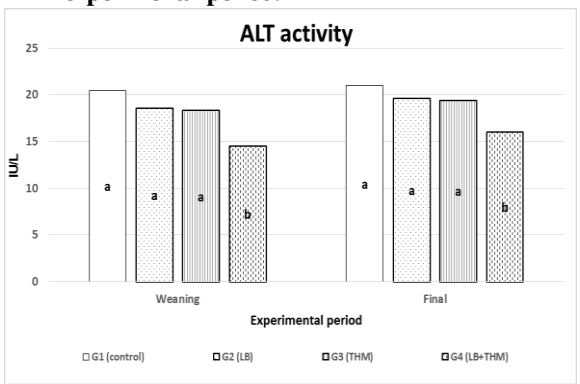

Fig. 2. Effect of Probax, thyme and their combination on ALT activity in serum of calves during the experimental period.

Immunoglobulins concentration

As affected by treatment, results illustrated in figures 3-5 revealed non-significant differences at 0 time (birth). On day 90 of age, calves in G3 and G4 showed higher (P<0.05) concentrations of IgG, IgM, and IgA than in G1, being the highest in G4. However, in G3 and G4, calves showed a slight decrease in IgG, and a marked increase in IgM and IgA by advancing calf age, in an opposite pattern to those in G1 and G2.

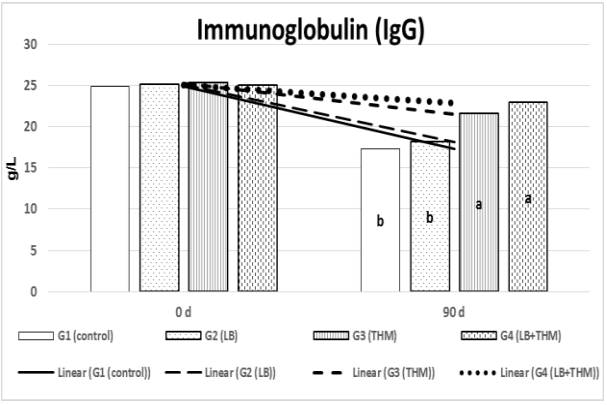

Fig. 3. Effect of Probax, thyme and their combination on immunoglobulin (IgG) in serum of calves during the experimental period.

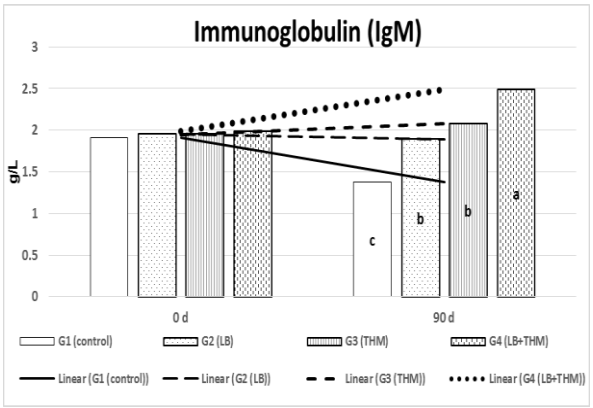

Fig. 4. Effect of Probax, thyme and their combination on immunoglobulin (IgM) in serum of calves during the experimental period.

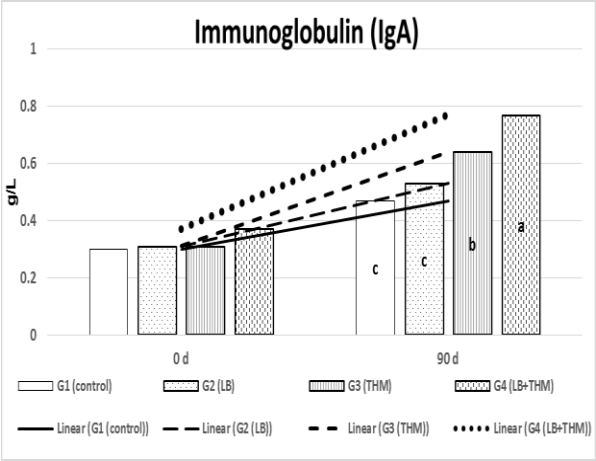

Fig. 5. Effect of Probax, thyme and their combination on serum IgA concentration of calves at calving and 90 d of age.

