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Supplementation of overripe pulp extract and green peel extract or powder of banana fruit peel (musa. cavendish) to diets of neonatal dairy calves: Effects on haematological, immunological and performance characteristics

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Abstract

The present study investigated the effects of overripe pulp and green peel extract and powder of banana fruit (*Musa. cavendish*) on haematological, biochemical, immunological, health, and performance of Holstein dairy calves. In all, 40 newborn calves were randomly divided into four groups of 10 animals. In the control group, animals received no banana meal. In group 1, calves were supplemented with 2 g (dry matter)/kg body weight/day of overripe banana pulp extract. The calves in group 2 were supplemented with 1 g (dry matter) of overripe banana pulp extract/kg body weight/day and 1 g (dry matter) of green banana peel extract/kg body weight/day. The animals in group 3 were supplemented with 2 g/kg body weight/day of green banana peel powder. The feeding period of calves on the tested supplements was 5 days. Blood samples and other evaluations were taken on day 0 (at birth, before supplementation) and on days 7, 15 and 30. Just a trend towards better average daily weight gain was seen in groups 2 and 3 than others ($p = 0.073$). Significant group and sampling time interactions were seen for the quantities of RBC (group 1 was lower than other groups at day 30), MCV (group 3 was lower than other groups at day 30) and MCH (group 1 was higher than other groups at day 30) ($p < 0.05$). A trend towards significance in values of IgG (group 1 was lower than other groups at days 15 and 30) and bilirubin (higher values at day 7 in groups 1 and 2 than control, higher amounts at days 15 and 30 in groups 3 and 2 than control, respectively) was also observed. In conclusion, banana supplementation in neonatal calves had beneficial effects on the values of RBC, MCV, MCH, bilirubin, IgG and average daily weight gain in dairy calves.

KEYWORDS
banana, calf, haematology, health, immunology, performance

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1 | INTRODUCTION

Initial growth and health of calves in the first 60 days of life are the most important subject of their future production especially milk production. The growth rate of neonates is dependent on their health status (McGrath, 2016). Thus, the maintenance of their health, especially in the first 2–3 months of age, has substantial impacts on the future dairy herd production and the economic status of a herd (Ghosh et al., 2010).

The nutrition of calves is an important factor in their health. For this reason, the diet of calves has been supplemented with many feed additives. Also, herbs are being used recently (Ghosh et al., 2010). The immunomodulatory effects of fruits, vegetables and other plant-based food products have been documented (Matsuda et al., 2006). The advantages of using herbs and botanicals for feeding farm animals may be related to the improvement of feed intake, the stimulation of immunity, their antioxidant, anti-bacterial, antiviral, anthelmintic, anti-inflammatory and coccidiostatic effects (Ghosh et al., 2010). These effects have been attributed to the secondary important metabolites of medicinal plants such as flavonoids, terpenoids, polyphenols and phenolic compounds (Mohsien, 2017). The antioxidants of herbs may reduce the incidence of morbidity and mortality by reducing oxidative damage which helps improve the pre-weaning calf performance (McGrath, 2016).

Banana (*Musa. cavendish*) is one of the most important tropical fruits, which belongs to the order of Zingiberales, the family of Musaceae and genus Musa (Singh et al., 2014). It has different cultivars and is cultivated in many tropical and subtropical countries. About 37% of its production is in Asia and the Pacific (Nayar, 2010). Banana can be classified into commercial and non-commercial cultivars. The non-commercial ones are also referred to as indigenous varieties because their cultivation for export or trade is rare (Anyasi et al., 2015). Non-commercial bananas which are also cultivated in the south, the east and the southeast of Iran can be utilized as animal food. The use of natural products in the ration of food for animals results in the reduction of the presence of chemical residues in human foods (Gregory et al., 2015). All different parts of the banana plant including fruits, peels, etc., have medicinal utilizations (Chabuck et al., 2013). Many studies have shown that banana pulp and peel are rich in antimicrobial and antibiotic compounds (Chabuck et al., 2013; Mohsien, 2017; Okechukwu et al., 2012; Yasmin & Saleem, 2014) which also have been used for blood haemoglobin production and are effective in cases of anaemia (Mohsien, 2017).

