Research Article

Administration of Bee Pollen, *Nigella sativa* Oil, and their Combination as a Strategy for Improving Growth Performance, Immunity, and Health Status of Newborn Friesian Calves During the Suckling Period

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Abstract | This study aimed to evaluate the effect of dietary supplementation of bee pollen (BP), black seed oil (BS, *Nigella sativa*), or their combination on growth performance, immunity, and health status of females of newborn Friesian calves. A total of 20 newborn calves (37.51±0.92 kg LBW) were divided into 4 groups. During the suckling period, animals in the 1st group were fed a starter without any addition (G1, control). Animals in G2, G3, and G4 were fed the same starter supplemented with BP (10g/kg), BS oil (10g/kg), or their combination (5g Bp+5g BS oil), respectively. Results showed that all immunoglobulin types (IgG, IgM, and IgA), total protein, albumin, globulin, glucose, total lipids, total cholesterol, and total antioxidant capacity in blood serum, and RBCs, WBCs, Hb, and PCV increased ($P<0.05$) in treated than in the control calves, being the highest with the combination treatment. Urea-N and creatinine concentrations, and AST and ALT activities decreased ($P<0.05$) in treated compared with the control calves, with the lowest values for the combination treatment. The treated calves were heavier with higher weight gains than the control one, being the highest with the combination treatment. In conclusion, addition of a combination of 5g bee pollen and 5g black seeds oil/kg in the starter during the sucking period improves live body weight, weight gain, immunity, kidney and liver functions, and antioxidant status of female newborn Friesian calves. This administration is considered a good strategy for raising dairy calves in dairy farms.

Keywords | Friesian calves, Bee pollen, Black seeds, Immunoglobulins, Hematology, Blood constituents.

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INTRODUCTION

Feed additives are important materials that can improve the efficiency of feed utilization and increase animal productivity. Modern animal production requires the use of safe and effective additives to stimulate feed consumption and destroy harmful microorganisms of the diet in addition to be used as rumen manipulators to increase animal productivity (Ahmed et al., 2009). The dietary fiber content is an important factor in reducing the digestion of fat and impeding the absorption of cholesterol in the intestine. Also, the volatile fatty acids reduce the cholesterol synthesis and alters lipoprotein metabolism (Cara et al., 1992). The induces of cellular damage is affected by the presence of reactive oxygen species (ROS) and antioxidants which present in low concentration can prevent or reduce the extent of oxidative destruction (Halliwell, 1990).

Bee pollen (BP) is produced from apiculture and contains substances with nutritional valuable such as protein, lipid, carbohydrates (glucose, fructose, and sucrose), vitamins, minerals, enzymes, or co-enzymes, essential amino acids, poly unsaturated fatty acids (linolenic, palmitic, and linoleic acids), and carotenoids (Aliyzicioglu et al., 2005; Izuta et al., 2009; Xu et al., 2009). Increasing level of BP treatment increases the immune response by increasing count of leukocytes, particularly, lymphocyte count, and improves most hematological variables and hepatic functions (El-Neney et al., 2014).

Several plant species extracts become popular used in many pharmaceutical and food processing applications according to its content of essential oils (Cowan, 1999). Different plants such cereals and medicinal herbs contain molecules having free radical-scavenging characteristics such as phenolic, nitrogen compounds, and terpenoids (Cai, et al., 2003; El-Sisy et al., 2018). *Nigella sativa* (NS) has pharmacologically positive effects against diabetic, cancer, immunomodulation, microbes, inflammation, and oxidant. It also has a protective effect on liver, kidney, and gastrointestinal tract (Desai et al., 2015). Thymoquinone in essential oil of NS has potent antioxidant action (Butt and Sultan, 2010) and the usage of NS oil showed impacts on maintenance of blood homeostasis, improvement of the general health status, and prevention of health problems in Friesian calves during the suckling period (Abd El-Hafeez et al., 2014).

