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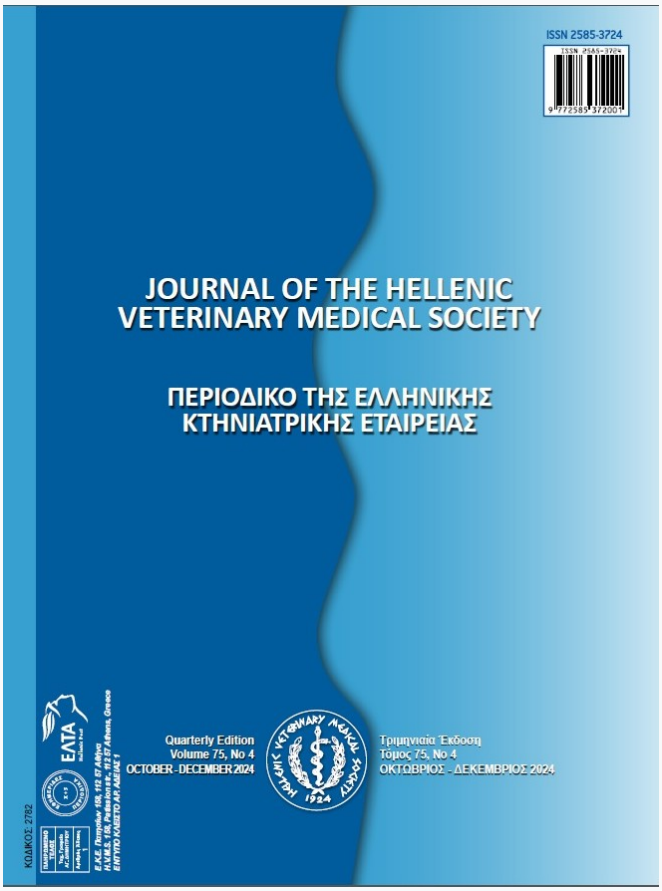

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Effect of propolis supplementation on productive performance and immunity response of suckling Friesian calves

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ABSTRACT: Objective of this study was to investigate the effect of propolis supplementation on productive performance and blood profile of suckling Friesian calves. Eighteen newly born male Friesian calves with average live body weight of 26.11±0.53 kg are assigned into three similar groups after colostrum. Calves were unsupplementedin G1 and served as control, or supplemented with propolis at the levels of 0.5 and 1.0 g/head/day in G2 and G3, respectively. The best values of all nutrients digestibility and feeding values were recorded to G3 followed by G2, whereas G1 had the poorest values. Ruminal TVFA's, acetate and propionate concentrations and serum total protein, globulin, albumin and glucose concentrations were significantly higher (P<0.05), however, ruminal pH value and ammonia-N and butyrate concentrations and serum urea-N, creatinine, AST and ALT concentrations were significantly lower (P<0.05) in G3 followed by G2 and vice versa in G1. Also, G3 had the highest immunoglobulins (IgG, IgA and IgM), haemoglobin concentration (HGB) and Haematocrit (a packed-cell volume) percentage (PCV), counts of leucocytes (WBC's) and their differential (lymphocytes, monocytes, neutrophil, eosinophil and basophil), erythrocyte (RBC's) and erythrocyte indices including mean cellular volume (MCV), mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC) and red cell distribution width (RDW) and platelet indices expressed as platelet count (PLT), procalcitonin (PCT), mean platelet volume (MPV) and Platelet Distribution Width (PDW) concentrations followed by G2, while G1 had the lowest concentrations (P<0.05). Weaning weight, total weight gain and average daily gain were significantly higher (P<0.05) in G3 followed by G2, while the lower values were in control ones (G1). In takes of TDN and DCP were significantly higher (P<0.05) in G3 compared to G1 with insignificant differences with G2. Feed conversion ratio was significantly (P<0.05) better with G3 compared G1, while G2 differ significantly in DM and CP/kg gain and insignificantly in TDN and DCP/kg gain with both G1 and G3. Feed cost was higher significantly (P<0.05), but feed cost of gain was lower significantly (P<0.05) in G3 compared to G1, and insignificantly with G2. While, G3 recorded significantly (P<0.05) the highest revenue of weight gain, net revenue, relative net revenue and economic efficiency followed by G2, but G1 had the lowest values. In conclusion, propolis supplements at the level of 1 g/calf/day for suckling calves led to significant improvements on productive performance and blood profile

***Keywords*:** Suckling calves; propolis; digestibility; rumen fermentation; blood parameters; feed intake; weight gain; feed conversion; economic efficiency.

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INTRODUCTION

Propolis or bee glue is a resinous mixture that honey bees produce by mixing saliva and beeswax with exudate gathered from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the beehive. Propolis is used for small gaps (around 6 mm (¼ in) or less), while gaps larger than the bee space (around 9 mm (⅜ in)) are usually filled with burr comb. Its color varies depending on its botanical source, with dark brown as the most common. Propolis is sticky above 19 °C (66 °F), while at lower temperatures, it becomes hard and brittle (Wagh, 2013). Propolis contains more than 300 biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, fatty acids, vitamins, and amino acids (Park et al., 2000; Alencar et al., 2007; Morsy et al., 2015). Propolis contains a range of biologically active compounds like phenol compounds, flavonoids (primuletin, chrysine, tecochrysine, akacetine, galangine, morin, robinetin), terpenes, lipid-wax substances, bioelements, vitamins (A, D, F, K, E, B1 , B2 , B5 , B6 , B12, C, H, P), enzymes (alpha and beta amylase), amino acids, sterols, steroids, plant steroids, plant sterols (ergosterol, stigmasterol, steroidal saponins, steroidal alkaloids) (Marcucci, 1995; Sahinler and Kaftanoglu, 2005). Apart from resins, constituting about 50% of the propolis, and wax, constituting about 30% of its content, propolis also contains essential oils, pollen and other organic components, constituting, respectively 10%, 5% and 5% of its content (Górecka et al., 2014). The main components of propolis are: resin (50-70%), oil and wax (30-50%), pollen (5-10%) and other chemical compounds including: amino acids, minerals, sugars, vitamins B, C and E, flavonoids, phenol, as well as aromatic compounds (Bankova et al., 2000; Russo et al., 2002). Raw propolis is typically composed of 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollens and 5% other organic substances (Kosalec et al., 2004; Bankova et al., 2000). The major components detected in propolis were the flavone acacetin (76.359 mg/g), caffeic acid (21.358 mg/g), p-coumaric acid (16.911 mg/g), naringenin (11.34 mg/g), chrysin (9.86 mg/g), quinic acid (7.285 mg/g), quercetin (6.223 mg/g), ferulic acid (5.11 mg/g), apigenin (4.686 mg/g), luteolin (4.394 mg/g), kaempferol (4.043 mg/g), hesperidin (2.089 mg/g), vanillic acid (1.647 mg/g) and protocatechuic acid (1.158 mg/g) (Karagecili et al., 2023).