Bananas contain high levels of minerals such as potassium and phosphorus. The pulp and the peel possess various antioxidants including phenolic compounds such as catechin, epicatechin, lignin, tannins, anthocyanins, vitamins (A, B, C and E) and β-carotene. 40% of the total weight of fresh bananas is peel which is considered as a rich source of protein, crude fat, lipid, dietary fibre, pectin, micronutrients, polyunsaturated fatty acids and essential amino acids. There are various bioactive compounds including terpenoids, flavonoids, carotenoids, alkaloids, sterols, triterpenes, tannins and glycosids in the peel. Furthermore, ripe peel also includes other compounds, such as catecholamines, cyanidin and delphinidin. These compounds were used as antioxidants, antidiabetic, anti-inflammatory and antibiotic against various Gram-positive and Gram-negative bacteria. The amounts of phenolic compounds and antioxidant properties are higher in the peel in comparison with the pulp. The amounts of phenolic compounds and flavonoids in the peel decrease with maturity (Mohsien, 2017; Sulaiman et al., 2011). In banana pulp, catecholamines such as norepinephrine, tryptophan, indole compounds, pectin, dopamine and serotonin have been found. Banana lectin (BanLec) which is abundantly present in the pulp of mature fruits is a novel member of the family of jacalin-related lectins which is highly stable and protease-resistant. BanLec is expressed just in the pulp and the roots of bananas (Chauhan et al., 2016). Banana peels have been used for production of by-products such as protein, enzymes, pectins, biomass, ethanol and methane. Also, it has been utilized as food for livestock (González-Montelongo et al., 2010).

BanLec can cause immune response and it has been shown that it is a powerful mitogen for murine and human T cells. It has the potency of stimulating macrophage activities, inhibiting HIV-1 reverse transcriptase activity and suppressing cancer cell proliferation. In addition, a pronounced IgG4 response against BanLec was found in human serum (Peumans et al., 2000; Singh et al., 2014). BanLec might be used as a potent protein for oral anti-hapten immunization in humans (Koshte et al., 1992).

Different studies have been conducted on the effects of various parts of banana plant supplementation in humans, small and large laboratory animals, chickens and even in prawns. In these studies, the effects of adding banana meal to ration have been evaluated on health, growth performance, feeding behaviour, milk production, carcasses characteristics, haematological, biochemical and immunological factors, diseases like metabolic disorders and wound healing (Abel et al., 2015; Akinlolu et al., 2015; Atzingen et al., 2013; Idoko & Oladiji, 2014; Nambi-Kasozi et al., 2016; Rattanavichai et al., 2015; Wu et al., 2015). In vitro studies also have been done for assessing the antimicrobial effects of bananas (Chabuck et al., 2013; Gregory et al., 2015; Marie-Magdeleine et al., 2014; Mohsien, 2017; Neuwirt et al., 2015).

To the best of our knowledge, there is just one study on calves which has evaluated the effect of dietary supplementation of bananas on immunocyte populations (Matsuda et al., 2006). Hence, this study was conducted to investigate the effects of dietary supplementation of extract of overripe banana pulp, extract of green banana peel with extract of overripe banana pulp together, and powder of green banana peel on haematological, biochemical, immunological, oxidants/antioxidants variables, health and performance of Holstein dairy calves.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The duration of study was 7 months, from 18 August 2017 to 18 March 2018 in a dairy herd with about 210 calves per year at the

suburbs of Mashhad (northeast of Iran). This herd consists of pure bred animals of Holstein breed. The herd was totally restricted in open-shed housing with no access to pasture. The ingredients of dry cow ration in both far off dry period and close up dry period are shown in Tables 1 and 2. The ration was balanced according to NRC recommendations (National Research Council, 2001).

The cows were dried 2 months before the expected time of parturition and they were transferred to a dry cow barnyard. Fifteen days before the expected time of parturition, the cows were moved to a straw bedded maternity pen. Immediate assistance was given to cows with dystocia. After parturition, the umbilicus of each calf was treated with povidone iodine and they were allowed to remain with their dams until the umbilicus dried off. Then the calves were weighed. Then their sex was recorded and they were transferred to individual pens bedded with straw. Within the first 6 hr of life, the calves were fed dam's colostrum by nipple bottle in amounts of 10% of their body weight and the colostrum feeding was repeated every 8 hr for 48 hr. Then, herd milk was replaced for feeding twice daily (2 kg every 12 hr) until 70 days of life. Calf starter (started from 72 hr of life) consisted of corn (17.5%), barley (20%), corn pulp (21%), soya (17%), bran (17.5%), bicarbonate (2%), calcium carbonate (2%), NaCl (1%) and supplement (2%). The supplement contained vitamin A (1,000,000 IU), vitamin D3 (250,000 IU), vitamin E (3,000 IU), calcium (253,422 mg), magnesium (42,300 mg), manganese (3,000 mg), zinc (3,000 mg), copper (1,700 mg), iodine (100 mg), selenium (100 mg), antioxidant (1,000 mg) and organic copper (100 mg) in each kilogram of dry matter. After transferring them to an individual pen, the animals had free access to clean drinking water. The calves were weaned at 70 days of life. The heifer calves were mainly used as herd replacements.