Newborn calves being the foundation of replacement animals that can be an important asset for the farmer to generate the income of dairy farms (Anjum et al., 2012). The first few weeks of age is the most critical interval in newborn calf's life, during this period they are exposed to several health and welfare problems which may be led to increase their mortality rate to about 10% in dairy calves (Raboisson et al., 2013). The first month of the buffalo calf's life is very critical period and the calf mortality is high by 20% during this period compared to remaining animal life (Sreedhar et al., 2010). During the first fourteen days of newborn calf's life they were exposed to several stresses because they suffer from change in environmental condition including diet, handling stress, and low immunity, which increases diarrhea infection (Quezada-Mendoza et al., 2011), morbidity and mortality rates (Ribeiro et al., 2009). At the first stage of calf's life, it essentially treats as monogastric animal and early weaning impairs growth performance and decrease their resistance to bacterial infections (Söderholm and Perdue, 2001). Many researchers indicated a relationship between pre-weaning average daily gain (ADG) and the long-term productivity of dairy calves, this finding emphasizes the importance of early nutrition (Soberon et al., 2012; Soberon and Van Amburgh, 2013).

The aim of the present study was to evaluate the effect of oral administration of newborn Friesian female calves with bee pollen, *Nigella sativa* (black seeds), or both on their growth performance, immune and blood constituents.

MATERIALS AND METHODS

This study was conducted within the framework of scientific cooperation between Animal Production Department, Faculty of Agriculture, Tanta University and Animal Production Research Institute, Agricultural Research Center, Egypt, and was streamlined with animal ethical standards (No AY 2019-2020/Session 6/2020.01.13).

Animals and feeding system

A total of 20 newborn female Friesian calves were used in this study. After calving, newborn calves were immediately fed colostrum from their mothers using a feeding bottle at a level from 1.5-2 kg/calf, then calves suckled directly their mothers during the 1st three days after calving. On Day 4 post-suckling the colostrum, calves were artificially suckled whole milk of their dams (10% of LBW) at 6 a.m. and 6 p.m. for 6 weeks. The milk amount was weekly decreased by about 1% of LBW up to the age at weaning (35 weeks of age). Starting from the 3rd week of age, calves were fed *ad libitum* on starter, berseem hay, and rice straw beside the amount of whole milk. Table 1 shows the chemical analyses of starter, berseem hay, and rice straw. Drinking water was available as a free choice to animals.

The experimental plan

At the beginning of the experimental period, 20 newborn calves (average LBW of 37.92±0.63 kg at 1st week of age) were divided into four groups, according to their LBW (5 animals in each). All animals were kept under the same feeding system, as mentioned above, but differed in dietary supplementation. During the suckling period (from one up to15 weeks of age), the control group (G1) was fed starter without additives. G2, G3, and G4 were fed the same starter supplemented with 10g commercial Egyptian bee pollen (BP), 10 g black seed oil (BS) (Abdel-Raouf et al., 2018), or a combination (5g BP + 5g BS oil) per kg, respectively.

Evaluation of growth performance, morbidity, and mortality

Initial live body weight at birth and weaning was individually recorded for calves in each group, then total and average weight gains were calculated during the suckling

Table 1: Chemical composition (on dry matter basis) of starter, berseem hay, and rice straw fed to Friesian calves in all experimental groups.

Feedstuff	DM%	CP%	CF%	EE%	NFE%	OM%	Ash%
Starter	88.98	19.24	5.63	2.97	64.71	92.55	7.45
Berseem hay	87.39	13.22	27.18	0.67	46.12	87.19	12.81
Rice straw	88.25	2.68	38.72	1.32	39.15	81.87	18.13

DM: Dry matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen free extract.

Table 2: Serum immunoglobulins (IgG, IgM, and IgA) concentrations in Friesian calves in the experimental groups. (Mean ± SE)

Immunoglobulin type	Control group	Treatment group		
	G1	G2	G3	G4
IgG (g/L)	11.95±0.15^{c}	13.47±0.18^{b}	13.35±0.27^{b}	15.70±0.13^{a}
IgM (g/L)	1.73±0.07^{c}	2.69±0.07^{b}	2.63±0.08^{b}	3.37±0.08^{a}
IgA (g/L)	0.55±0.03^{c}	0.73±0.03^{b}	0.71±0.04^{b}	0.93±0.03^{a}

$^{a, b, and\ c}$: Significant group differences at P<0.05.
G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter.

period. During the experimental period, no mortality cases were recorded, and no diseases (pneumonia, diarrhea, and enteritis) were detected.

Blood samples

Blood samples were collected from all newborn calves at weaning via the jugular venipuncture. Blood samples were divided into two portions, the first was used in determining the hematological parameters while the second portion was centrifuged at 3000 rpm for 20 min to obtain the serum samples which were stored at -20°C till analysis.