Propolis may be used as a feed additive in animal production and meet current consumer expectations about safety and toxicity of animal products. Propolis is a natural bee product, it is rich in plant phenolic compounds that confer important biological properties such as antibacterial actions, cytostatic, hepatoprotective activities (Banskota et al.,2000), antimicrobial, anti-inflammatory, immunomodulatory properties (Bankova et al., 2000), antioxidant, antibacterial (Marcucci et al., 2001), antibiotic (Cottica et al., 2011), antiparasitic and antiseptic properties in its structure is antiviral (Vynograd et al., 2000), antifungal (Ota, et al., 2001), analgesic and tissue regenerative (De Castro, 2001). In addition, Stangaciu (1999) reported the positive effects and preventive properties of propolis on dangerous pathogenic bacteria's and viral infections. The beneficial effect of propolis on immune response and growth performance in both broiler (Ziaran et al., 2005) and layer (Galal et al., 2008) have been reported.

Many researchers reported that propolis can efficiently be used in ruminant feeding to promote and accelerate growth and development as a safe and natural alternative to antibiotics. Lambs receiving propolis at the dose of 150 µL/day had 2.94kg greater weight gain in the trial which lasted 42 days compared to the control group (Cécere et al., 2021). Abd-Allah and Daghash (2019) stated that the use of propolis as a feed additive in Egyptian Buffalo calves had a positive effect on the growth and live weight gain of calves, which the weaning weights of the calves fed control ration plus 50 mg propolis/head/day was 7.7 kg greater than the control group. In a similar study carried out on Simmental calves and lasted 21 days after birth, it was reported that 4 ml of EEP (Propolis ethanol extract) group had 213,9 g greater in average daily gain compared to the control group (Kupczyński et al., 2012). Zawadzki et al. (2011) examined the effects of propolis administration on finishing performance and carcass traits of Nellore bulls and stated that the supplementation of propolis extract on bull's diets significantly increased daily weight gain (1.17 kg) compared to control group (0.87 kg). Daily weight gains were higher for the male and female Holstein Friesian calves for propolis groups compared to control ones (Yucel et al., 2015).

Active immunity in calves is gradually acquired after birth. Calves are therefore extremely vulnerable to external disease factors when they are born. Colostrum protects calves by providing passive immunity with a high content of antibodies (immunoglobulins),

but the content of colostrum varies due to various factors, and in some cases, colostrum may not be enough, or calves may not consume sufficient levels of colostrum. It has been stated that the mortality rate is high and the risk of pneumonia is twice as high in newborn calves when Immunoglobulin (IgG) level is lower than 10 g/L (Kozat, 2019). In various studies, propolis has been reported to affect the Immunoglobulin levels of animals. Shedeed et al. (2019) reported that with the supplementation of propolis, a significant increase was observed in blood Immunoglobulin (IgA) levels in sheep compared to the control group, but there was no change in IgM and IgG levels. In a similar study, propolis administration was reported to significantly increase blood IgA levels in lambs (Cécere et al., 2021). In a study conducted on Hanwoo calves for 90 days suckling period, with different feed additives (propolis, illite, neomycin (antibiotic)) on some blood parameters and the highest Immunoglobulin (IgA, IgM, IgG) levels were reported in the propolis applied group (Sarker and Yang, 2010).

Dairy calves are exposed to several pathogens, and if their immune system is not sufficiently strengthened, the disease incidence can be high. Yucel et al. (2015) reported the beneficial effect of propolis tincture (2 mL/d) on the prevention of diarrhea in calves, which consequently affected their performance in a positive way. Propolis has been shown to treat numerous diseases, in veterinary medicine, and has had positive effects on animal health (Kadhim et al., 2018). Because of the high occurrence of gastrointestinal disorders and their effect on calf morbidity and mortality, dairy systems have increased the inclusion of additives that have a positive effect on the control of diarrhea. Because of its antimicrobial, anti-inflammatory, antioxidant, antiviral, and immunomodulatory properties (Alencar et al., 2007), propolis has a high potential to be used in dairy systems as an additive, improving animal health and performance.

The objective of this study was to investigate the effect of propolis supplementation on the digestibility, rumen fermentation activity, blood biochemical, immunity response, haematological profile, growth performance, feed intake, feed conversion ratio and economic efficiency of suckling Friesian calves.

MATERIALS AND METHODS

The current work was carried out at Karada Animal Production Research Station belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture. The experiments were performed according to the guidelines of a local ethics committee for animal care and welfare (Number 08/2016 EC).