In all, 40 newborn Holstein dairy calves were selected for the study. The animals were randomly divided into four groups of 10. The groups were homogeneous for parity of dams, sex and time of birth. In the control group, animals received no banana meals. In group 1, calves were supplemented with 2 g (dry matter)/kg body weight/day of overripe banana pulp extract for 5 days. Calves in group 2 were supplemented with 1 g (dry matter) of overripe banana pulp extract/kg body weight/day and 1 g (dry matter) of green banana peel extract/kg body weight/day for 5 days. In group 3, animals were supplemented

TABLE 1 The ingredients of dry cow ration in both far off and close up periods

Gradient	Far off dry period ration (%)	Close up dry period ration (%)
Alfalfa hay	17.79	13.71
Corn silage	25.22	19.45
Wheat straw	31.16	13.73
Barley grain rolled	10.37	16
Corn grain ground dry	5.73	16.68
Wheat bran	7.7	6.14
Fish meal	1.27	–
$CaCo_3$	0.23	–
Salt	0.24	–
Canola meal	–	2.67
Soy meal, expellers	–	6.59
Anionic salt	–	3.92
TMS	–	1.11
Vitamin/mineral supplement[a]	0.29	–
Total	100	100
Calculated dry matter intake	14 kg	17 kg

[a]Vitamin/mineral supplement contain/kg: Vit A 1,000,000 IU, Vit D3 300,000 IU, Vit E 10,000 IU, Ca 6,118 mg, P 1,500 mg, Mg 5,000 mg, Mn 1,000 mg, Zn 1,000 mg, Cu 500 mg, Se 50 mg, Iodine 50 mg, Fe 1,000 mg, Co 5 mg and antioxidant 1,000 mg.

TABLE 2 The nutrients composition of dry cow rations in both far off and close up periods

Nutrients composition	Far off dry period	Close up dry period
Total % DMI	13.97	17
Forage (%)	74.34	46.89
Concentrate (%)	25.66	53.11
NEL3× (Mcal)	17.71	25.204
NEL3× (Mcal/kg)	1.27	1.482
Protein		
RUP (kg)	0.53	0.871
RUP (% DM)	3.77	5.128
RDP (kg)	0.87	1.468
RDP (% DM)	6.20	8.635
CP (kg)	1.39	2.339
CP (% DM)	9.97	13.763
NDF (% DM)	52.78	38.225
ADF (% DM)	32.29	22.00
NFC (% DM)	29.82	42.596
EE (% DM)	2.42	2.741
Ca/P ratio	1.51	1.719
DCAD (mEq/kg DM)	185.31	−131.834
Absorbable Ca (gr/day)	0.064	0.089
Dietary Ca (%)	0.456	0.528
Absorbable P (gr/day)	0.042	0.052
Dietary P (%)	0.302	0.307
Mg (%)	0.207	0.241
Cl (%)	0.521	0.895
K (%)	1.431	1.210
Na (%)	0.147	0.034
S (%)	0.155	0.321

Note: Concentrate, ADF, Acid Detergent Fibre; CP, Crude Protein; DCAD, Dietary Cation–Anion Difference; EE, Ether Extract; NDF, Neutral Detergent Fibre; NEL, net energy for lactation; NFC, Non-Fibre Carbohydrates; RDP, Rumen-Degradable Protein; RUP, Rumen-Undegradable Protein.

with 2 g/kg body weight/day of green banana peel powder for 5 days. The extract or powder was mixed with milk or warm water and was administered to the calves orally in a milk bottle. All other aspects of their diets were identical for all groups including the control group.

2.2 | Preparation of banana products

Ripe and completely green Bananas (*Musa. cavendish*) were purchased locally from a banana local market without any ethylene gas exposure and were stored at 20°C for 24 hr before extraction.

2.2.1 | Preparation of green banana peel extract

Green bananas were rinsed thoroughly in tap water, surface sterilized with 70% alcohol and then they were rinsed by distilled water to remove any contaminants. Peels were manually separated from the pulp and they were put into 70°C water for 20 s to inactivate polyphenol oxidases. The peels were cut into small pieces using a sharp knife and they were dried in an oven at 60°C for 38 hr. Then, the dried peel was ground into a powder with an industrial grinder. The milled peel was mechanically stirred for 2 hr (1 g in 10 ml distilled water) in a vacuum evaporator under reduced pressure at 60°C. After extraction, the extract was centrifuged for 15 min at 3,500 × rpm. The supernatant containing the water-soluble extracts was transferred into 50 ml falcon tubes and it was stored at −70°C until the experiment started.

2.2.2 | Preparation of overripe banana pulp extract

Yellow bananas were left at room temperature until peels became yellow brown and the edible portion became leaky (overripe). The peels were separated from the pulps by hand. The flesh was weighed, cut into appropriate sizes and mixed with a threefold weight of deionized water in a vacuum evaporator under reduced pressure at 60°C for 10 hr. The homogenate was centrifuged at I,800 *g* for 15 min. The obtained supernatant was transferred into 50 ml falcon tubes and was stored at −70°C until the experiment started.

2.2.3 | Preparation of green banana peel powder

The green peels were manually separated from the pulp and were cut into small pieces. Pieces were shade-dried for about 2 weeks and then they were crushed to make a coarse powder in a pulverizer. The powder was stored in cold, dry and dark place until the experiment started.