Analytical procedures

Hemoglobin concentration and packed cell volume (PCV%) as hematological variables in the whole blood of calves, were directly determined using Mission® Plus kit (C132-3031, USA) according to Henry (2001). Count of red (RBCs) and white (WBCs) blood cells were recorded using a hemocytometer. Concentrations of total proteins (Henry, 1964), albumin (Doumas et al., 1971), glucose (Trinder, 1969), total lipids (Zöllner and Kirsch, 1962), total cholesterol (Richmond, 1973), and kidney function markers (creatinine and urea) were determined in blood serum according to Henry (1974) and Patton and Crouch (1977), respectively.

The concentration of globulin was computed by subtraction of albumin from total protein concentration. The liver function enzymes, including serum AST and ALT activities, were determined according to Reitman and Frankel (1957). Serum concentration of immunoglobulin (G, M, and A) was determined according to Killingsworth and Savory (1972). Total antioxidant capacity was determined as the methods described by Koracevic et al. (2001).

Statistical analysis

Statistical analysis was performed by SPSS program (IBM SPSS, 2017) using one-way ANOVA analysis to study the effect of treatment. The significant differences were performed by Duncan Multiple Range Test (Duncan, 1955) at a level of P<0.05. The rates of change for each parameter in each treatment group relative to the control group are shown in the figures.

RESULTS AND DISCUSSION

Immune response

Different types of serum immunoglobulins (IgG, IgM, and IgA) concentrations in calves at weaning were affected significantly by treatment (Table 2). Results showed that all types of serum immunoglobulins were significantly (P<0.05) increased in treatment groups (G2, G3, and G4) compared with the control (G1). The combination treatment (G4) showed significantly (P<0.05) the highest values, but the differences in immunoglobulins between G2 and G3 were not significant.

Results cleared that the highest rate of increase in serum immunoglobulins of calves in G2, G3, and G4 relative to G1 (control) was recorded for IgM level and the lowest for IgG level. In this respect, the combination treatment showed the highest rate of increase in all immunoglobulin types, being the highest for IgM (Fig. 1). These results indicated similarity in the beneficial impacts of either BP or BS alone, but their synergetic effect was recorded on the immune response of calves at weaning.

Similar to our results in calves, the dietary addition of BS oil during the suckling period caused a marked increase in blood immunoglobulin concentration of buffalo calves

Table 3: Hematological parameters of Friesian calves in the experimental groups at weaning. (Mean± SE)

Item	Control group	Treatment group		
	G1	G2	G3	G4
RBCs (x10⁶/mm³)	8.19±0.13[c]	9.20±0.15[b]	9.13±0.19[b]	10.18±0.22[a]
WBCs (x10³/mm³)	9.59±0.16[c]	10.56±0.16[b]	10.48±0.23[b]	11.67±0.16[a]
Hb (g/dL)	10.23±0.15[c]	11.40±0.16[b]	11.41±0.29[b]	12.72±0.19[a]
PCV (%)	30.01±0.34[c]	35.23±0.28[b]	35.20±0.51[b]	38.70±0.35[a]

[a, b, and c]: Significant group differences at P<0.05.

G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter.

RBCs: Red blood cells. WBCs: White blood cells. Hb: Hemoglobin. PCV: Packed cell volume.

Table 4: Concentration of total proteins, albumin, globulin, and glucose in blood serum of Friesian calves in the experimental groups. (Mean ± SE)

Item	Control group G1	Treatment group		
		G2	G3	G4
Total protein (mg/dL)	5.59±0.08[c]	6.91±0.11[b]	6.88±0.14[b]	8.31±0.17[a]
Albumin (mg/dL)	3.02±0.10[c]	3.71±0.12[b]	3.66±0.07[b]	4.09±0.09[a]
Globulin (mg/dL)	2.57±0.11[c]	3.19±0.13[b]	3.22±0.11[b]	4.22±0.15[a]
Glucose (mg/dL)	42.76±1.07[c]	47.90±1.01[b]	47.80±0.87[b]	52.71±0.92[a]

[a, b, and c]: Significant group differences at P<0.05.

G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter.

