International Journal of Research Studies in Agricultural Sciences (IJRSAS)
Volume 4, Issue 9, 2018, PP 22-33
ISSN No. (Online) 2454–6224
DOI: http://dx.doi.org/10.20431/2454-6224.0409004
www.arcjournals.org

Blood Biochemical, Haematological, Immunity Response and Diarrhea Incidence of Early Weaning Friesian Calves Supplemented with Bee Pollen and Black Seeds

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Abstract: *The current work was carried out to investigate the effect of bee pollen (BP) and black seeds (BS) as natural additive on blood biochemical and haematological, immunity response and diarrhea incidence of early weaning Friesian calves. Twenty-four newly born Friesian calves (12 males and 12 females) with average live body weight of 31 ± 0.26 kg were suckled their dam's colostrum for three days and divided into six similar groups (2 males and 2 females in each group) according to their live body weight and month of birth. The experimental period lasted 180 days over three consecutive periods (60 days each). All claves were fed a basal diet consisted of whole milk, calf starter and fresh berseem during suckling period and on calf starter and fresh berseem during 1st post weaning period or concentrate feed mixture, berseem hay and rice straw during 2nd post weaning period. Group1 given their diet without any additives and served as control, while G2 and G3 groups were supplemented with 5 or 10g BP/head/day in their diets, respectively. While, the diets of G4 and G5 groups were supplemented with 5 or 10 g BS/head/day, respectively. Lastly, G6 group was supplemented with 2.5 g BP + 2.5 g BS/head/day.*

Results showed that G3 and G5 showed the highest concentrations of total protein and globulin in blood plasma followed by G2, G4 and G6, while G1 the lowest concentrations during suckling, 1st and 2nd post weaning periods. While, albumin concentration was nearly for the different groups. Plasma creatinine, urea-N concentrations as well as AST and ALT enzymes were significantly lower (P<0.05) in G3 and G5 compared to G1, while in G2, G4 and G6 were intermediate with insignificant differences during the different periods. The counts of white blood cells, lymphocytes, monocytes and granulocytes in blood of Friesian calves improved significantly (P<0.05) with BP and BS additives with the best results in G3 and G5 compared to G1 during experimental periods. Groups 3 and 5 recorded significantly (P<0.05) the highest RBC's count, HGB concentration and HCT percentage followed by G2, G4 and G6, while G1 had the lowest values during the three periods. The high levels of BP and BS additives in G3 and G5 recorded significantly (P<0.05) the highest values of MCV, MCH, MCHC and RDW, while the lowest values were in G1 during the different periods. Groups 3 and 5 (10 g BP and BS) recorded significantly (P<0.05) the highest values of PLT, PCT, MPV and PDW, while G1 had the lowest values during the different stages. Groups 3 and 5 recorded significantly (P<0.05) the highest immunoglobulins (IgG, IgM and IgA) concentrations in plasma followed by G2, G4 and G6, while the lowest values were in G1 during the 3rd and 6th months of age. The BP and BS additive decreased significantly (P<0.05) the percentage of diarrhea incidence during suckling and 1st post weaning periods with the lowest value in G5 and decreased gradually with advancing age from the birth to the fourth month of age. In conclusion, the high level of BP and BS additives (10 g/head/day) improved blood biochemical, haemotological and immunoglobulins as well as reduced diarrhea incidence of suckling and post weaning Friesian calves.

Keywords: *Friesian calves, bee pollen, black seeds, blood biochemical and haematological, immunity response and diarrhea incidence.*

1. INTRODUCTION

Raising young calves is a labor intensive and expensive segment of livestock production. Weaning of calve sat an early age is practiced to reduce the cost and labor of feeding **(Owen and Larson, 1982; Quigley *et al*., 1991).** Bee pollen is apicultural product composed of nutritionally valuable substances **(Aliyazicioglu *et al*., 2005).** Bee pollen is rich in protein (25%), essential amino acids, oil (6%), containing more than 51% poly unsaturated fatty acids of which 39% linolenic, 20% palmitic and 13% linoleic acid, 11 enzymes or co-enzymes and also abounds with carbohydrate (35-61%, mainly glucose, fructose and sucrose), lipid, more than 12 vitamins, 28 minerals **(Echigo and Yanami, 1986; Xu *et al*., 2009)** and carotenoids **(Izuta *et al*., 2009).** Another very interesting bee product is propolis, which has antibiotic properties and may improve growth performance, feed efficiency and feed intake of animals **(Sarker and Yang, 2010).** These effects may be due to the content of antioxidants, vitamins, minerals, phenolic constituents and enzymes **(El-Hanoun *et al.*, 2007).** The immunity responsiveness represented in leukocytes counts and mainly on lymphocytes increased significantly with increasing BP levels. Hematological parameters and liver functions have been improved due to bee pollen treatment **(El-Neney and El-Kholy, 2014). Butt and Sultan (2010)** concluded that *Nigella sativa* oil contains thymoquinone, which has potent antioxidant effect. The mechanism(s) underlying the protective effect of *Nigella sativa* oil on the testis might be due to its direct cytoprotective effect and/ or indirect antioxidant and androgen like activities that reported by **Wahba (2011).** *Nigella sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant properties **(Desai *et al*., 2015).** The use of *Nigella sativa* oil for suckling calves was effective in maintaining blood homeostasis, improving general health status and for prevention and treatment of health problems in suckling Friesian calves **(Abd El-Hafeez *et al*., 2014).**

Objective of this study was to investigate the effect of bee pollen and black seed additives on blood biochemical and haematological, immunity response and diarrhea incidence of early weaning Friesian calves.