2.2.4 | Measuring dry matter

The moisture content of the extracts was calculated on the basis of weight loss after the sample had been heated in an oven at 105°C for 8 hr. The DM content of pulp and peel extracts were 45.86% and 8.5%, respectively.

2.3 | Blood sampling

The blood samples were taken on day 0 (at birth) and on days 7, 15 and 30 through the jugular vein with the aid of disposable syringes. 2.5 ml of blood were transferred into EDTA-3K tubes for haematological analysis and haemolysate preparation and 7.5 ml was transferred to plain tubes for serum separation. As soon as collection, all tubes were placed on ice and were immediately transferred to the laboratory. The blood in the plain tubes was allowed to clot at room temperature and then it was centrifuged for 15 min at 1,800 g for serum separation. The serum was aliquot into 1.5 ml microtubes and the sample code was written on them. The serum was frozen at −20°C until analysis.

2.4 | Evaluation of Heamogram and Leukogram

Complete blood count was performed using an automated haematology analyzer (Nihon Kohden, Cell Tac α, MEK 6450k, Tokyo, Japan). The blood smear was stained by Giemsa colour, and then differential leukocyte counts were performed on 100 WBC.

2.5 | Biochemical profile analysis

Glucose (Glu), albumin (Alb), total cholesterol (Chol), blood urea nitrogen (BUN), creatinine (Creat) and total bilirubin (bil) concentrations plus the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were measured by commercial colorimetric kits (Pars Azmoon, Tehran, Iran) using an auto analyzer (Mindray, BS-200E, Shenzhen, China). The inter assay and intra assay of all measurement methods were less than 5%. The control serum (Centronorm, centronic GmbH, Wartenberg, Germany) was used for controlling measurement accuracy. The plasma protein and fibrinogen amounts were determined by refractometry and heat precipitation methods, respectively.

2.6 | Measurement of immunological variables

The amounts of Immunoglobulin G (IgG, assay range: 2–600 μg/ml, sensitivity: 1.03 μg/ml), Interferon gamma (IFN-γ, assay range: 5–2,000 pg/ml, sensitivity: 2.35 pg/ml), tumour necrosis factor alpha (TNF-α, assay range: 10–30,000 ng/L, sensitivity: 5.56 ng/L), interleukin 4 (IL-4, assay range: 1–280 ng/L, sensitivity: 0.54 ng/L) and interleukin 10 (IL-10, assay range: 5–2,000 ng/L, sensitivity: 2.52 ng/L) were measured in the serum samples by enzyme-linked immunosorbent assay (ELISA) (Bioassay Technology Laboratory kits, Shanghai, China) using ELISA automatic washer and reader (Bio Tek, ELx-50, Winooski, USA, Bio Tek, ELx-800, Winooski, USA).

2.7 | Evaluating the calves performance and treatment duration

Body weight was recorded on days 0 (at birth), 7, 15, 22 and 30 (end of the trial) and the mean daily and total weight gains were calculated. Daily observation was made of each calf for diarrhoea and pneumonia signs and duration of treatments (days) for each disease was recorded.

2.8 | Statistical analysis

The data were subjected to repeated measure analysis of variance (ANOVA) using the PROC MIXED of SAS 9.2 (SAS institute Inc.). Normality of all variables was evaluated by the PROC UNIVARIATE. The outcome variables with Shapiro–Wilk values of ≥0.05 were considered as normal (MCV, FRAP, phosphorous, sodium, glucose, cholesterol, treatment duration) and all the other variables were transformed using a natural logarithmic transformation to reach a normal distribution. The time of sampling (0, 7, 15 and 30 day(s) relating to birth), group (control, groups 1, 2 and 3), sex and parity of dams were used as independent effects and all measured variables were considered as dependent. The calves were included as the random effect and the error term. The mathematic model was defined by the following equation:

$$Y_{ijklm} = \mu + G_i + T_j + S_k + P_l + (\mathrm{GT})_{ij} + (\mathrm{GS})_{ik} + (\mathrm{GP})_{il} + C_m + \mathrm{e}_{ijklmn}$$

where Y_{ijklm} = a dependent variable of calf with factors *ijkl* in calf *m*; μ = overall mean, $G_{i=}$ group $i = 0$ (Control, no treatment) or 1 (Group 1, receiving 2 g (dry matter)/kg body weight/day of overripe banana pulp extract) or 2 (Group 2, receiving 1 g (dry matter)/kg body weight/day of overripe banana pulp extract) 3 (Group 3, receiving 2 g/kg body weight/day of green banana peel powder)], $T_{j=}$ time of sampling [$j = 1$ (0 day) or 2 (7th day) or 3 (15th day) or 4 (30th day)], S_k = sex [$k = 1$ (female) or 2(male)], P_l = parity of dams ($l = 1$ (1st) or 2 (2nd) or 3 (3rd) or 4 (4th or more)], $(\mathrm{GT})_{ij}$ = interaction between group and time, $(\mathrm{GS})_{ik}$ = interaction between group and sex, $(\mathrm{GP})_{il}$ = interaction between group and parity of dams, $C_{m=}$ random calf effect and $e_{ijklmn=}$ random error term.