Discussion

With the great attention of the recent usage of herbal extracts, as natural antioxidants, besides the using Probax in improving animal production, the objective of the current study is evaluation of the effect of thyme extract, as a natural antioxidant, direct feed microbes (*Lactobacillus acidophilus*), as a probiotic, or their combination on growth performance, rumen function, blood biochemicals and enzymes activities, as markers of lipid-profile, liver and kidney functions, and hematological parameters and immunoglobulins as markers of health status and immunity of Friesian newborn calves. The present results indicated that dietary supplementation of calf diets with THM (G3) or its combination with Probax (G4) showed the highest growth performance parameters in terms of increasing LBW, weight gain, and feed intake as compared to the control group (G1). In comparison with G1, the rate of increase in LBW of calves was 15.6 and 28.9% in G4, and 13.8 and 25.2% in G3 at weaning and 150 days of age (Final stage). Also, total weight gain increased in G3 and G4 by 33.1 and 35.2% at 4-105 days, 84.3 and 98.4% at 106-150, and 49.0 and 54.9% during the entire experimental period (4-150 days of age), respectively. Calves in G4 showed the highest increase in dry matter intake, being 26.0, 17.4, and 19.8% as compared to controls at 4-105, 106-150, and 4-150 days of age, respectively. The corresponding values of calves in G3 were 25.0, 9.5, and 13.6%, respectively. Although some authors found a non-significant effect of Lactobacillus bacteria additives on daily gain (Timmerman *et al.*, 2005), or THM supplementation on final body weight of calves (Vakili *et al.*, 2013; Darabighane *et al.*, 2016), the obtained results indicated a beneficial effect of THM alone or in a combination with Probax on growth performance of calves. In agreement with the present results, Agazzi *et al.* (2014) found clear improvement in growth performance of different animal species (mono-gastric or ruminant) as affected by dietary supplementation of lactic acid bacteria. Wafa *et al.* (2021) found that calves fed a diet containing 40 mg THM extract/kg showed the highest LBW and total weight gain during the suckling period and at weaning. In Holstein calves (Froehlich, 2016) and Holstein Friesian calves (Ozkaya *et al.*, 2017), a significant increase in calve final body weight was found as affected by THM addition in their rations. Seifzadeh *et al.* (2016) reported an increase in the final body weight of Holstein suckling calves treated with Probax and THM as compared to the control group. The improvement observed in growth performance of calves in G4 fed a combination of THM and Probax in this study may be attributed to that probiotics can improve ration digestibility in ruminants (Ayad *et al.*, 2013), and herbal extracts have positive effects on nutrient digestibility in cattle (Benchaar *et al.*, 2008). Also, Probax has beneficial effects, as pathogenic bacteria colonization inhibition, which improves the balance of gut microorganisms and increases the digestion efficiency leading to better growth performance (Schneider *et al.*, 2004; Corcionivoschi *et al.*, 2010). Also, dietary supplementation of THM essential oil showed significant improvement in digestibility coefficients of dry and organic matter by calves (Ebrahimi *et al.*, 2018). In buffalo calves, Ibrahim (2016) recorded an increase in dry matter and protein digestibility of diet containing lactic bacteria. In dairy heifers, a significant increase in nutrient digestibility attributed to dietary addition of probiotics was reported by Ghazanfar *et al.* (2015).

According to the obtained results improving BW and gain observed in calves of G3 and G4 associated with increasing concentration of VFA in the rumen liquor of calves at the post-weaning stage. It is of interest to note that the increase in VFAs concentration was in parallel with increasing protozoal count in G3 ($P \geq 0.05$) and G4 ($P < 0.05$). Meanwhile, the insignificant differences in the ruminal pH value may reflect maintenance of the ruminal fermentation conditions by Probax and THM combination, which resulted in a non-significant decrease in ammonia-N concentration. Values of ruminal pH in the present study, being around the normal range (5.5-7.0) were described as critical in maintaining the ruminal fibre digestion (Olson, 1997; Garrett *et al.*, 1999). These results are in harmony with several authors, who found that the supplementation of diet with different bacteria strains that live naturally in the digestive tract had a significant effect on increasing protozoal count in rumen liquor and improving rumen function (Soto *et al.*, 2010; Ripamonti *et al.*, 2011). In this respect, beneficial effects on rumen fermentation and general animal performance of feeding ruminant on diet supplemented with herbal extract were reported by Beauchemin *et al.* (2007) and Benchaar *et al.* (2008). The role of lactobacillus bacteria which produced the lactic acid and short-chain fatty acids in the rumen may improve the rumen microflora activity. Also, the short-chain fatty acids can improve rumen function by decreasing pH and stimulating the epithelial cells in rumen (Reid *et al.*, 2003).