2. 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 during in co-operation with Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University the period from January to August 2014.

2.1. Experimental Animals

Twenty-four newly born Friesian calves including 12 males with average live body weight of 31.67±0.31 kg and 12 females with average live body weight of 30.33±0.31 kg were used after suckling their dam's colostrum for three days. Calves were divided into six similar groups (2 males and 2 females in each group) according to live body weight.

2.2. Experimental Rations

The whole experimental period was lasted for 180 days where it staged for consecutive three periods (60 days each), the first one was serves as suckling period form the birth until the weaning at 60 days of age (suckling period), the second one was extended from 61 to 120 days post weaning and third period was continued from 121 to 180 days of age. All claves were fed a basal diet consisted of whole milk, calf starter and 2nd cut fresh berseem during suckling period, calf starter and 3rd cut fresh berseem during the second period or concentrate feed mixture, berseem hay and rice straw during the third one. The group (G1) was unsupplemented and served as control, while G2 and G3 groups were supplemented with 5 or 10 g BP/head/day and G4 and G5 groups were supplemented with 5 or 10 g BS/head/day, respectively, and lest group G6 was supplemented with 2.5 g BP + 2.5 g BS/head/day.

2.3. Management Procedures

Calves were fed individually their allowance during the suckling and post weaning periods to cover their nutritional requirements according to **NRC (2001)**. During suckling period, calves were suckled the whole milk in plastic buckets in two equal parts at 7 a.m. and 4 p.m., calf starter (CS) once time at

8 a.m. and fresh berseem (FB) at 11 a.m. During post weaning period, CS or concentrate feed mixture (CFM) was offered two times daily at 8 a.m. and 4 p.m., fresh berssem or berseem hay (BH) once daily at 9 a.m., RS was given at 11 a.m. Bee pollen and black seeds additives were supplemented in milk during suckling period or on calf starter or concentrate feed mixture during post weaning period. Fresh water was free available for calves all the day round. Calf starter was consisted (as fed) of 20% soybean meal, 5% linseed cake, 34% ground yellow corn grain, 20% wheat bran, 15% rice bran, 3% molasses, 2% limestone and 1% common salt. While, CFM consisted (as fed) of 30% undecorticated cottonseed cake, 5% linseed cake, 25% wheat bran, 22% ground yellow corn grains, 12% rice bran, 3% molasses, 2% limestone and 1% common salt. Composition of whole milk was 3.90% fat, 3.16% protein, 5.02% lactose, 8.90% solids not fat (NFS), 12.8% total solids (TS) and 0.71% ash. Chemical composition of different feedstuffs are shown in Table (1).

Table1. *Chemical analysis of feedstuffs and basal diets (% on dry matter basis)*

Item	DM %	Composition of DM %					
		OM	CP	CF	EE	NFE	Ash
Feedstuffs:							
Calf starter	91.58	90.68	18.43	5.96	3.91	62.38	9.32
Concentrate feed mixture	91.60	88.66	17.54	11.73	3.15	56.24	11.34
2nd cut fresh berseem	15.45	87.97	15.87	24.35	3.60	44.15	12.03
3rd cut fresh berseem	16.30	87.60	15.42	24.70	3.45	44.03	12.40
Berseem hay	90.10	88.19	14.32	27.87	2.84	43.16	11.81
Rice straw	89.85	83.85	2.52	30.47	2.10	48.76	16.15
Basal rations							
Suckling period	18.08	92.03	21.06	6.66	17.14	47.17	7.97
1st post weaning period	35.00	89.60	17.38	12.52	3.75	55.95	10.40
2nd post weaning period	90.88	87.80	14.32	19.38	2.90	51.20	12.20

2.4. Blood Samples

Blood samples were taken at the last week of each period from the jugular vein of each calves by clean sterile needle in clean dry glass tubs using heparin as an anticoagulant in two parts. The first part was used for determined hematological parameters. The second part was centrifuged for 15 minutes at 4000 rotations per minute to obtain plasma. Plasma samples were kept in deep freezer at -20 °C till chemical analysis was carried out.

2.5. 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 Daibley, 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**)** levels 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 and granulocytes) was conducted as described by **Coles (1986).**

2.6. Biochemical Analysis

Total protein and albumin concentrations 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, plasma samples were analyzed for determinations blood activities of aspartate amino transferase (AST) and alanine amino 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.