The results were expressed as means ± standard errors of means (*SEM*) in each group. The effect of independent factors was considered significant at $p < 0.05$, whereas a trend towards significance was noted for $0.05 < p < 0.1$. In parameters which had some significant differences between the groups at time 0 (day 0) (MCV, monocyte, total protein and bilirubin), the effect of time 0 was considered as a covariate and all of the analysis were corrected based on this.

3 | RESULTS

The treatment had no significant effect on the measured variables ($p < 0.05$) and just a trend towards significance was observed for the average daily weight gain ($p = 0.073$). Age (time of sampling) had a significant effect on the values of most measured variables ($p < 0.05$) and a trend towards significance was observed for IL-4 and WBC ($p = 0.058$ and $p = 0.077$, respectively). No significant effect was seen for IL-10, TNF-α, IFN-γ, PCV, monocyte, haemoglobin and BUN. The significant group and the sampling time interactions were seen for the quantities of RBC, MCV and MCH ($p < 0.05$). A trend towards significance in the values of IgG and bilirubin was observed ($p = 0.052$ and $p = 0.058$, respectively), while there were no significant effects for the other variables (Tables 3–7).

No significant differences were detected in the total weight gain and the mean body weight between the trial groups, although the values were higher in test group three than the other three groups. Moreover, there was no significant difference in treatment duration (day) among trial groups (mean days of treatment were 2.61 ± 0.64 in control group, 1.43 ± 0.69 in group 1, 1.78 ± 0.71 in group 2 and 2.03 ± 0.69 in group 3).

4 | DISCUSSION

In the present study, average daily gain (ADG) was higher in the calves of group 3 in comparison with the control group and in calves of group 2 in comparison with group 1. In agreement with our results, in an earlier study (Ibrahim et al., 2000), the highest average daily live weight gain was reported in cattle supplemented with banana diet and it was attributed to the high starch and energy content of bananas. In contrast to these results, in the study of Renaudeau et al. (2014) on pigs, higher ADG was reported in the control group. They suggested that the lower crude protein content of banana meal resulted in lower ADG in the treatment group.

The number of RBC on day 30 of life was significantly higher in calves of groups 2, 3 and in the control group in comparison with group 1 (Table 7). Banana pulp and peel are good sources of iron, which plays an important role in haemoglobin production and helps in the treatment of anaemia (Anhwang et al., 2008; Mohapatra et al., 2010). Also, banana pulp and peel are good sources of antioxidants that protect RBCs from oxidative damages (Sundaram et al., 2011). These data are in disagreement with our results that show no effect of treatments on RBC counts in comparison with control group and also with the result obtained from calves in group 1 which show a lower number of RBC compared with the control group. Therefore, the reason for different results in the present study in comparison with previous studies is not clear. In calves supplemented with both overripe banana pulp and unripe banana peel extract and also those supplemented with unripe banana peel powder, the number of RBC was higher than the animals supplemented with pulp extract. This may be related to the higher quantity of antioxidants in peel which prevents structural and functional components of cells from free radicals. It was reported that oxidative stress may have a role in pathogenesis of anaemia (Rajabian et al., 2017). In the study of Sundaram et al. (2011) on *Musa paradisiac*, they reported that the banana peel compared to the pulp, contains high levels of micronutrient

TABLE 3 The effect of treatments (banana pulp extract, pulp extract + peel extract and peel powder) on haematological parameters (Means and *SE*) between trial groups

Parameter	Control	Group 1[a]	Group 2[b]	Group 3[c]	*SE*[d]	Age	Group	Age × group
TP (g/dl)	6.11	6.18	6.29	6.46	0.15	S	NS	NS
Fib (mg/dl)	368/78	399.19	393	431.69	35.99	S	NS	NS
PCV (%)	27.20	26.80	26.04	25.25	1.31	NS	NS	NS
RBC (10^6/μl)	7.54	7.45	7.36	7.31	0.36	S	NS	S
Hb (g/dl)	9.41	9.12	9.04	8.77	0.46	NS	NS	NS
MCV (fl)	34.40	34.32	34.61	33.23	0.64	S	NS	S
MCH (pg)	12.51	12.99	12.39	12.08	0.40	S	NS	S
MCHC (g/dl)	34.83	34.75	34.72	34.82	0.17	S	NS	NS
WBC (10^3/μl)	10.49	10.62	11.35	11.33	0.95	T	NS	NS
Neut (10^3/μl)	4.05	4.43	5.53	5.22	0.58	S	NS	NS
Lymph (10^3/μl)	5.47	5.46	4.92	5.10	0.52	S	NS	NS
Eos (10^3/μl)	0.06	0.03	0.05	0.02	0.02	S	NS	NS
Mono (10^3/μl)	0.77	0.63	0.70	0.77	0.13	NS	NS	NS
Band (10^3/μl)	0.11	0.05	0.12	0.19	0.05	S	NS	NS
Plt (10^5/μl)	3.89	3.96	4.30	5.05	0.37	S	NS	NS

Note: Eos, eosinophil; Fib, fibrinogen; Hb, haemoglobin; Lymph, lymphocyte; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; Mono, monocyte; Neut, neutrophil; NS, not significant effect; PCV, packed cell volume; Plt, platelet; RBC, red cell count; S, significant effect ($p < 0.05$); T, trend for significant effect ($0.05 < p < 0.1$); TP, total protein; WBC, white blood cell count.