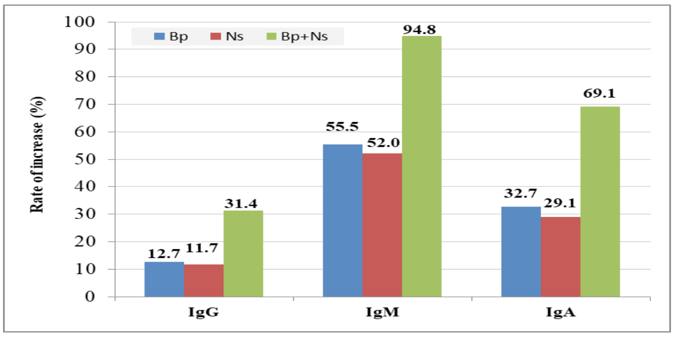

Figure 1: Rate of increase in serum immunoglobulin concentrations at weaning in treatment groups relative to control one. (G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter).

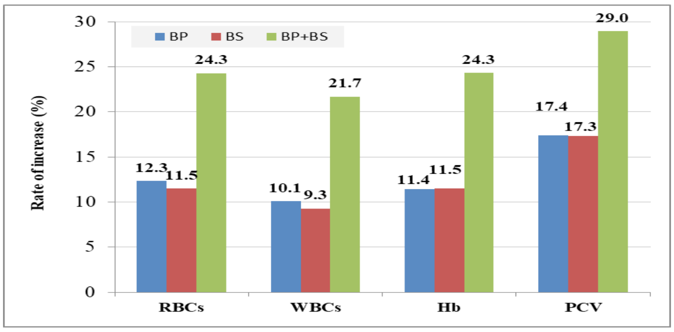

Figure 2: Rate of increase in hematological parameters at weaning of calves in treatment groups relative to control one. (G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter). RBCs: Red blood cells. WBCs: White blood cells. Hb: Hemoglobin. PCV: Packed cell volume.

(Khattab et al., 2011), improved the general health status, and reduced diseases (Abd El-Hafeez et al., 2014). Also, BS had an immune simulative effect and was reported as a dietary additive for increasing viability in fish (Dorucu et al., 2009). In male Wistar rats, serum IF-γ, IL-12, and TNF-αlevels were significantly increased by NS seeds (30 g/kg and 50 g/kg) in the diet as compared to the control diet (Mahmoud et al., 2021). These improvements may be due to the antioxidant properties of NS oil which contains a substance called Nigellone that can modulate and regulate the early activation steps in the acquired immune response of bovine calves (Abd El-Hafeez et al., 2014).

On the other hand, BP has been documented as a significant nutritional additive with several therapeutic actions, as immunomodulatory activity (Khalifa et al., 2021). The inclusion of BP in the broiler diets (1.5%) during the 1st three weeks of age increased IgM concentration (De Oliveira et al., 2013) and this impact may be attributed to that BP was found to protect the health of the intestinal tract of broiler and rabbits (Wang et al., 2007; Attia et al., 2011a, b), respectively. The aforementioned increase in immunoglobulin (IgG, IgM, and IgA) could be credited to B-lymphocytes stimulation. Consequently, the immunoglobulins will elevate initiating and immune response through helper T-cells, cytotoxic T-cells, and C8 T- cells (El-Gaafrawy et. al., 2000; Tizard, 2004) which may lead to perfect immunocompetent calves.

Hematological parameters

The effect of treatments on hematologic variables of calves in the experimental groups at weaning is shown in Table 3. Hematological parameters including RBCs, WBCs, Hb, and PCV were significantly (P<0.05) improved by BP, BS, or their combination as compared to the control, being significantly (P<0.05) higher for the combination treatment than either BP or BS alone. The differences in hematological parameters between G2 and G3 were not significant. Normal hematological parameter values were obtained in our study of Friesian calves during the suckling period (Wafa et al., 2021). The obtained results regarding the hematological parameters of calves cleared that the rate of increase was nearly similar for all hematological parameters studied of calves in treatment groups relative to control. The combination treatment showed the highest rate of increase in all parameters relative to the control versus similar impacts of calves fed diet supplemented with either BP or BS alone (Fig. 2).