2.7. Immunoglobulins Determination

The concentrations of immunoglobulins IgG, IgM and IgA concentrations in the blood plasma samples were measured at 3 and 6 months of age using the quantitative ELISA Bovine (IgG, IgM and IgA), ELISA Quantitation Kit, Bethyl laboratories, UK.

2.8. Statistical Analysis

The data were analyzed using general linear models procedure adapted by **IBM SPSS Statistics (2014)** 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 $P<0.05$.

3. Results and Discussions

3.1. Blood Plasma Biochemical

3.1.1. Plasma Proteins Concentration

The effect of BP and BS additives on plasma total protein, albumin and globulin concentrations of male and female Friesian calves during suckling, 1st and 2nd weaning periods are presented in Table (2). The highest concentrations of total protein and globulin in blood plasma during suckling, 1st and 2nd weaning periods were found in G3 and G5, followed by G2, G4 and G6, while the lowest concentrations were detected in G1 ($P<0.05$). However, albumin concentration was nearly for the different groups. These results indicated that the increase in plasma total protein due to the BP and BS additives occurred in globulin. Plasma total protein, albumin and globulin concentrations were nearly similar for male and female calves. The interactions between experimental groups and sex of calves in plasma total protein and globulin concentrations were significant ($P<0.05$). These results agreed with those obtained by **Hedia, *et. al*. (2007) and Attia *et al*. (2011a,b and 2014b)** they showed that treatment with BP caused marked increases in plasma total protein and albumin. Bee pollen additive in 25 g d^{-1} and polysaccharides in 5 g d^{-1} in milk replacer of calves slightly increased serum TP and ALB content (**Zhang *et al*., 2010).** Also, total protein and globulin were increased significantly ($P\leq0.05$) in blood of lambs fed black cumin seeds **(Shams Al-dain and Jarjeis, 2015)**. The serum protein level was significantly ($P<0.05$) higher than those of the control group **(Dorucu *et al*., 2009).**

3.1.2. Kidney Functions

Results in Table (2), showed that kidney function in terms of plasma creatinine and urea-N concentrations of male and female Friesian calves during suckling, 1st and 2nd weaning periods improved with BP and BS additives. Plasma creatinine and urea-N concentrations during suckling, 1st and 2nd weaning periods were significantly lower ($P<0.05$) in G3 and G5 compared to G1, while in G2, G4 and G6 were intermediate with insignificant differences ($P>0.05$). Plasma creatinine and urea-N concentrations were nearly similar for male and female calves. The interaction between groups and sex of calves in plasma creatinine and urea-N concentrations were significant ($P<0.05$). Plasma creatinine and urea-N concentrations are within the normal level for cattle being 1-2 and 10-30 mg/dl for creatinine and urea-N, respectively **(Kaneco *et al*., 2008).** Bee pollen protects the kidneys and can lower the level of blood urea nitrogen in rats **(Hu *et al*., 2003). Shams Al-dain and Jarjeis (2015)** found that bloods urea-N decreased significantly in lambs fed black cumin seeds. On the other side, *Nigella sativa* (*N. sativa*) had no effect on the kidney function of rates which evidence of normal urea and creatinine level in blood **(Dollah *et al*., 2013).**

3.1.3. Liver Enzymes Activity

Liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity during suckling, 1st and 2nd weaning periods improved with BP and BS additives as presented in Table (2). The activity of AST and ALT enzymes in blood plasma were significantly lower ($P<0.05$) in G3 and G5 compared to G1. However, there were no significant difference ($P>0.05$) in the activity of AST and ALT in plasma between male and female calves. The interaction between groups and sex of

calves in plasma AST and ALT activity are significant (P<0.05). Plasma AST and ALT activity are lower than the critical levels of AST and ALT being 70 and 45 U/L, respectively **(Kaneco *et al*., 2008).** Liver functions have been improved due to bee pollen treatment **(El-Neney and El-Kholy, 2014).** Nigella sativa up to the dose of 1 g/kg supplemented for a period of 28 days resulted no changes in liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in rates **(Dollah *et al*., 2013).**

Table2. *Blood plasma biochemical of male and female calves for different groups and periods*