[a]Overripe banana pulp extract supplemented group.

[b]Overripe banana pulp extract + green banana peel extract supplemented group.

[c]Green banana peel powder supplemented group.

[d]Standard error.

TABLE 4 The effect of treatments (banana pulp extract, pulp extract + peel extract and peel powder) on biochemical parameters (Means and *SE*) between trial groups

Parameter	Control	Group 1[a]	Group 2[b]	Group 3[c]	*SE*[d]	Age	Group	Age × Group
Alb (g/dl)	3.22	3.33	3.26	3.23	0.09	S	NS	NS
BUN (mg/dl)	36.86	30.35	33.43	35.24	2.35	NS	NS	NS
Creat (mg/dl)	1.51	1.52	1.52	1.54	0.07	S	NS	NS
Glu (mg/dl)	108.99	110.27	120.06	104.15	4.22	S	NS	NS
Chol (mg/dl)	76.92	92.39	95.43	86.81	6.80	S	NS	NS
Bilirubin (mg/dl)	0.02	0.08	0.12	0.07	0.01	S	NS	T
ALT (IU/L)	8.71	8.93	8.21	8.79	0.90	S	NS	NS
AST (IU/L)	26.22	23.62	27.65	27.93	2.97	S	NS	NS

Note: Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Chol, Cholesterol; Creat, Creatinine; Glu, glucose; NS, not significant effect; S, significant effect ($p < 0.05$); T, trend for significant effect ($0.05 < p < 0.1$).

[a]Overripe banana pulp extract supplemented group.

[b]Overripe banana pulp extract + green banana peel extract supplemented group.

[c]Green banana peel powder supplemented group.

[d]Standard error.

especially ascorbate, catechin, gallocatechin and dopamine all of which act as antioxidants and enhance RBC resistance to oxidative stress. Furthermore, in the unripe stage, activities of phenolic compounds and antioxidant enzymes are higher and by fruit maturation and ripening these amounts decrease. In agreement with those findings, it was shown that unripe peel extracts have higher inhibition on erythrocyte haemolysis than ripe peel and leaky ripe peel extracts (Sundaram et al., 2011).

The results for MCH value are completely opposite to the results for RBC number. In calves of group 1, MCH values were more than the amounts in calves of the other three groups on day 30 of life (Table 7). Both the pulp and the peel are rich in vitamin C and iron

TABLE 5 The effect of treatments (banana pulp extract, pulp extract + peel extract and peel powder) on immunological parameters (Means and *SE*) between trial groups

Parameter	Control	Group 1[a]	Group 2[b]	Group 3[c]	*SE*[d]	Age	Group	Age × Group
IgG (μg/ml)	112.05	95.79	122.53	112.15	11.88	S	NS	T
TNFα (ng/L)	455.65	391.51	464.84	468.14	42.02	NS	NS	NS
IFNɣ (pg/ml)	399.22	358.17	417.91	401.08	25.02	NS	NS	NS
IL-4 (ng/L)	66.36	66.24	72.21	64.00	5.99	T	NS	NS
IL-10 (ng/L)	436.98	362.81	460.64	416.40	62.27	NS	NS	NS

Note: IFNɣ, interferon ɣ; IgG, immunoglobulin G; IL-10, interleukin 10; IL-4, interleukin 4; NS, no significant effect; S, significant effect ($p < 0.05$); T, trend for significant effect ($0.05 < p < 0.1$); TNFα, tumour necrosis factor α.

[a]Overripe banana pulp extract supplemented group.

[b]Overripe banana pulp extract + green banana peel extract supplemented group.

[c]Green banana peel powder supplemented group.

[d]Standard error.

TABLE 6 The effect of treatments (banana pulp extract, pulp extract + peel extract and peel powder) on total weight and average daily gain (Means ± *SE*) between trial groups

Parameter	Control	Group 1[1]	Group 2[2]	Group 3[3]	*SE*[4]	Age	Group	Age × Group
Total weight gain (kg)	4.467 ± 0.17	4.646 ± 0.16	4.722 ± 0.16	4.844 ± 0.18	0.17	S	NS	NS
Average daily gain (kg)	0.38 ± 0.09[ab]	0.23 ± 0.08[ac]	0.42 ± 0.09[bd]	0.39 ± 0.08[cd]	0.08	S	T	NS
Mean body weight (kg)	44.6 ± 1.64	46.44 ± 1.71	47.86 ± 1.72	48.36 ± 1.72	1.69	S	NS	NS

Note: Means within rows lacking a common superscript had trend towards significance ($0.05 < p < 0.1$)

NS, no significant effect; S, significant effect ($p < 0.05$); T, trend for significant effect ($0.05 < p < 0.1$).