In agreement with our results, Khattab et al. (2011) reported an improvement in hematological traits of calves treated with NS oil in comparison with control calves. It is well known that the main role of WBCs is protecting the animal body from diseases induced by bacteria, viruses, and fungi. The obtained increase in the WBC count of calves in the treatment groups may upregulate the immune response of calves by T-cell lymphocytes. T-cells can attack malignant cells or cells infected with viruses, while B-cells can produce and release antibodies. In this context, BP treatment was reported to increase immunity responsiveness by increasing leukocyte counts, mainly lymphocytes, and health status by improving RBCs count, Hb concentration, and PCV value (El-Neney et al., 2014). It was reported that RBCs, Hb, PCV, lymphocytes, and eosinophils were increased significantly in lambs fed the BS diet (Zaki et al., 2015). Also, dietary supplementation of 0.6% BP increased WBCs, heterophils, and lymphocytes in chicks (Farag and El-Rayes, 2016). In chicks, the highest RBCs, Hb, and PCV were obtained by feeding 0.6% PB in the diet (Farag and El-Rayes, 2016).

Protein and carbohydrate metabolites

Protein metabolites namely total protein, albumin, and globulin, as well as carbohydrate metabolite (glucose) in the blood serum of calves at weaning, were affected significantly (P<0.05) by treatment, being higher in treatment groups (G2, G3, and G4) than in G1(control group). Calves in G4 showed significantly (P<0.05) higher values of protein and glucose metabolites in comparison with G2 and G3 (Table 4).

These results indicated positive impact of all treatments, being the highest on albumin and the lowest on glucose concentration. The rate of increase in protein and glucose metabolites was maximized by the combination treatment. However, BP or BS alone showed a lower rate of increase in this regard (Fig. 3).

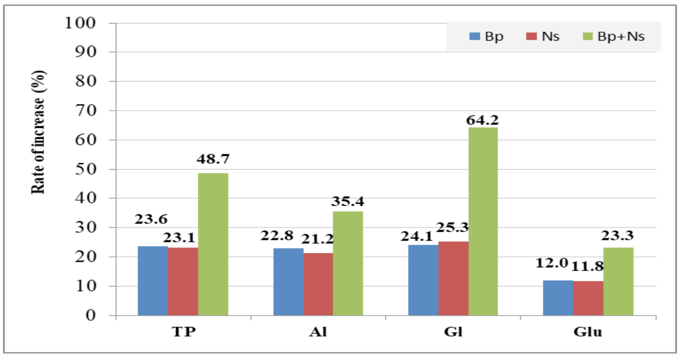

Figure 3: Rate of increase in serum total protein (TP) and carbohydrate metabolites [albumin (Al), globulin (Gl), glucose (Glu)] at weaning of calves in treatment groups relative to control one. (G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter).

Blood total protein and its fractions can be used as indicators to reflect the nutritional status and the physiological changes of animals (Boyd, 1984). Based on these findings, each of PB and BS had a positive impact on protein and carbohydrate metabolism, with a synergetic impact of their combination on animal metabolism. These results are in agreement with other reports. In lambs, feeding on diet supplemented with black cumin seed oil (CUMO) significantly (P<0.001) increased concentrations of total protein and globulin (Obeidat, 2020). Also, concentrations of total protein and globulin were significantly higher by feeding BS-diet as compared to control diet (Zaki et al., 2015).

BP treatment markedly increased plasma total protein and albumin in rabbit male (El-Hanoun et al., 2007; Attia et al., 2011 a,b and 2014). Milk replacer supplemented with 25 g BP increased concentration of total protein and albumin in serum of calves (Tu et al., 2015). In contrast, plant essential oils treatment did not effect on blood glucose level (Chaves et al., 2008; Tassoul and Shaver, 2009; Yang et al., 2010).

Lipid profile and kidney function

The concentration of total lipids and total cholesterol, as indicators of lipid profile, and the concentration of urea and creatinine, as kidney function markers, in the blood serum of calves are presented in Table 5. Total lipids and total cholesterol concentrations significantly (P<0.05) increased by all treatments compared with control, being significantly the highest in G4. On the other hand, the concentration of urea and creatinine were significantly (P<0.05) decreased by all treatments compared with the control, being significantly the lowest in G4.