In accordance with the present results regarding the improvement in hematological parameters of only calves fed Probax and THM combination (G4), it was reported that plant extracts, as THM, can be used to improve hematological parameters (Lakhani *et al.*, 2019) than animal health being in a perfect condition. In this respect, Hb concentration, RBCs and PCV at weaning were improved in calves by dietary supplementation with THM at a level of 40 mg/kg LBW (Wafa *et al.*, 2021). Feeding male and female calves on diet with Probax raised the haemoglobin concentration, PCV and red blood cells count, and decreased the white blood cell count (Maroccolo *et al.*, 2013).

Contrary, Ebrahimi *et al.* (2018) reported a clear increase in blood white cells count in newborn calves treated with THM oil.

Concerning the positive effect of treatment on blood biochemicals of calves fed THM and Probax combination diets (G4), the present results indicated that increasing serum TP was associated with an increase in both AL and GL. There is a relationship between blood protein level in animals and their nutritional status so it can be used as an indicator for ruminant nutrition (Kumar *et al.*, 1981). In this respect, the Concentration of serum total proteins at weaning increased ($P<0.05$) for calves fed a diet supplemented with 40 mg THM extract/kg LBW (Wafa *et al.*, 2021). The presented data showed a similar trend as described by Seifzadeh *et al.* (2016), who reported a worthily increase in Holstein's calves blood total protein that attributed with the addition of Probax or THM in the ration. While Froehlich (2016) indicated that dairy calves fed a diet containing THM essential oil, blood serum protein was slightly increased. In our study, all supplements (Probax, THM, or their combination) increased serum glucose concentration, being the highest in calves fed a combination of Probax and THM. Similarly, Ebrahimi *et al.* (2018) reported that feeding Brown Swiss calves on diet with THM essential oils led to an increase in blood glucose concentration. Seifzadeh *et al.* (2016) found that the concentration of blood glucose was increased in Holstein calves fed on diet containing Probax bacteria or THM. Maroccolo *et al.* (2013) found a slight increase in blood glucose of dairy calves fed on diet with Probax. Vakili *et al.* (2013) reported a slight increase in blood glucose concentration of Holstein calves fed on ration with THM.

In harmony with the present results concerning the observed reduction in creatinine concentration, some studies showed that feeding newborn calves on a diet that contained Probax (Maroccolo *et al.*, 2013) or THM essential oils (Ebrahimi *et al.*, 2018) decreased ($P<0.05$) the concentrations of blood creatinine. Although some authors indicated that the treatment of calves with phytogenic (Lakhani *et al.*, 2019) or THM (Vakili *et al.*, 2013; Biricik *et al.*, 2016) did not affect blood urea concentration, the present study revealed a significant increase in urea concentration in calves fed THM or its combination with Probax. This finding is in association with an insignificant reduction in ammonia-N concentration in rumen liquor of calves in G3 and G4. The recent results of Wafa *et al.* (2021) indicated that dietary supplementation of THM extract (40 mg/kg BW) increased urea and decreased creatinine in blood of calves at weaning. Concerning the observed alleviation in total cholesterol and triglycerides in calves fed diet supplemented with Probax and THM combination (G4), a similar trend of increase in total cholesterol and triglycerides concentrations were observed by Wafa *et al.* (2021) in weaned calves fed diet containing 40 mg THM extract/kg LBW. Seifzadeh *et al.* (2016) showed that feeding newborn dairy calves on diet containing Probax or THM led to an increase in the blood cholesterol concentration. Ebrahimi *et al.* (2018) recorded increased blood triglycerides when neonatal buffalo calves were fed on a diet supplemented with Probax. In Holstein's calves (Vakili *et al.*, 2013), concentration of total cholesterol and triglycerides in blood were increased non-significantly in calves fed on a diet supplemented with THM. The present results of blood biochemical indicated beneficial effects of the combination (Probax+THM) on increasing metabolism of protein, carbohydrates and lipids, consequently growth performance parameters of calves in G4

As such, we studied the safety of using Probax and THM combinations for improving the growth rate of calves in terms of kidney and liver function. Besides the observed reduction in serum creatinine, as a kidney function marker, in calves of G3 (THM alone) and G4 (Probax and THM), also a pronounced reduction in serum AST and ALT activities were observed only on calves fed the combination diet. The average of ALT and AST activity in our study are within a normal range reported by Gaffar (1994) in Friesian calves and is inconsistent with the results of Wafa *et al.* (2021), who noticed a significant reduction in AST and ALT activities in blood serum of calves fed the diet with 40 mg THM extract/kg LBW during the suckling period. These findings may indicate a synergic effect of Probax with THM on the elimination of the load on liver function by decreasing AST and ALT activities in G4. On the other hand, immune response in terms of immunoglobulin concentrations in the blood serum of calves at the post-weaning period was also evaluated in this study. The obtained results in this concern indicated improving the concentration of all immunoglobulin types (IgG, IgM, and IgA) of calves fed a diet containing THM (G3) or Probax and THM combination (G4) as compared to G1 (control) or those fed diet containing Probax alone (G2). Qiao *et al.* (2013) stated that herbs can improve the immunity system by increasing IgG, IgM and IgA concentrations. The present results of immunoglobulin concentrations are in good agreement with Lakhani *et al.* (2019), who recorded that humoral immune response increased by feeding buffalo calves on plant phytogenic. Dietary supplementation of THM extracts significantly improved total health scores in calves during the suckling period (Wafa *et al.*, 2021).