[1]Overripe banana pulp extract supplemented group.

[2]Overripe banana pulp extract + green banana peel extract supplemented group.

[3]Green banana peel powder supplemented group.

[4]Standard error.

but as Mohapatra et al. (2010) and others reported the amount of iron in pulp is (0.75 ± 0.22–0.83 ± 0.19) higher than its amounts in the peel (0.61 ± 0.22). Moreover, the tannin content of pulp (1,259 ± 29.2 mg/100 g) was 1.26% lower than its amount in peel (1,670 ± 21.2 mg/100 g). Tannin interferes with iron absorption and so it influences the nutritional value of certain plants like banana (de Angelis-Pereira et al., 2016). Based on these findings, the lower level of MCH in calves supplemented with the peel (extract or powder) may be related to the lower content of iron and higher content of tannin in the peel.

On day 30 of life, the amounts of MCV in calves of the control group and the calves of group 1 were higher than the calves of group 3. This result is in accordance with the results obtained from MCH and shows the physiological changes indicating that the lower size of RBC coincides with lower content of haemoglobin (Mohri et al., 2007).

The amounts of bilirubin in calves of the control group were lower than its amounts in calves of group 2 on days 7 and 30, calves of group 1 on day 7 and calves of group 3 on day 15. In contrast to our results from the comparison between control group and treatment groups, Eleazu and Okafor (2015) reported a significant reduction in the amounts of total and conjugated bilirubin in diabetic rats fed unripe plantain pulp and attributed this to the enhancement of liver function. Furthermore, the amounts of bilirubin in the calves of group 3 in comparison with the calves of group 2 were lower on days 7 and 30. Cytochrome P450 1A2 (MROD) significantly contributes to the metabolism of bilirubin (Zaccaro et al., 2001) and a decline in MROD activity in the murine hepatocytes under the influence of banana supplement has been reported (Chatuphonprasert & Jarukamjorn, 2012) which is in agreement with our results. In addition, the amounts of unsaturated fatty acids (linolenic, linoleic and oleic acids) in both banana pulp (2,779 mg/kg D.M.) and peel (3,858 mg/kg D.M.) are high (Oliveira et al., 2008) and it was shown that unsaturated fatty acids decline the rate of bilirubin conjugation, which may be due to the inhibition of glucuronyl transferase (Hargreaves, 1973) and this report is also in agreement with our results.

On days 15 and 30, the values of IgG in calves of group 1 were lower than the values of control group and group 2. Furthermore, the amount of IgG on day 15 in calves of group 3 was lower than in

TABLE 7 The effect of treatments (banana pulp extract, pulp extract + peel extract and peel powder) on parameters (Means ± *SEM*) with a significant age and group interaction in calves

Age	Control	Group 1[1]	Group 2[2]	Group 3[3]
RBC (10^6/μl)				
Day 0	6.84 ± 40.64^{a}	7.00 ± 0.43^{a}	6.86 ± 0.44^{a}	6.46 ± 0.43^{a}
Day 7	7.38 ± 40.64^{a}	7.53 ± 0.43^{a}	7.14 ± 0.44^{a}	7.08 ± 0.43^{a}
Day 15	7.71 ± 40.64^{a}	7.90 ± 0.43^{a}	7.32 ± 0.44^{a}	7.50 ± 0.43^{a}
Day 30	8.24 ± 40.64^{a}	7.35 ± 0.43^{b}	8.13 ± 0.44^{ac}	8.18 ± 0.43^{ac}
MCV (fL)				
Day 0	38.57 ± 0.72^{a}	36.59 ± 0.84^{a}	38.74 ± 0.75^{a}	38.13 ± 0.84^{a}
Day 7	36.16 ± 0.72^{a}	35.33 ± 0.84^{a}	36.7 ± 0.75^{a}	35.71 ± 0.84^{a}
Day 15	34.75 ± 0.72^{a}	33.7 ± 0.84^{a}	34.94 ± 0.75^{a}	33.56 ± 0.84^{a}
Day 30	32.3 ± 0.72^{a}	33.91 ± 0.84^{a}	32.17 ± 0.75^{ab}	30.42 ± 0.84^{b}
MCH (pg)				
Day 0	13.43 ± 0.74^{a}	12.62 ± 0.78^{a}	13.34 ± 0.79^{a}	13.25 ± 0.78^{a}
Day 7	12.92 ± 0.74^{a}	12.30 ± 0.78^{a}	12.90 ± 0.79^{a}	12.57 ± 0.78^{a}
Day 15	12.34 ± 0.74^{a}	11.48 ± 0.78^{a}	12.22 ± 0.79^{a}	11.80 ± 0.78^{a}
Day 30	11.35 ± 0.74^{a}	15.54 ± 0.78^{b}	11.09 ± 0.79^{ac}	10.70 ± 0.78^{ac}
Bilirubin (mg/dl)				
Day 0	0.49 ± 0.02^{a}	0.68 ± 0.01^{a}	0.44 ± 0.02^{a}	0.43 ± 0.01^{a}
Day 7	0.04 ± 0.02^{a}	0.11 ± 0.01^{b}	0.16 ± 0.02^{c}	0.09 ± 0.01^{ab}
Day 15	0.01 ± 0.02^{a}	0.07 ± 0.01^{ab}	0.09 ± 0.02^{ab}	0.1 ± 0.01^{b}
Day 30	0.01 ± 0.02^{a}	0.07 ± 0.01^{ab}	0.1 ± 0.02^{b}	0.04 ± 0.01^{a}
IgG (μg/ml)				
Day 0	96.63 ± 14.31^{a}	115.52 ± 15.18^{a}	100.73 ± 15.44^{a}	109.37 ± 15.18^{a}
Day 7	102.63 ± 14.31^{a}	87.99 ± 15.18^{a}	110.91 ± 15.44^{a}	107.18 ± 15.18^{a}
Day 15	134.44 ± 14.31^{ac}	96.83 ± 15.18^{b}	165.20 ± 15.44^{c}	124.01 ± 15.18^{ab}
Day 30	114.49 ± 14.31^{a}	82.82 ± 15.18^{b}	113.27 ± 15.44^{a}	108.04 ± 15.18^{ab}