Table 5: Concentrations of lipid profile and kidney function markers in blood serum of Friesian calves in the experimental groups. (Mean ± SE)

Item	Control group G1	Treatment group		
		G2	G3	G4
Lipid profile				
Total lipids (mg/dL)	468.25±6.44[c]	510.87±4.64[b]	509.18±5.35[b]	550.44±5.16[a]
Total cholesterol (mg/dL)	80.22±1.13[c]	87.06±1.07[b]	86.83±0.91[b]	93.81±0.72[a]
Kidney function markers				
Urea (g/dL)	3.25±0.11[a]	2.66±0.10[b]	2.68±0.11[b]	2.18±0.13[c]
Creatinine (g/dL)	1.67±0.06[a]	1.24±0.05[b]	1.25±0.08[b]	0.96±0.04[c]

[a, b, and c]: Significant group differences at $P<0.05$.

G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter.

Table 6: Activity of liver enzymes and concentration of total antioxidant capacity in blood serum of Friesian calves in the experimental groups. (Mean ± SE)

Item	Control group G1	Treatment group		
		G2	G3	G4
AST (IU/l)	44.73±0.86[a]	38.41±0.66[b]	38.78±0.96[b]	31.86±0.86[c]
ALT (IU/l)	20.86±0.47[a]	16.02±0.48[b]	16.17±0.56[b]	12.44±0.48[c]
TAC (mmol/L)	2.12±0.08[c]	2.64±0.11[b]	2.61±0.10[b]	3.13±0.12[a]

[a, b, and c]: Significant group differences at $P<0.05$.

G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. TAC: Total antioxidant capacity.

Table 7: Live body weight and weight gain of Friesian calves in experimental groups during the suckling period. (Mean ± SE)

Item	Control group G1	Treatment group		
		G2	G3	G4
Initial LBW (kg)	37.64±2.48	37.50±1.86	37.14±1.87	37.74±1.67
LBW at weaning (kg)	77.30±2.10[c]	95.42±1.94[b]	95.52±1.83[b]	113.94±2.34[a]
Total gain (kg)	39.66±2.71[c]	57.92±3.18[b]	58.38±2.85[b]	76.20±0.95[a]
Daily gain (kg/d)	0.38±0.03[c]	0.55±0.03[b]	0.56±0.02[b]	0.73±0.01[a]

[a, b, and c]: Significant group differences at $P<0.05$.

G2: 10g BP/kg starter. G3: 10g BS oil/kg starter. G4: 5g BP + 5g BS oil/kg starter.

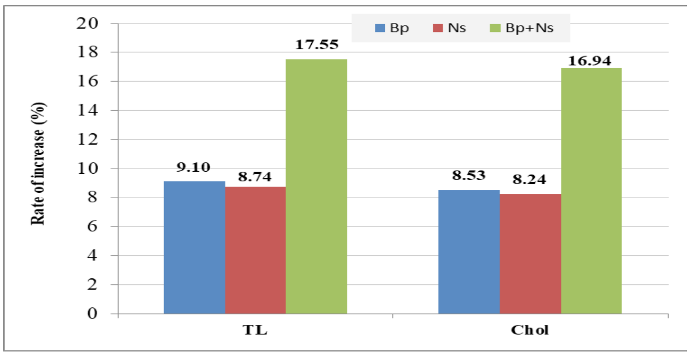

Figure 4: Rate of increase in total lipids (TL) and total cholesterol (Chol) in blood serum at weaning of calves in treatment groups relative to control one.

These results showed pronounced effects of BP, BS, or their combination on increasing lipid profile (Fig. 4) and improving kidney function of calves by lowering the concentration of urea and creatinine (Fig. 5). The combination treatment showed double impacts than BP or BS alone, clearing a synergic effect on lipid profile and kidney function.

In our study, we found normal values of total lipids, total cholesterol, creatinine, and urea in the blood serum of suckling calves (Wafa et al., 2021) and cattle (Kaneko et al., 2008). Our results are in parallel with those reported by several authors, who found that, the diets containing CUMO decreased serum cholesterol levels ($P < 0.001$) of lambs compared with the control diet (Obeidat, 2020). A decrease in serum cholesterol and triglycerides levels in dairy cows fed a basal diet supplemented with 25 mg CUMO per kg LBW was reported (Salem et al., 2019). The essential oils have a hypocholesterolemic effect by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (Srinivasan and Sambaiah, 1991; Lee et al., 2004), the key of the cholesterol synthetic pathway regulation by its active phenolic contents. BP was found to protect the kidneys by lowering the level of urea-N in the blood of rats (Hu et al., 2003). Also, feed black cumin seeds significantly decreased urea-N in blood of lambs (Zaki et al., 2015). In rats, Shalaby et al. (2016) found unchanged urea and creatinine levels in the blood of group fed on BS as compared to control group, indicating normal kidney function.