Also, Darabighane *et al.* (2016) indicated that THM oil decreased the incidence of diarrhea cases and improve the animal health of Holstein's calves. Wafa *et al.* (2021) reported that immunoglobulin types (G, A, and M), within three days after calving, improved in blood of calves as affected by THM treatment (40 mg/kg B.W). This treatment lowered ($P<0.05$) the reduction in IgG and IgM levels after calving as compared to the control.

Amirghofran *et al.* (2012) indicated that THM extract treatment had a positive role in resisting infections and immune-related diseases. Several authors noticed that the concentrations of blood immunoglobulins in dairy Friesian calves were decreased with age progress (Panivivat *et al.*, 2004; Ježek *et al.*, 2012; Wafa, 2017). This trend was observed for immunoglobulin concentrations in G1 and G2. However, in calves of G3 or G4 dietary supplementation with THM alone or Probax+THM decreased the reduction rate in IgG, and increased IgM and IgA with age progress. Similarly, Froehlich (2016) and Ozkaya *et al.* (2017) indicated that blood immunoglobulins (types G, M and A) of calves increased with THM treatment as compared to control calves.

CONCLUSION

In conclusion, dietary supplementation of Lactobacillus bacteria (50 mg) and thyme extract (40 mg)

combination improved growth performance, rumen function, hematological parameters, blood biochemical, and immunity status of Friesian calves from 3 to 150 days.

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تأثير البروباكس ، مستخلص الزعتر و الخليط بينهما على أداء النمو ، وظيفة الكرش و مكونات الدم لعجول الفريزيان

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أجريت هذه الدراسة بهدف تقييم تأثير إضافة البروباكس (اللاكتوبسلس) ، مستخلص الزعتر وخليطهما على الأداء الإنتاجي ، وظيفة الكرش ومكونات الدم الهيماتولوجية والبيوكيميائية ومناعة العجول الفريزيان حديثة الولادة من الولادة حتى الفطام وعند 150 يوم من العمر. تم إختيار 20 عجل بقري فريزيان ذكور عند عمر ثلاثة أيام بمتوسط وزن الجسم 35.07±0.56 كجم وتم تقسيمها إلى أربعة مجاميع متساوية (5 عجول في كل مجموعة). تم تغذية العجول على لبن كامل من الأمهات على وجبتين ثم تم تقديم العلف البادئ ودريس البرسيم بعد 7 أيام من العمر وهي مجموعة الكنترول وفي المجموعة الثانية تم تغذية العجول على عليقة الكنترول مضافا إليها 50 ملجم من البروباكس لكل كجم من وزن الجسم وفي المجموعة الثالثة تم إضافة 40 ملجم من مستخلص الزعتر لكل كجم من وزن الجسم وفي المجموعة الرابعة تم إضافة 50 ملجم من البروباكس مع 40 ملجم من مستخلص الزعتر لكل كجم من وزن الجسم. أظهرت النتائج حدوث تحسن معنوي عند مستوى معنوية (0.05 ٪) في معدل نمو العجول المغذاة على عليقة مضافا لها خليط من البروباكس ومستخلص الزعتر في المجموعة الرابعة ، وكذلك حدث تحسن في مستوى الأحماض الدهنية الطيارة وعدد البروتوزوا في سائل الكرش ، وأيضا تحسن عدد كريات الدم الحمراء ونسبة الهيموجلوبين والهيماتوكريت والبروتين الكلي والألبيومين والجلوبيولين والجلوكوز واليوريا والكوليستيرول الكلي والدهون الثلاثية والجلوبيولينات المناعية من (نوع G, M, A) في سيرم الدم في حين إنخفض كل من الكرياتينين وإنزيمات الكبد (ALT, AST). يستخلص من هذة الدراسة أن معاملة العجول البقري حديثة الولادة بإضافة خليط من البروباكس ومستخلص الزعتر بمعدل 50 و 40 ملجم/كجم وزن جسم يمكن أن يحسن معدل النمو ووظيفة الكرش والمكونات الهيماتولوجية والبيوكيميائية والمناعة في الدم عند الفطام وعند عمر 150 يوم.