Items	Experimental groups						MSE
	G1	G2	G3	G4	G5	G6	
Suckling period:							
Total protein (g/dl)	6.54^{c}	6.78^{bc}	7.27^{a}	6.85^{bc}	7.30^{a}	7.09^{ab}	0.07
Albumin (g/dl)	3.11	3.19	3.28	3.19	3.25	3.25	0.05
Globulin (g/dl)	3.42^{c}	3.59^{bc}	3.99^{a}	3.66^{bc}	4.05^{a}	3.84^{ab}	0.05
Creatinine (mg/dl)	1.31^{a}	1.24^{ab}	1.16^{b}	1.25^{ab}	1.15^{b}	1.21^{ab}	0.02
Urea-N (mg/dl)	21.18^{a}	20.17^{ab}	18.53^{b}	20.12^{ab}	18.41^{b}	19.41^{ab}	0.37
AST (U/L)	41.97^{a}	40.34^{ab}	37.05^{b}	40.23^{ab}	36.82^{b}	38.81^{ab}	0.75
ALT (U/L)	18.93^{a}	18.15^{ab}	16.67^{b}	18.10^{ab}	16.57^{b}	17.47^{ab}	0.34
1st post weaning period:							
Total protein (g/dl)	6.52^{c}	6.74^{bc}	7.14^{a}	6.78^{b}	7.17^{a}	6.99^{ab}	0.06
Albumin (g/dl)	2.95	3.00	2.98	2.96	2.91	2.98	0.04
Globulin (g/dl)	3.58^{c}	3.75^{bc}	4.17^{a}	3.82^{bc}	4.25^{a}	4.01^{ab}	0.06
Creatinine (mg/dl)	1.37^{a}	1.30^{ab}	1.19^{b}	1.29^{ab}	1.18^{b}	1.25^{ab}	0.02
Urea-N (mg/dl)	22.08^{a}	20.73^{ab}	19.04^{b}	20.67^{ab}	18.92^{b}	19.95^{ab}	0.38
AST (U/L)	44.16^{a}	41.46^{ab}	38.08^{b}	41.35^{ab}	37.84^{b}	39.89^{ab}	0.77
ALT (U/L)	19.87^{a}	18.66^{ab}	17.14^{b}	18.61^{ab}	17.03^{b}	17.95^{ab}	0.35
2nd post weaning period:							
Total protein (g/dl)	6.22^{c}	6.35^{c}	6.81^{a}	6.47^{bc}	6.89^{a}	6.69^{ab}	0.06
Albumin (g/dl)	2.89	2.88	2.93	2.94	2.99	3.00	0.05
Globulin (g/dl)	3.34^{c}	3.48^{bc}	3.88^{a}	3.53^{bc}	3.90^{a}	3.68^{ab}	0.05
Creatinine (mg/dl)	1.46^{a}	1.33^{ab}	1.22^{b}	1.33^{ab}	1.21^{b}	1.28^{ab}	0.02
Urea-N (mg/dl)	23.43^{a}	21.29^{ab}	19.56^{b}	21.23^{ab}	19.43^{b}	20.49^{ab}	0.39
AST (U/L)	46.35^{a}	42.58^{ab}	39.11^{b}	42.47^{ab}	38.87^{b}	40.97^{ab}	0.79
ALT (U/L)	20.91^{a}	19.16^{ab}	17.60^{b}	19.11^{ab}	17.49^{b}	18.44^{ab}	0.36

a, b, c: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

3.2. Blood Haematology

3.2.1. White Blood Cells (Wbc's) and Differential Leukocytes

White blood cells (WBC's) and differential leukocytes counts in blood of male and female Friesian calves during the suckling, 1st and 2nd periods as affected by BP and BS additives are shown in Table (3). The counts of white blood cells, lymphocytes, monocytes and granulocytes in blood of male and female Friesian calves during the suckling, 1st and 2nd periods improved significantly (P<0.05) with BP and BS additives with the best results for the high level (10 g/head/day) for both BP and BS. There were significant (P<0.05) differences in the counts of white blood cells, lymphocytes, monocytes and granulocytes among the different groups and G3 and G5 recorded the higher counts, however G1 had the lower counts. However, the percentages of lymphocytes, monocytes and granulocytes were nearly similar for the different groups. Moreover, the counts of white blood cells, lymphocytes, monocytes and granulocytes and percentages of lymphocytes, monocytes and granulocytes were nearly similar in blood of male and female calves. The interaction between experimental groups and sex of calves were significant (P<0.05) in the counts of white blood cells, lymphocytes, monocytes and granulocytes, but were insignificant (P>0.05) in the percentages of lymphocytes, monocytes and granulocytes. The counts of WBC's, lymphocytes, monocytes and granulocytes in blood obtained in this study are within the normal ranges obtained by **Jezek *et al.* (2011)** being 6.0-15.6 x10^{3}/ µl for WBC's, 5-10 x10^{3}/ µl for lymphocytes, 0.5-1.7 x10^{3}/ µl for monocytes and 1-3 x10^{3}/ µl for granulocytes. White blood cells (WBC) play a major role in defending

the body against disease-producing bacteria, viruses and fungi. There are three main types, lymphocyte, monocyte and granulocyte, each of which performs a specific function. T and B cells of lymphocytes are very important in regulating the immune response. T cells attack virus infected and malignant cells, whereas B cells produce and release antibodies, or protein substances, which bind to infectious agents and help in preventing them from damaging the body. A deficiency in any type of normal white blood cell may result in an increased susceptibility to infections. The immunity responsiveness represented in leukocytes counts and mainly on lymphocytes increased significantly with increasing BP levels **(El-Neney and El-Kholy, 2014).** Chicks fed 0.6% bee-pollen diet found to have highest white blood cells, hetrophils and lymphocytes (**Farag and El-Rayes, 2016). Shams Al-dain and Jarjeis (2015)** found that lymphocytes and esinophilis cells increased significantly in lambs fed black cumin seeds.