Experimental animals and groups

Eighteen newlyborn male Friesian calves with an average live body weight of 26.11±0.53 kg are assigned into three similar groups after suckled their dam's colostrum. Calves were unsupplemented in G1 and served as control, or supplemented with propolis at the levels of 0.5 g/calf/day in G2 or 1.0 g/head/day in G3. Propolis was dissolved in the ethanol at the rate of 1 g in 2 ml. Ethanolic extract of propolis was analysed by Shimadzu GC-MS Ultra Gas Chromatograph Mass Spectrometer (Shimadzu, Kyoto,Japan). *Chemical composition* of ethanolic extract of propolis assessed by GC-MS as trimethylsilyl ethers derivatives *(%* of total ion current, TIC) was aliphatic acids (3.0% palmitic, 0.9% stearic, 4.0% oleic, 1.6% tetracosanoic, 0.3% succinic, 1.3% lactic and 0.3% piruvic), aromatic acids (0.2% benzoic, 0.5% trans-coumaric, 0.3% Caffeic, 0.2% ferulic and 0.4% dimethoxycinnamic), esters (0.5% ethyl palmitate, 1.2% ethyl oleate, 0.9% isopentenyl caffeate, 1.3% dimethylallyl caffeate, 1.1% dodecyl caffeate, 3.1% tetradecyl caffeate, 0.3% tetradecenyl caffeate, 4.7% hexadecyl caffeate, 0.60% benzyl caffeate and 0.2% phenylethyl caffeate), sugars (6.1% D-glucose, 3.1% sorbose, 3.1% fructose, 1.6% sucrose and 0.2% mannitol), flavonoids (1.1% pinocembrin, 0.7% galangin, 0.8% chrysin, 0.6% pinostrobin, 0.3% pinobanksin and 1.1% 3-O-acetylpinobanksin), triterpenic alcohols (1.2% lanosterol, 7.1% cycloartenol, 4.8% triterpenic alcohol of amyrine type and 4.7% ß-amyrine) and others (2.7% phosphoric acid, 0.5% tricosane and 1.8% glycerol octadecyl ether) (Christov et al., 1998).

Management procedures

Calves were housed in separate pins at night time and free outside during day. Calves had been fed their allowance of milk, starter, and berseem hay all through the suckling duration (105 days) to meet their nutritional requirements, as shown in Table 1 according to NRC (2001). Calves were artificially fed milk in plastic bucket twice daily at 7 a.m. and 7 p.m., calf starter was given once daily at 9 a.m. and berseem hay once time at 11 a.m. Ethanolic extract of propolis was supplemented in the whole milk once time daily during the morning suckling. Compositions of ingredients and basal diet are shown in Table (2).

Table 1: Allowances of milk, starter and berseem hay per day for suckling calves.

Age	Milk	Starter	Berseem hay
1-3 days	**Suckling their dam's colostrum**		
		Kg/day	
4-7 days	3.5	-	-
Week 2	4.0	0.10	-
Week 3	4.5	0.25	0.1
Week 4	5.0	0.25	0.1
Week 5	5.0	0.50	0.2
Week 6	4.5	0.50	0.2
Week 7	4.0	0.75	0.3
Week 8	3.5	0.75	0.3
Week 9	3.0	1.00	0.4
Week 10	2.5	1.00	0.4
Week 11	2.0	1.25	0.5
Week 12	1.75	1.25	0.5
Week 13	1.5	1.50	0.6
Week 14	1.25	1.50	0.6
Week 15	1.0	1.75	0.7
Total	**318.50**	86.45	34.30
Average	3.03	0.82	0.33

- Composition of milk used to be 3.80% fat, 3.20% protein, 4.93% lactose, 8.85% solids not fats (NFS), 12.65% total solids (TS) and 0.72%ash.
- Starter: 15% soya bean meal, 10% linseed cake, 34% ground corn grain, 20% wheat bran, 15% rice bran, 3% molasses, 2% limestone and 1% common salt.

Table 2: Chemical analysis of ingredients and basal diet.

Items	DM%	Composition of DM%									
		OM	CP	CF	EE	NFE	Ash	NDF	ADF	ADL	NFC
Milk	12.24	93.86	24.92	00.00	29.74	39.20	6.14	00.00	00.00	00.00	39.20
Starter	90.83	91.45	17.32	5.87	3.38	64.88	8.55	19.80	6.85	1.45	50.95
Berseem hay	90.15	87.16	12.46	27.92	2.45	44.33	12.84	50.25	38.30	3.35	22.00
Basal diet	33.81	91.18	18.29	8.97	10.10	53.82	8.82	21.01	11.67	1.47	41.78

- NFC = 100 - CP - EE - Ash - NDF (NRC, 2001).
- DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fiber; EE: ether extract; NFE: nitrogen free extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, NFC: non fiber carbohydrates.

Live body weight

Calves were weighed weekly in the morning before feeding from birth to weaning and the weekly weight gain and average daily gain were calculated for each animal.

Digestibility trials

Three digestibility trials have been carried out the 12th week of suckling period to determine nutrient digestibility and feeding values using of acid insoluble ash (AIA) as a natural marker (VanKeulen and Young, 1977). Samples of milk had been analyzed using Milko-Scan (model133B), calf starter, berseem hay and feces were analyzed according to the techniques of AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were further determined in feeds according to Van Soest et al. (1991). The digestibility coefficients of all nutrients were calculated by the equations of Schneider and Flatt (1975).

$$DM\ digestibility\ \% = 100 - \left(100 \times \frac{AIA\ \% \in feed}{AIA\ \% \in feces}\right)$$

$$Nutrient\ digestibility\ \% = 100 - \left(100 \times \frac{AIA\ \% \in feed}{AIA\ \% \in feces}\right) \times \left(\frac{Nutrient\ \% \in feces}{Nutrient\ \% \in feed}\right)$$

The total digestible nutrients (TDN) and digestible crude protein (DCP) were calculated by the equation of McDonald *et al.* (1995).

TDN = (CP*CPD + CF*CFD + EE*EED*2.25 + NFE*NFED)/100

DCP = (CP*CPD)/100

CPD: Crude protein digestibility, CFD: crude fiber digestibility, EED: ether extract digestibility, NFED: nitrogen free extract digestibility.