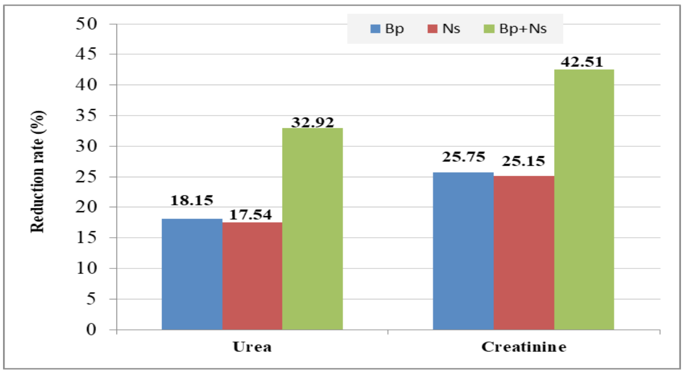

Figure 5: Reduction rate of urea, and creatinine in blood serum at weaning of calves in treatment groups relative to control one.

Liver function and total antioxidant capacity

Data in Table 6 indicated that the activity of enzymes (AST and ALT), as a liver function marker, was significantly decreased ($P<0.05$), while the total antioxidant capacity (TAC) level was significantly decreased in the blood serum of calves by all treatments compared with control. The lowest AST and ALT activities and the highest total antioxidant capacity were recorded in G4.

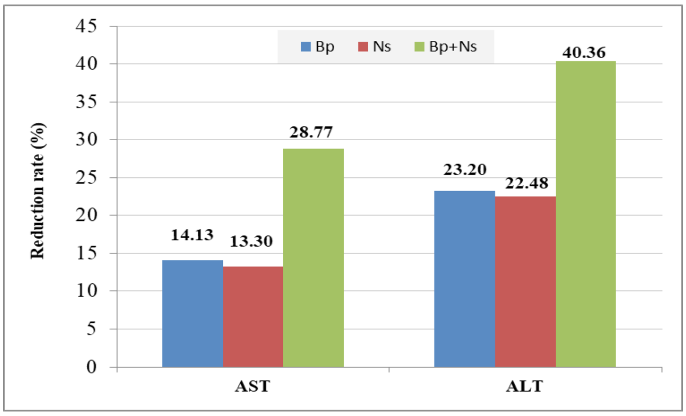

Figure 6: Reduction rate in activity of AST and ALT in blood serum at weaning of calves in treatment groups relative to control one.

The present results indicated beneficial impacts of either BP or BS alone on improving liver function (Fig. 6) and the antioxidant status of calves (Fig. 7). On the other hand, the combination treatment of BP and BS showed positive synergic effects of both BP and BS on liver function and antioxidant status of calves (Figs. 6 and 7).

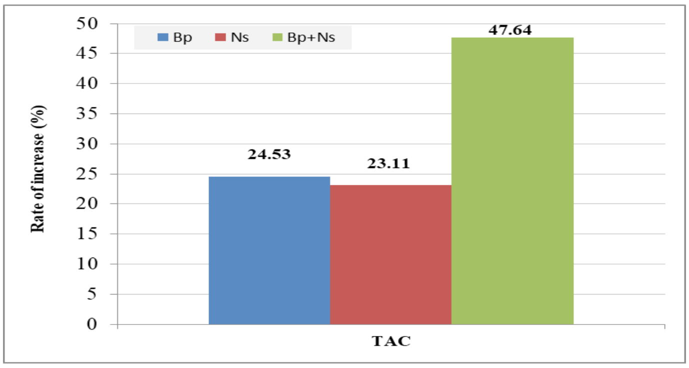

Figure 7: Rate of increase in serum total antioxidant capacity (TAC) at weaning of calves in treatment groups relative to control one.

The obtained values of AST and ALT activities in blood of calves in our study decreased as compared to the critical levels (70 and 45 U/L), respectively, as reported by Kaneko et al. (2008). In agreement with the present results, activity of serum ALT was significantly lower in lambs fed diets supplemented with plant essential oils compared with the controls (Darwish et al., 2021). However, addition of NS (1 g/kg) in the diet for 28 days had no effect on the activity of AST and ALT in rates (Shalaby et al., 2016). Also, BP treatment was found to enhance the liver function in rabbits (El-Neney et al., 2014).