Table3. *White blood cells and differential leukocyte counts for different groups during suckling, 1st and 2nd post weaning periods.*

Items	Experimental groups						MSE
	G1	G2	G3	G4	G5	G6	
Suckling period:							
WBC's (x10^3/µl)	9.67^c	10.12^c	11.67^a	10.44^{bc}	11.65^a	11.16^{ab}	0.16
Lymphocytes (x10^3/µl)	7.74^c	7.97^c	9.16^a	8.21^{bc}	9.14^a	8.90^{ab}	0.14
Lymphocytes (%)	79.77	78.51	78.96	78.41	79.66	80.01	0.53
Monocytes (x10^3/µl)	0.85^b	0.98^{ab}	1.13^a	1.05^{ab}	1.14^a	1.06^{ab}	0.03
Monocytes (%)	8.96	9.74	9.61	9.97	9.78	9.53	0.29
Granulocytes (x10^3/µl)	1.08^b	1.17^{ab}	1.38^a	1.18^{ab}	1.37^a	1.20^{ab}	0.04
Granulocytes (%)	11.27	11.75	11.81	11.07	11.81	10.81	0.32
1st post weaning period:							
WBC's (x10^3/µl)	10.12^c	10.52^{bc}	13.67^a	10.92^{bc}	13.85^a	11.74^b	0.27
Lymphocytes (x10^3/µl)	6.93^b	7.30^b	9.31^a	7.50^b	9.41^a	8.12^{ab}	0.25
Lymphocytes (%)	67.68	68.91	68.04	67.98	68.29	68.77	1.25
Monocytes (x10^3/µl)	1.09^b	1.11^b	1.53^a	1.25^{ab}	1.59^a	1.29^{ab}	0.05
Monocytes (%)	10.94	10.64	11.14	11.69	11.37	11.18	0.40
Granulocytes (x10^3/µl)	2.10^b	2.11^b	2.84^a	2.17^b	2.85^a	2.32^{ab}	0.11
Granulocytes (%)	21.38	20.46	20.82	20.33	20.34	20.05	0.95
2nd post weaning period:							
WBC's (x10^3/µl)	10.40^c	11.00^{bc}	12.98^a	11.21^{bc}	13.04^a	11.97^b	0.23
Lymphocytes (x10^3/µl)	6.98^b	7.21^b	8.28^a	7.36^b	8.32^a	7.94^{ab}	0.15
Lymphocytes (%)	67.30	65.58	63.88	66.36	63.73	65.81	0.61
Monocytes (x10^3/µl)	1.52^b	1.65^{ab}	2.03^a	1.69^{ab}	2.04^a	1.81^{ab}	0.06
Monocytes (%)	14.62	14.99	15.62	15.32	15.64	15.99	0.40
Granulocytes (x10^3/µl)	1.90^b	2.14^{ab}	2.66^a	2.16^{ab}	2.69^a	2.22^{ab}	0.09
Granulocytes (%)	18.09	19.43	20.50	18.33	20.63	18.20	0.55

a, b, c: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

3.2.2. Red Blood Cells Count (Rbc's), Haemoglobin Concentration (Hgb) and Haematocrit Percentage (Hct)

Results in Table (4) revealed that BP and BS additives showed significant (P<0.05) effect on red blood cells count (RBC's), Haemoglobin concentration (HGB) and Haematocrit percentage (HCT) during the suckling, 1st and 2nd weaning periods. Groups 3 and 5 recorded significantly (P<0.05) the highest RBC's count, HGB concentration and HCT percentage followed by G6, then G2 and G4, while G1 had the lowest values during the suckling, 1st and 2nd weaning periods. While, RBC's count, HGB concentration and HCT percentage were nearly similar for male and female calves during the different periods without significant difference (P>0.05). The interaction between experimental groups and sex of calves in RBC's count, HGB concentration and HCT percentage during the suckling, 1st and 2nd weaning periods were significant (P<0.05). The RBC's count, HGB concentration and HCT percentage in blood increased with the progress age from 2 to 4 months and tended to decrease at 6 months of age. The count of RBC's 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, HGB

concentration and the percentage of HCT are within the normal ranges in cattle obtained by **UCDAVIS (2001)** being 8-13 g/dl and 23-35%, respectively. These results are in agreement with those obtained by **Shams Al-dain and Jarjeis (2015)** who found that red blood cells count, hemoglobin concentration and packed cell volume increased significantly (P≤0.05) in blood of lambs fed black cumin seeds. Chicks fed 0.6% bee-pollen diet were found to have highest red blood cells, hemoglobin concentration and packed cells volume **(Farag and El-Rayes, 2016).** The treatment with BP caused to significant increase in hemoglobin (Hb), RBC's count and hematocrit percentage **(El-Neney and El-Kholy, 2014).** Anemia is defined by **Benjamin (1978)** as a decrease below normal in RBC, Hb and PCV.