Samples of rumen liquor:

Rumen liquor samples were collected from calves at the 12th week of suckling period using a stomach tube at three hours after morning feeding and filtered through two layers of cheesecloth. Ruminal pH was determined using Orian 680 digital pH meter. The concentration of ammonia-N was determined using magnesium saturated solution according to AOAC technique (2000). The concentration of TVFA's was determined in the rumen liquor using the steam distillation according to the technique of Warner (1964). The VFA fractions were determined in rumen liquor according to method of Filípek and Dvořák (2009).

Samples of blood:

Blood samples were taken at the 12th week of suckling period d from the jugular vein of each calf by clean sterile needle in tow clean dry glass tubs. The first tube contained drop of heparin as an anticoagulant and used for determined hematological parameters. The second tube was allowing the blood to clot by leaving it undisturbed at room temperature for 15-30 minutes and centrifuging at 1000-2000 r.p.m for 10 minutes in a refrigerated centrifuge. The supernatant serum immediately transferred into a clean tube using a pipette. The samples should be maintained at -20 °C until analysis.

Biochemical analysis:

Total protein and albumin concentrations in serum were determined using commercial kits supplied by Randox (Randox Laboratories Ltd, Crumlin, Co, Antrim, UK) according to Henry et al. (1974). Globulin concentration was estimated by subtracting the values of albumin from the corresponding values of total protein per sample. Also, serum samples were analyzed for determinations blood activities of aspartate transaminase (AST) and alanine transaminase (ALT) according to Hafkenscheid and Dijt (1979) and creatinine concentration according to Chasson et al. (1961) using commercial kits (Bio-Merieux Laboratory Reagents and Products, France) according to the manufacturer procedure.

Determination of immunoglobulins

The concentrations of immunoglobulins IgG, IgM, and IgA in blood serum samples were determined using a quantitative ELISA Bovine (IgG, IgM and IgA), ELISA Quantitation Kit from Bethyl laboratories in the United Kingdom.

Haematological analysis

Haematological analysis was performed by Medonic Vet. Hematology Analyzer (Medonic CA 620, Sweden) directly after the samples were received by the research laboratory and within 1-2 hours after samples were collected. The haematological indices of the collected blood samples were analyzed using standard methods. Haemoglobin (HBG) concentration was determined using the cyanomethaemoglobin method (Blaxhall and Daisley, 1973), total erythrocyte and leucocytes (RBC and WBC) counts were done using an improved Neubouer haemocytometer according to techniques described by Jain (1993). Hematocrit (HCT) level in the blood was determined using the microhaematocrit centrifuge technique (Dacie and Lewiz, 1984). The mean cellular volume (MCV), mean cellular haemoglobin (MCH) and mean cellular haemoglobin concentration (MCHC) were evaluated from the results of RBC, WBC, Hb and HCT according to the methods given by Baker and Silverton (1982). Red cell distribution width (RDW), platelet count (PLT), procalcitonin (PCT), mean platelet volume (MPV) and Platelet Distribution Width (PDW) were determined according to according to Drew et al. (2004). The differential leucocytes count (lymphocytes, monocytes, neutrophil, eosinophil and basophil) was conducted as described by Coles (1986).

Feed conversion ratio

Feed conversion ratio was calculated as the required amounts of DM, TDN, and DCP per kilogram of weight gain as follows:

DM (kg/kg gain) = DM intake / ADG

TDN (kg/kg gain) = TDN intake / ADG

DCP (kg/kg gain) = DCP intake / ADG

Where, ADG is average daily gain

Economical efficiency

Economical efficiency parameters included feed

cost, cost of gain, revenue of gain, net revenue and economic efficiency have been calculated according to the prices of year2020. Prices per kg have been 8 LE for starter, 4.5 LE for berseem hay, 8.5 LEfor milk, 1500 LE for propolis, 65 LE for ethanol and 90 LE for weight gain. Relative net revenue and economic efficiency was calculated as follows:

Relative net revenue %= net revenue of G2 or G3 * 100/net revenue of G1.

Economic efficiency %= net revenue * 100/feed cost.

Statistical analysis

The data were analyzed using general linear models procedure adapted by IBM SPSS Statistics (2020) for user's guide with one-way ANOVA. Significant differences in the mean values among dietary treatments were analyzed by Duncan's tests within SPSS program set at the level of significance P<0.05.

RESULTS

Nutrients digestibility and feeding values

The influences of dietary supplementation with propolis on nutrient digestibility coefficients and feeding values of suckling calves diets are shown in Table (3). There were significant (P<0.05) difference in digestibility coefficients of DM, OM, CP, CF, EE and NFE among different groups in favor of the propolis groups. The best values were recorded to G3 supplemented with 1 g propolis followed by G2 received 0.5 g propolis, whereas unsupplemented G1 had the poorest values. Feeding values as TDN and DCP were significantly (P<0.05) elevated with propolis supplemented in G2 and G3 by 2.22, 4.37% TDN and 0.39 and 0.78% DCP, respectively, compared to the control ones (G1).

Rumen liquor parameters:

The effect of propolis supplementation on rumen fermentation activity of suckling calves is presented in Table 4. The pH value and ammonia-N and butyrate concentrations decreased significantly (P<0.05) with increasing the level of propolis, which the lowest values were detected in G3 followed by G2, whereas the highest values were in G1. However, total VFA, acetate and propionate concentrations were higher significantly (P<0.05) with high propolis level in G3 followed by low propolis level in G2, while unsupplemented ones G1 had the lower concentrations.

Blood serum biochemical

The metabolic profile of blood serum biochemical of suckling calves has been found to benefit from propolis supplementation as presented in Table 4. G3 (1 g propolis) showed significantly (P<0.05) the higher concentrations of serum total protein, globulin and glucose followed by G2 (0.5 g propolis), whereas G1 had the lower concentrations. Whereas, serum albumin concentration in G3 was higher significantly (P<0.05) compared to G1 with insignificant differences with G2. In addition, propolis supplementation as well as with increasing propolis level improved the kidney and liver functions, which the concentrations of urea-N, creatinine, AST and ALT were significantly (P<0.05) lower in G3 followed by G2, but G1 had the higher values.