In accordance with the observed increase in antioxidant status of calves, serum TAC level and catalase activity, as an antioxidant enzyme, was significantly increased in male Wistar rats by feeding NS seeds (30-50 g/kg) compared with the control diet (Mahmoud et al., 2021). Also, a positive impact of dietary addition of essential oils was observed on the blood oxidative stability by reducing MDA level and increasing activity of GPx (Salim et al., 2019).

Generally, NS seeds have revealed strong antioxidant free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. This property may be due to the high content of phenolic and flavonoid compounds in NS seeds (Mahmoud et al., 2021). In this context, Adetuyi and Ibrahim (2014) found DPPH functions are associated with phenolic and flavonoid components. On the other hand, Lee et al. (2016) investigated the antiviral activity of BP and showed anti-influenza activity by isolating a few bioactive components (one alkaloid and six flavonoids) of BP. Khalifa et al. (2021) documented the BP as a significant nutritional additive with antioxidant and hepatoprotective properties.

Growth performance

Although the live body weight of calves at birth was nearly similar in all groups, calves were significantly (P<0.05) heavier in treatment groups than in the control one, being the heaviest in G4. Also, total and average daily gain were significantly (P<0.05) higher in treatment than in control group, being the highest in G4 (Table 7).

These results indicated similar impacts of either BP or BS on LBW and the gain of calves during the suckling period. The weaning weight of calves in G2 and G3 was higher by about 23% relative to the control calves. Also, total and daily weight gain were increased by about 47% in G2 and G3 relative to control. The corresponding values regarding the calves in G4 were 47 and 92%, respectively.

Animal body weight is commonly used for monitoring nutritional status and growth performance (Ndlovu et al., 2007). As indicated in our study, several authors mentioned that feeding calves with feed additives as natural antioxidants significantly increased weaning body weight, total weight gain, and average daily gain as compared to the control (Ahmed et al., 2009; Sarker et al., 2009). In this respect, buffalo calves fed on the diet supplemented with BS oil grew faster than those fed on the control diet (Khattab et al., 2011). In growing calves, growth performance parameters including LBW and weight gain were increased in calves fed black cumin meal (BCM) compared with the control diet (Abdel-Magid et al., 2007). LBW and weight gain of lambs treated with BCM, were higher than the control animals, indicating that lambs fed on the BCM150 were more efficient in converting nutrients to growth (Obeidat, 2020). Feeding diet containing NS cake increased LBW and the average daily gain of growing lambs (Taha, 2017; Retnani et al., 2019). Barbarine lambs fed diets supplemented with *Nigella sativa* seeds in low or high concentrate diets increased growth rate (Cherif et al., 2018). In Zaraibi goats, dietary supplementation with NS improved final LBW and average daily gain (Habeeb and Tarabany, 2012). Recently, Mahmoud et al. (2021) found that feeding male Wistar rats on diet containing BS seed significantly improved the final LBW and feed conversion ratio as compared to control.

The growth performance promotion could be due to the nutritional value of key NS components that contain high fatty acid percentages and essential amino acids (Atta, 2003). Moreover, NS exerts an enhancing effect on digestive enzymes (Platel and Srinivasan, 2000) and gastrointestinal motility (Hannan et al., 2019) and thus improves feed utilization and feed conversion ratio.

CONCLUSION

In conclusion, dietary supplementation of a combination of 5g bee pollen and 5g black seeds oil/kg during the sucking period improves live body weight, weight gain, immunity, kidney and liver functions, and antioxidant status of female newborn Friesian calves. This administration is considered a good strategy for raising dairy calves in dairy farms.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

NOVELTY STATEMENT

There are several article studied the effect of bee pollen or black seeds supplementation effects on animal performance but the novel point in the present study is the combination treatment of bee pollen and black seeds during the sucking period of newborn Friesian calves which is considered a good strategy for raising female dairy calves in dairy farms.

AUTHOR'S CONTRIBUTION

All authors were contributed to design the experimental work. El-Nagar, H.A. and Mandouh, M.S. conducted the experimental procedures and data collection. El-Hais A.M., Bayoumi, A.H. and Attia, K.A. performed the sample preparations and chemical analysis, Wafa, W.M. conducted the statistical analysis and critically revised the manuscript.

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