Table4. *Red blood cells count, haemoglobin concentration and haematocrit percentage for different groups during suckling, 1st and 2nd post weaning periods*

Items	Experimental groups						MSE
	G1	G2	G3	G4	G5	G6	
Suckling period:							
RBC's (x10^6/µl)	6.06^{c}	6.38^{bc}	7.45^{a}	6.53^{bc}	7.53^{a}	7.06^{ab}	0.14
HGB (g/dl)	8.95^{c}	9.80^{bc}	11.02^{a}	9.84^{bc}	11.06^{a}	10.19^{ab}	0.18
HCT (%)	24.23^{c}	26.59^{bc}	30.51^{a}	27.08^{abc}	30.69^{a}	28.89^{ab}	0.39
1st post weaning period:							
RBC's (x10^6/µl)	7.40^{c}	7.84^{bc}	9.11^{a}	7.98^{bc}	9.15^{a}	8.37^{b}	0.13
HGB (g/dl)	10.63^{b}	10.95^{ab}	12.87^{a}	10.70^{b}	12.97^{a}	11.68^{ab}	0.29
HCT (%)	28.39^{b}	30.46^{ab}	33.86^{a}	30.30^{ab}	33.73^{a}	31.81^{ab}	0.38
2nd post weaning period:							
RBC's (x10^6/µl)	6.56^{c}	7.15^{bc}	8.20^{a}	7.07^{bc}	8.27^{a}	7.40^{b}	0.14
HGB (g/dl)	9.50^{b}	10.52^{ab}	11.63^{a}	10.50^{ab}	11.71^{a}	10.81^{ab}	0.28
HCT (%)	27.58^{b}	29.59^{ab}	33.64^{a}	28.06^{b}	33.18^{a}	30.58^{ab}	0.32

a, b, c: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

3.2.3. Erythrocyte Indices

Erythrocyte indices expressed as mean cellular volume (MCV)**,** mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC) and red cell distribution width (RDW) in blood of calves are shown in Table (5). There were significant differences (P<0.05) in the values of MCV, MCH, MCHC and RDW among the different groups. The high levels of BP and BS additives in G3 and G5 recorded significantly (P<0.05) the highest values of MCV, MCH, MCHC and RDW followed by G2, G4 and G6, while the lowest values were in G1 during the suckling, 1st and 2nd weaning periods. While, the values of MCV, MCH, MCHC and RDW in blood were nearly similar for male and female calves during the different periods without significant difference (P>0.05). The interaction between experimental groups and sex of calves in MCV, MCH, MCHC and RDW values during the suckling, 1st and 2nd weaning periods were significant (P<0.05). The values of MCV, MCH, MCHC and RDW in blood increased with the progress age from 2 to 4 months and tended to decrease at 6 months of age. 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. The variation in various erythrocytic indices may be attributed to variable RBC size, its oxygen carrying capacity in connection with age and physiologic state. The difference due to technique variance cannot also be ruled out **(Farooq *et al*., 2011).** Many of hematological parameters are influenced by many factors like breed, age, sex, seasonal variations, lactation, pregnancy, health and nutrition status **(Aengwanich, 2002; Al-Shami, 2007; Mohammed *et al*., 2007).**

Table5. *Erythrocyte indices for different groups during suckling, 1st and 2nd post weaning periods*

Items	Experimental groups						MSE
	G1	G2	G3	G4	G5	G6	
Suckling period:							
MCV (fl)	32.78^{c}	35.03^{bc}	39.54^{a}	35.39^{bc}	39.81^{a}	37.84^{ab}	0.55
MCH (pg)	12.78^{c}	13.73^{bc}	15.50^{a}	13.87^{bc}	15.60^{a}	14.83^{ab}	0.22
MCHC (g/dl)	28.61^{c}	30.43^{bc}	34.34^{a}	30.74^{bc}	34.58^{a}	32.87^{ab}	0.48
RDW (%)	14.66^{c}	15.67^{bc}	17.68^{a}	15.83^{bc}	17.80^{a}	16.93^{ab}	0.24