Table 3: Effect of propolis supplementation on nutrients digestibility and feeding values of the experimental diets.

Items	Treatments			SEM	*p*-value
	G1	G2	G3		
Digestibility coefficients %					
DM	68.02^{c}	70.14^{b}	72.41^{a}	0.68	0.004
OM	69.57^{c}	71.58^{b}	73.98^{a}	0.69	0.004
CP	68.21^{c}	70.33^{b}	72.43^{a}	0.66	0.008
CF	59.56^{c}	61.91^{b}	63.92^{a}	0.69	0.005
EE	74.15^{c}	76.34^{b}	78.41^{a}	0.68	0.007
NFE	72.15^{c}	74.23^{b}	76.29^{a}	0.66	0.006
Nutritive values %					
TDN	73.66^{c}	75.88^{b}	78.03^{a}	0.70	0.006
DCP	12.50^{c}	12.89^{b}	13.28^{a}	0.12	0.008

^{a,b,c} Values in the same row with different superscripts differ significantly at P<0.05.

- G1: control, G2: 0.5 g propolis/calf/day, G3: 1 g propolis/calf/day.

- DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fiber, EE: ether extract, NFE: nitrogen free extract, TDN: total digestible nutrients, DCP: digestible crude protein, SEM: standard error of mean.

Immunity response

Blood serum immunity of suckling calves as affected by propolis supplementation is shown in Table 4. Concentrations of immunoglobulins (IgG, IgA and IgM) increased with supplemental propolis with significant (P<0.05) differences among the different groups. High supplemental propolis level in G3 (1 g) had the highest immunoglobulins (IgG, IgA and IgM) concentrations followed by low level in G2 (0.5 g), while the control ones G1 had the lowest concentrations (P<0.05).

Blood haematological profile

Data for haematological parameters in blood of suckling calves are shown in Table 5. Prppolis supplement as well as increasing its level led to increase in all blood haematological parameters with significant (P<0.05) differences among the different groups. Haemoglobin concentration (HGB) and Haematocrit (packedcell volume) percentage (PCV) increased significantly (P<0.05) with propolis supplements, and G3 showed the higher values followed by G2, but the lower values were done in G1. Also, G3 (1 g propolis) recorded the highest counts of leucocytes (WBC's) and their differential (lymphocytes, monocytes, neutrophil, eosinophil and basophil) followed by G2 (0.5 g propolis), whereas the lowest values were detected in unsupplemented G1 ones. Erythrocyte (RBC's) and erythrocyte indices including mean cellular volume (MCV), mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC) and red cell distribution width (RDW) in blood of calves were significant differ (P<0.05) among the different groups and increased with propolis supplement. Erythrocyte and erythrocyte indices were significantly (P<0.05) higher in G3 followed by G2, while the G1 control ones had the lower values. Platelet indices expressed as platelet count (PLT), procalcitonin (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) in blood of calves increased significantly (P<0.05) with propolis supplements. There were significant differences (P<0.05) in the values of PLT,

Table 4: Effect of propolis supplementation on rumen liquor parameters, blood serum biochemical and immunity response of suckling calves in different groups

Item	Experimental groups			SEM	*p*-value
	G1	G2	G3		
Rumen liquor parameters					
pH value	6.62^{a}	6.45^{b}	6.30^{c}	0.05	0.004
NH3-N (mg/dL)	14.23^{a}	12.60^{b}	10.82^{c}	0.54	0.006
TVFAs (mM)	102.2^{c}	126.8^{b}	145.3^{a}	6.76	0.003
Acetate (mM)	47.3^{c}	60.9^{b}	72.1^{a}	3.60	0.001
Propionate (mM)	29.5^{c}	41.4^{b}	50.7^{a}	3.08	0.001
Butyrate (mM)	20.5^{c}	17.8^{b}	15.5^{a}	0.73	0.001
Serum biochemical					
Total protein (g/dL)	6.60^{c}	6.82^{b}	7.05^{a}	0.07	0.004
Albumin (g/dL)	3.15^{b}	3.23^{ab}	3.32^{a}	0.03	0.017
Globulin (g/dL)	3.45^{c}	3.59^{b}	3.73^{a}	0.04	0.001
Glucose (mg/dL)	63.68^{c}	65.92^{b}	68.25^{a}	0.73	0.007
Creatinine (mg/dL)	1.18^{a}	1.04^{b}	0.87^{c}	0.05	0.005
Urea-N (mg/dL)	16.84^{a}	14.55^{b}	12.21^{c}	0.74	0.006
AST (U/L)	49.20^{a}	45.65^{b}	41.84^{c}	1.15	0.003
ALT (U/L)	23.75^{a}	21.15^{b}	18.70^{c}	0.78	0.002
Immunity response					
IgG (mg/dL)	20.91^{c}	23.29^{b}	26.14^{a}	0.83	0.005
IgM (mg/dL)	2.37^{c}	2.61^{b}	2.87^{a}	0.08	0.003
IgA (mg/dL)	0.67^{c}	0.74^{b}	0.81^{a}	0.02	0.003

^{a,b,c} Values in the same row with different superscripts differ significantly at P<0.05.

- G1: control, G2: 0.5 g propolis/calf/day, G3: 1 g propolis/calf/day.

- TVFAs: total volatile fatty acids, NH3-N: ammonia nitrogen, AST: aspartate transaminase, ALT: alanine transaminase, IgG: immunoglobulin G, IgM: immunoglobulin M, IgA: immunoglobulin A, SEM: standard error of mean.

PCT, MPV and PDW among the different groups. G3 recorded significantly (P<0.05) the highest values of PLT, PCT, MPV and PDW followed by G2, while the lowest values were in G1.