1st post weaning period:							
MCV (fl)	37.04^{c}	39.79^{bc}	44.38^{a}	39.89^{bc}	44.55^{a}	42.56^{ab}	0.61
MCH (pg)	14.45^{c}	15.59^{bc}	17.39^{a}	15.64^{bc}	17.46^{a}	16.68^{ab}	0.24
MCHC (g/dl)	32.34^{c}	34.56^{bc}	38.55^{a}	34.66^{bc}	38.69^{a}	36.78^{ab}	0.52
RDW (%)	16.56^{c}	17.79^{bc}	19.85^{a}	17.84^{bc}	19.92^{a}	19.04^{ab}	0.27
2nd post weaning period:							
MCV (fl)	35.07^{c}	37.38^{bc}	42.11^{a}	37.67^{bc}	42.28^{a}	40.39^{ab}	0.59
MCH (pg)	13.68^{c}	14.65^{bc}	16.50^{a}	14.77^{bc}	16.57^{a}	15.83^{ab}	0.23
MCHC (g/dl)	30.62^{c}	32.47^{bc}	36.58^{a}	32.73^{bc}	36.73^{a}	35.09^{ab}	0.50
RDW (%)	15.69^{c}	16.72^{bc}	18.83^{a}	16.84^{bc}	18.90^{a}	18.07^{ab}	0.26

a, b, c: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

3.2.4. Platelet Indices

The effect of BP and BS additives on platelet indices expressed as platelet count (PLT), procalcitonin (PCT), mean platelet volume (MPV) and Platelet Distribution Width (PDW) in blood of calves are shown in Table (6). There were significant differences (P<0.05) in the values of PLT, PCT, MPV and PDW among the different groups. Group 3 (10 g BP) and G5 (10 g BS) recorded significantly (P<0.05) the highest values of PLT, PCT, MPV and PDW followed by G2, G4 and G6, while the lowest values were in G1 during the suckling, 1st and 2nd weaning periods. While, the values of PLT, PCT, MPV and PDW in blood were nearly similar for male and female calves during the different periods without significant difference (P>0.05). The interaction between experimental groups and sex of calves in PLT, PCT, MPV and PDW values during the suckling, 1st and 2nd weaning periods were significant (P<0.05). The values of PLT, PCT, MPV and PDW in blood increased with the progress age from 2 to 4 months and tended to decrease at 6 months of age. Platelet indices values in blood obtained in this study are within the normal ranges obtained by **UCDAVIS (2001)** being 233-690 x10^3/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. 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).**

Table6. *Platelet indices for different groups during suckling, 1st and 2nd post weaning periods*

Items	**Experimental groups**						**MSE**
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	
Suckling period:							
PLT (x10^3/µl)	282.49^{c}	306.71^{bc}	346.61^{a}	303.82^{bc}	341.27^{a}	324.43^{ab}	4.04
PCT (µg/l)	0.159^{c}	0.170^{bc}	0.189^{a}	0.172^{bc}	0.192^{a}	0.183^{ab}	0.003
MPV (fl)	4.61^{c}	4.93^{bc}	5.51^{a}	4.98^{bc}	5.56^{a}	5.32^{ab}	0.08
PDW (fl)	52.15^{c}	55.73^{bc}	62.91^{a}	56.31^{bc}	62.86^{a}	60.21^{ab}	0.87
1st post weaning period:							
PLT (x10^3/µl)	301.25^{c}	325.47^{bc}	367.04^{a}	321.14^{bc}	361.88^{a}	344.01^{ab}	4.09
PCT (µg/l)	0.175^{c}	0.188^{bc}	0.209^{a}	0.190^{bc}	0.212^{a}	0.203^{ab}	0.003
MPV (fl)	5.16^{c}	5.54^{bc}	6.20^{a}	5.60^{bc}	6.25^{a}	5.99^{ab}	0.09
PDW (fl)	59.97^{c}	63.77^{bc}	71.98^{a}	64.43^{bc}	71.93^{a}	68.89^{ab}	0.99
2nd post weaning period:							
PLT (x10^3/µl)	298.23^{c}	322.73^{bc}	352.34^{a}	314.58^{bc}	354.75^{a}	337.23^{ab}	4.65
PCT (µg/l)	0.168^{c}	0.181^{bc}	0.201^{a}	0.181^{bc}	0.204^{a}	0.195^{ab}	0.003
MPV (fl)	4.98^{c}	5.35^{bc}	5.98^{a}	5.40^{bc}	6.03^{a}	5.78^{ab}	0.08
PDW (fl)	56.85^{c}	60.45^{bc}	68.22^{a}	61.07^{bc}	68.17^{a}	65.30^{ab}	0.94

a, b, c: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

3.3. Immunoglobulins Concentration

Immunoglobulins (IgG, IgM and IgA) concentrations in blood plasma of calves at 3 and 6 months of age are shown in Table (12) and Figs. (34-36). The concentrations of IgG, IgM and IgA in plasma of

G3 and G5 were significantly higher (P<0.05) than that of G1, whereas in G2, G4 and G6 were intermediate with insignificant differences. Moreover, the concentrations of IgG, IgM and IgA in plasma decreased with advancing age from 3 to 6 month of age. The values of IgG, IgM and IgA in plasma of G3 and G5 increased by 24.14, 22.66; 23.91, 21.74 and 28.79, 25.76% at 3 months and 25.29, 23.74; 28.05, 25.00 and 63.64, 54.55% at 6 months of age compared to control group (G1), respectively. Bee pollen can be included in broiler diets at 1.5% until 21 days of age to increase IgM levels **(Oliveira *et al*., 2013).** Black seed oil additive increased plasma immunoglobulin concentration regarding to calves, performance during the suckling period **(Khattab *et al*., 2011).**

Table7. *Immunity response for different groups at 3rd and 6th month of age*

Age (month)	Experimental groups						MSE
	G1	G2	G3	G4	G5	G6	
At 3 months:							
IgG (g/L)	20.30^{b}	22.80^{ab}	25.20^{a}	22.50^{ab}	24.90^{a}	23.10^{ab}	0.51
IgM (g/L)	2.30^{b}	2.60^{ab}	2.85^{a}	2.55^{ab}	2.80^{a}	2.65^{ab}	0.06
IgA (g/L)	0.66^{b}	0.77^{ab}	0.85^{a}	0.75^{ab}	0.83^{a}	0.79^{a}	0.02
At 6 months:							
IgG (g/L)	15.50^{b}	17.50^{ab}	19.42^{a}	17.26^{ab}	19.18^{a}	17.74^{ab}	0.40
IgM (g/L)	1.64^{b}	1.87^{ab}	2.10^{a}	1.83^{ab}	2.05^{a}	1.92^{ab}	0.04
IgA (g/L)	0.22^{b}	0.30^{ab}	0.36^{a}	0.28^{ab}	0.34^{a}	0.31^{a}	0.01

a, b: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

3.4. Diarrhea Incidence

Diarrhea incidence of male and female Friesian calves as affected by BP and BS additives is shown in Table (8). The BP and BS additive decreased significantly (P<0.05) the percentage of diarrhea incidence during suckling and 1st weaning periods. Control group (G1) without additive recorded the highest percentage of diarrhea incidence followed by G2, G4 and G6 then G3, while the lowest percentage detected with G5 for suckling and 1st weaning period as well as the whole period. Also, the percentage of diarrhea incidence decreased gradually with advancing age from the first month to the fourth month of age. Moreover, the percentage of diarrhea incidence was nearly similar for male and female calves and tended to increase in female during the 1st, 2nd and 4th months of age and in male during the 3rd month of age. The interaction between treatments and sex of calves on diarrhea incidence were significant (P<0.05). Bee pollen appears promising because it protects intestinal tract health **(Attia *et al.*, 2011a, 2011b). Wang *et al*. (2007)** used 1.5% BP in broiler diets and demonstrated that the BP had a trophic effect in the small intestine. The use of Nigella sativa oil for suckling calves was effective in improving general health status and for prevention and treatment of health problems in suckling Friesian calves **(Abd El-Hafeez *et al*., 2014).** The low cost and the immune simulative effect of black cumin seed, it is recommended to be used in fish feed to minimize the mortalities caused by some pathogens **(Dorucu *et al*., 2009).**

Table8. *Diarrhea disease incidence (%) of Friesian calves during suckling and 1st post weaning periods*

Items	Experimental groups						MSE
	G1	G2	G3	G4	G5	G6	
Suckling period:							
1st month	24.38^{a}	22.50^{ab}	18.75^{c}	20.48^{bc}	11.25^{d}	22.23^{ab}	0.96
2nd month	16.25^{a}	15.00^{ab}	12.50^{c}	13.65^{bc}	7.50^{d}	14.75^{ab}	0.64
Mean	**20.31^{a}**	**18.75^{ab}**	**15.63^{b}**	**17.06^{ab}**	**9.38^{c}**	**18.49^{ab}**	**0.75**
1st post weaning period:							
3rd month	10.16^{a}	4.06^{bc}	2.03^{c}	6.10^{b}	1.93^{c}	5.85^{b}	0.65
4th month	5.47^{a}	2.19^{c}	1.09^{d}	3.28^{b}	0.99^{d}	3.03^{bc}	0.34
Mean	**7.81^{a}**	**3.13^{bc}**	**1.56^{c}**	**4.69^{b}**	**1.46^{c}**	**4.44^{b}**	**0.40**
Overall mean	**14.06^{a}**	**10.94^{b}**	**8.59^{c}**	**10.88^{b}**	**5.42^{d}**	**11.46^{b}**	**0.60**

a, b, c, d: Values in the same row for each item with different superscripts differ significantly (P<0.05).

G1: control G2: 5 g BP G3: 10 g BP G4: 5g BS G5: 10 g BS G6: 2.5 g BP + 2.5 g BS

4. CONCLUSION

From the present results, it could be concluded that both bee pollen and black seeds additives at the level of 10 g/head/day for Friesian calves during suckling and post weaning periods led to an improvement of blood biochemical, haematological and immunity response and reduced diarrhea incidence.

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Citation: *.M.A. Gaafar, et al. (2018). Blood Biochemical, Haematological, Immunity Response and Diarrhea Incidence of Early Weaning Friesian Calves Supplemented with Bee Pollen and Black Seeds. International Journal of Research Studies in Agricultural Sciences (IJRSAS), 4(9), pp.22-33, http://dx.doi.org/10.20431/2454-6224.0409004*