Live body weight and weight Gain

Results of Table (6), showed that the birth weight was nearly equal for the different groups without any significant differences. Whereas, weaning weight, total weight gain and average daily gain were significantly higher (P<0.05) in G3 (1 g propolis) followed by G2 (0.5 g propolis), while the lower values were in control ones (G1). Weaning weight was increased significantly (P<0.05) by about 6.86 and 13.93%, respectively in calves fed diets supplemented with propolis in G2 and G3 compared with fed control diets in G1. Compared with the control group, there was a significant improvement (P<0.05) in body weight gain by 8.80 and 17.86% in G2 and G3, respectively compared with a control ones. Furthermore, average daily gain was significantly (P<0.05) increased by 8.70 and 17.84% in treated calves in G2 and G3, respectively compared with a control ones.

Feed intake

Daily feed intake in terms of DM, TDN, CP and DCP as affected by propolis supplementation during suckling period are shown in Table (6). The intakes of DM and CP were nearly the same for the different groups with insignificant differences (P>0.05). While, intakes of TDN and DCP in G3 were higher significantly (P<0.05) compared to G1 with insignificant (P>0.05) differences with G2. Feed intake by G2 and G3 increased by 69 and 104 g TDN and 5 and 11 g DCP compared with G1, respectively.

Feed conversion ratio

Feed conversion ratio for suckling Friesian calves as affected by propolis additive are shown in Table (6). Totally, propolis additive into the diets of calves led to significant (P<0.05) improvements in the feed conversion ratio expressed as DM, TDN, CP and DCP required per one kg live weight gain. Control group (G1) recorded significantly (P<0.05) the highest amounts (poorest one) of DM, TDN, CP and DCP/kg gain. Inversely, the feed conversion ratio was significantly (P<0.05) the best with G3 in comparison with the control (G1), while G2 differ significantly

Table 5: Effect of propolis supplementation on blood haematological profile of suckling calves in different groups.

Items	Experimental groups			SEM	*p*-value
	G1	G2	G3		
HGB (g/dl)	9.10^{c}	10.65^{b}	12.90^{a}	0.65	0.004
PCV (%)	26.60^{c}	29.40^{b}	32.75^{a}	0.98	0.003
Leucocyte and leucocyte differential					
Leucocyte (x10^{3}/µl)	10.65^{c}	12.30^{b}	14..60^{a}	0.61	0.001
Lymphocytes (x10^{3}/µl)	4.85^{c}	5.64^{b}	6.93^{a}	0.33	0.001
Monocytes (x10^{3}/µl)	0.80^{c}	0.90^{b}	1.01^{a}	0.03	0.005
Neutrophil (x10^{3}/µl)	4.41^{c}	5.05^{b}	5.84^{a}	0.23	0.004
Eosinophil (x10^{3}/µl)	0.49^{c}	0.56^{b}	0.63^{a}	0.02	0.004
Basophil (x10^{3}/µl)	0.11^{c}	0.15^{b}	0.20^{a}	0.01	0.001
Erythrocyte and erythrocyte indices					
Erythrocyte (x10^{6}/µl)	6.20^{c}	6.85^{b}	7.60^{a}	0.22	0.004
MCV (fl)	32.96^{c}	36.72^{b}	41.21^{a}	1.31	0.005
MCH (pg)	12.79^{c}	14.25^{b}	15.99^{a}	0.51	0.004
MCHC (g/dl)	28.78^{c}	32.06^{b}	35.98^{a}	1.14	0.005
RDW (%)	16.48^{c}	18.35^{b}	20.60^{a}	0.65	0.003
Platelet Indices					
PLT (x10^{3}/µl)	288.19^{c}	320.99^{b}	360.24^{a}	11.24	0.004
PCT (µg/l)	0.162^{c}	0.180^{b}	0.197^{a}	0.005	0.006
MPV (fl)	4.50^{c}	4.95^{b}	5.45^{a}	0.15	0.004
PDW (fl)	52.40^{c}	58.36^{b}	65.50^{a}	2.08	0.005

^{a,b,c} Values in the same row with different superscripts differ significantly at P<0.05.

- G1: control, G2: 0.5 g propolis/calf/day, G3: 1 g propolis/calf/day.

- HGB: Haemoglobin, PCV: packed cell volume (haematocrit), MCV: mean cellular volume, MCH: mean cellular haemoglobin, MCHC: mean cellular haemoglobin concentration: RDW: red cell distribution width, PLT: platelet count, PCT: procalcitonin, MPV: mean platelet volume, PDW: platelet distribution width.

(P<0.05) in DM and CP/kg gain and insignificantly (P>0.05) in TDN and DCP/kg gain with both G1 and G3.

Economic efficiency

The effect of BP and BS additives on economic efficiency of Friesian calves are presented in Table (6). Cost of feed as LE/day was higher significantly (P<0.05), but cost of gain as LE/kg gain was lower significantly (P<0.05) in G3 compared to G1, whereas in G2 insignificantly (P>0.05) with both G1 and G3. Meantime, differences in revenue of gain, net revenue, relative net revenue and economic efficiency among the different groups were significant (P<0.05). Which, G3 recorded significantly (P<0.05) the highest values of revenue of gain, net revenue, relative net revenue and economic efficiency followed by G2, but G1 had the lowest values.

DISCUSSION

The improvement in the digestibility coefficient of OM and CP may be due to that propolis has the ability to improve nutrient digestibility and absorption which stimulate the activities of saccharase, amylase and phosphatase (Marieke et al., 2005). Also, propolis contains benzoic and 4- hidoxibenzoic acid, which may improve the digestibility of such nutrients as protein and ash (Seven, 2008 and Seven et al., 2012). Additionally, Prado et al., (2010) observed improved DM, OM, NDF, TCHO and TDN digestibility coefficient values in buffaloes supplemented with propolis. Morsy et al. (2021) found that propolis supplementation increased (P=0.02) organic matter and crud protein digestibility compared to the control. Moreover, propolis can improve the digestion rate of fibrous and non-fibrous carbohydrates, and inhibit gas production in vitro by ruminal microorganisms (Stradiotti Ju-

Table 6: Effect of propolis supplementation on growth performance, feed intake, feed conversion ratio and economic efficiency.

Items	Experimental groups			SEM	*p*-value
	G1	G2	G3		
Growth performance					
Birth weight (kg)	25.67	26.17	26.50	0.53	0.830
Weaning weight (kg)	95.67^{c}	102.33^{b}	109.00^{a}	1.56	0.002
Total weight gain (kg)	70.00^{c}	76.16^{b}	82.50^{a}	1.45	0.003
Average daily gain (kg)	0.667^{c}	0.725^{b}	0.786^{a}	0.013	0.003
ADG improvement (%)	00.00^{c}	8.70^{b}	17.84^{a}	1.97	0.003
Feed intake (kg/calf/day)					
DMI	1.413	1.415	1.417	0.004	0.935
TDNI	1.04^{b}	1.073^{ab}	1.106^{a}	0.007	0.045
CPI	0.259	0.259	0.259	0.001	0.986
DCPI	0.177^{b}	0.182^{ab}	0.188^{a}	0.001	0.047
Feed conversion ratio (kg/kg gain)					
DM	2.12^{a}	1.91^{b}	1.85^{c}	0.04	0.001
TDN	1.56^{a}	1.48^{ab}	1.41^{b}	0.02	0.003
CP	0.388^{a}	0.357^{b}	0.330^{c}	0.01	0.001
DCP	0.265^{a}	0.252^{ab}	0.240^{b}	0.004	0.004
Economic efficiency					
Cost of feed (LE/day)	33.80^{b}	34.62^{ab}	35.43^{a}	1.61	0.015
Cost of gain (LE/kg gain)	50.80^{a}	47.44^{ab}	45.25^{b}	0.83	0.011
Revenue of gain (LE)	60.00^{c}	65.28^{b}	70.72^{a}	1.24	0.001
Net revenue (LE/day)	26.20^{c}	30.66^{b}	35.29 ^{a}	1.11	0.001
Relative net revenue %	00.00^{c}	17.55^{b}	35.27^{a}	3.92	0.001
Economic efficiency %	77.51^{c}	88.56^{b}	99.59^{a}	2.86	0.003

^{a,b,c} Values in the same row with different superscripts differ significantly at P<0.05.
- G1: control, G2: 0.5 g propolis/calf/day, G3: 1 g propolis/calf/day.
- ADG improvement % = ADG of G2 or G3 × 100/ADG of G1
- DMI: dry matter intake; TDNI: total digestible nutrients intake, CPI: crude protein intake; DCPI: digestible crude protein intake. DM: dry matter; TDN: total digestible nutrients, CP: crude protein; DCP: digestible crude protein.
- Prices in Egyptian pound (LE) during 2020 per kg were 8 LE for starter, 4.5 LE for berseem hay, 8.5 LEfor milk, 1500 LE for propolis, 65 LE for ethanol and 90 LE for weight gain.
- Relative net revenue %= net revenue of G2 or G3 * 100/net revenue of G1.
- Economic efficiency %= net revenue * 100/feed cost.

nior et al., 2004). Similarly, supplementing the diet of crossbred buffaloes with the chrysin equivalent of propolis enhanced nutrient digestibility (Costa Jr et al., 2012).

According to Stradiotti Júnior et al. (2004), propolis has a bacteriostatic action on gram-positive and some gram-negative bacteria, supposedly by modifying the bioenergetic state of the bacterial membrane and inhibiting its mobility, which refers to the functioning of ionophores, which can modify ruminal microbial fermentation. Stradiotti Júnior et al. (2004) stated that propolis extract contains natural nutrients contribute to regulating ruminal microbiota, which in diet supplemented with propolis in Holstein steers, the total SCFA was improved by propolis addition. In lactating cows, different concentrations of Brazilian propolis extracts contains different phenolic compounds reduced the ruminal NH3-N (Aguiar et al., 2014). Also, Ozturk et al. (2010) confirmed a reduction in ruminal NH3-N, when ethanolic extract of propolis was supplemented to diet.

For instance, the oral administration of ethanolic extract of Brazilian red propolis to Santa Inês ewes (3 g/ewe/day) during the flushing period increased concentrations of globulin, proteins, and blood glucose, while decreased triglycerides, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase (Morsy et al., 2016). Also, Hashem et al. (2013) found that the concentration of blood plasma glucose was enhanced by propolis supplementation. Denli et al. (2005) illustrated that the supplementation of propolis in diet caused a reduction in liver enzymes activities. Based on the results of Abd-Allah and Daghash (2019), propolis may have hepatoprotective effects or play a role in the prevention of liver injury. Hafez et al. (1983) and Abdel-Hamid et al. (1999) stated that ALT and AST secretions are accelerators to the rate of metabolism and protein biosynthesis in order to meet the increased requirements to synthesis new tissues.

Propolis is one of the natural substances which have positive immunomodulatory effects on animals, which mentioned the general improvement of the animals health and immunity that fed propolis (Orsatti et al., 2010). Several factors such as nutrition, dry weather, and health condition can have negative effects on Igs (A, G, M) by decreasing the cell-mediated immune response, which led to degradation efficiency of the thymus, lymph nodes, and spleen in sheep and goats (Abdalla et al., 2015; El-Hawy et al., 2018). Emtnan et al. (2005), who found that Ig concentration improved by supplementation of propolis to Baladi goats might be attributed to unknown compounds and biologically active compounds such as phenol compounds and flavonoids, which positively affect the humoral immune response (Yaghoubi et al., 2007). Furthermore, evidence exists for improved immune response in ruminants receiving propolis extract (Cecere et al., 2021; Slanzon et al., 2019). Sakker and Yang, (2010) study the effect of propolis as a natural feed additive (0.05% of diet) to the pre weaned Hanwoo Korean calves (from birth to 90 days), and found an enhancement of the immunoglobulins (IgG, IgA and IgM) concentrations with propolis.

The HGB concentration and the percentage of PCV are within the normal ranges of cattle being 8-13 g/dl and 23-35%, respectively (UCDAVIS, 2001). Although, the normal range is 24-46%, in my experience the PCV is usually in the upper 20s in adult cattle and slightly higher in calves (*Navarre, 2007).* White blood cells (leukocytes) are an important part of the body's defense against infectious organisms and foreign substances. To defend the body adequately, a sufficient number of white blood cells must receive a message that an infectious organism or foreign substance has invaded the body, get to where they are needed, and then kill and digest the harmful organism or substance (Dale, 2023). The obtained values of counts of leucocyte, lymphocytes, monocytes, neutrophil, eosinophil and basophil are within the normal values of cattle being 5.8-12.6, 3.7-7.6, 0.0-0.9, 2.3-6.8, 0.0-1.2 and 0.0-0.3x10^3/µl, respectively (UCDAVIS, 2001). The oral administration of ethanolic extract of Brazilian red propolis to Santa Ines ewes (3 g/ewe/day) during the flushing period increased total leukocytes (Morsy et al., 2013). The count of RBCs in whole blood obtained here in blood obtained in this study are within the normal range obtained by Jezek et al. (2011) being 5-11 x10^6 / µl. Also, the values of erythrocyte indices in blood of this study are within the normal range obtained by UCDAVIS (2001) being 30-50 fl for MCV, 12-18 pg for MCH, 26-39 g/dl for MCHC and 14-20% for RDW. Hashem et al., (2013) found that the dietary ethanolic extract of the Egyptian propolis enhanced hematopoiesis including the number of red blood cells, hematocrite value and hemoglobin concentration. The total WBCs and RBCs were increased while MCV was decreased when New Zealand white bucks fed on 150 mg/kg propolis (Elshama et al., 2015). In Hanwoo calves, the propolis powder (0.05%) supplemented with concentrate diets slightly increased WBCs (Sarker and Yang, 2010). Platelet in-

dices values in blood obtained in this study are within the normal ranges obtained by UCDAVIS (2001) being 233-690 x103 /µl for PLT, 0.15-0.40 µg/l for PCT, 4.5-7.6 fl for MPV and 50-80% for PDW. Platelets, or thrombocytes, are tiny disc-shaped cells which help prevent abnormal or excessive bleeding by forming clots. A deficiency of platelets can cause bleeding of the mucous membranes or other tissues, such as the skin. They are much smaller in size than other blood cells and they group together to form clumps, or a plug, in the hole of a vessel to stop bleeding (Sarker et al., 2010). Platelets play an important role in hemostasis (Bacha and Bacha, 2000; Despopouols and Silbernagl, 2003). This study mentioned the general improvement of the animal's health with propolis supplementation.

The beneficial effect of propolis on growth performance in suckling calves may be act as an antioxidant (Cottica et al., 2011) and antimicrobial (Bankova et al., 2000) leading to decreased growth of pathogenic bacteria responsible for growth depression resulted in better intestinal health and improved digestion and absorption. The increases in body weight and body weight gain in treated calves agree with Corlateanu (1976) found that calves treated with propolis have better growth performance than calves in control group. Besides, Hegazi and Abd El-Hady (1996) reported that propolis has positive effect on growth of livestock. Yucel et al. (2015) illustrated that propolis had significant effect on female calves for total and daily weight gain. Abd-Allah and Daghash (2019) stated that the use of propolis as a feed additive in Egyptian Buffalo calves had a positive effect on the growth and live weight gain of calves. The weaning weights of the calves fed control ration plus 50 mg propolis/head/day was 7.7 kg greater than the control group. Simmental calves and lasted 21 days after birth, it was reported that 4 ml of PEE (Propolis ethanol extract) group had 213,9 g greater average daily live weight gain compared to the control group (Kupczyński et al., 2012).

The amount of feed consumed by an animal in a certain period of time is an important factor. The optimum amount of feed consumes every day the greater will be the opportunity for increasing its daily production, which an increase in production obtained by higher feed intakes (McDonald et al., 1995). The solid residue from alcoholic extraction of brown propolis (RBP) treatments did not influence DM intake (Ítavo et al., 2019). In addition, studies have shown that propolis supplementation does not affect dry matter intake (Stradiotti Júnior et al., 2004; Stelzer et al., 2009). Mohsen et al. (2017) found that TDN and DCP intakes by suckling calves increased with bee pollen additive.

Feed conversion ratio was lower in animals fed diet with 5 g of solid residue from alcoholic extraction of brown propolis /kg DM in comparison to animals fed only basal diet (Ítavo et al., 2019). Hashem et al. (2017) who found that feed conversion was improved and live body weights and weight gain of rabbits were higher in the groups received diets contained propolis at 150 and 300 mg/kg compared to the control. Also, Attia et al. (2015) reported that all natural growth promoters including propolis improved feed conversion of rabbit does. Additionally, propolis is an alternative source to antibiotics in diet (Itavo et al., 2011), which may improve feed efficiency of animals (Sarker and Yang, 2010). Also, propolis has antimicrobial, anti-inflammatory, and immunomodulatory properties (Daneshmand et al., 2015) which allowing for better utilization of nutrients. Mohsen et al. (2017) found that feed conversion ratio by suckling calves improved with bee pollen additive.

Similar results of economic efficiency were found by El-Neney and El-Kholy (2014) who showed higher economic efficiency when added 400 mg BP to the diet of rabbits than that of control group. Mohsen et al. (2017) found that economic efficiency parameters of suckling calves improved with bee pollen additive.

CONCLUSION

From the present results, it could be concluded that propolis supplements at the level of 1 g/calf/day for the diet of suckling Friesian calves led to significant improvements on digestibility, feeding values, rumen fermentation activity, blood biochemical, immunity response, haematological parameters, growth performance, feed intake, feed conversion ratio and economic efficiency.

CONFLICT OF INTEREST

None declared

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