Note: Means within rows lacking a common superscript were significantly different ($p < 0.05$ or $0.05 < p < 0.1$).

[1]Overripe banana pulp extract supplemented group.

[2]Overripe banana pulp extract + green banana peel extract supplemented group.

[3]Green banana peel powder supplemented group.

calves of group 2. BanLec is a special lectin as it is highly immunogenic in man and causes a strong IgG4 antibody response (Koshte et al., 1990). In agreement with this, it was reported that BanLec is capable of inducing IgG4 formation, being as a T-cell mitogen, binding mannose and some of its oligosaccharides (Mo et al., 2001) and inducing Th1 cytokines production (Souza et al., 2013). Based on Singh et al.'s (2014) report, lectin content of pulp and roots of mature banana is high and it is one of the predominant proteins in ripe bananas (*Musa acuminata L.*) pulp, but it is absent in other tissues like peel. On the other hand, ripening increases the amount of lectin yield from 0.006 mg/g in normal ripeness stage to 0.2 mg/g in further ripening stage (Wearne et al., 2013). These reports are in contrast with our results that show higher values of IgG in calves of control group in comparison with calves of group 1. This may be attributed to the differences between the present study and the previous studies in relation to dose, duration and the route of banana supplementation.

Studies in human revealed that lectin binds to antigen binding sites of IgG and antibody response to lectin is predominantly IgG particularly IgG4 and much less IgG1 and IgA. Furthermore, as a result of interaction between lectin and C3, the normal tolerance induction is prevented and the immune response progress to IgG4 antibody production (Koshte et al., 1992). Also, proliferation of CD3+, CD4+ and CD8+ T cells, but not B cells or monocytes were resulted in response to both the recombinant and the natural forms of BanLec (Sansone et al., 2016).

The important point is that both rBanLec concentration and functional characteristics of its target cells determine the result of rBanLec stimulation (Marinkovic et al., 2017). Since, according to these previous studies, after administration of overripe banana pulp extract, the serum IgG level decreased because of the lectin bound to IgG; on the other hand, the number of B cell, secretion of IgG and complement activity are low in the first month of life (Chase et al., 2008; Hernández-Castellano et al., 2018), so the induction of

IgG response did not occur and the amounts of serum IgG in calves of group 1 were lesser than the other groups.

The dark spots on fruits in overripe banana are attributed to the presence of melanin. Wade et al. (1993) reported that dopamine oxidation by polyphenoloxidase produces black melanin in overripe bananas. Furthermore, Sidhu and Zafar (2018) attributed the decreased concentration of dopamine in overripening stage of banana pulp to its oxidation to quinones that may polymerize to melanin. The reduced levels of IL-1β and IL-6 attributed to the immunosuppressive effects of melanin (Avramidis et al., 1998; ElObeid et al., 2017; Kunwar et al., 2012; Liu & Nizet, 2009; Mohagheghpour et al., 2000; Tajima et al., 2019) in overripe pulp may be the other reason of the lower levels of IgG in the calves of group 1. Endogenous IL-1 beta (not IL-1 alpha) is required in T-cell-dependent antibody production (Nakae et al., 2001). Beside, in vitro studies showed IL-6 as a B-cell growth factor and inducer of plasma cell differentiation and in vivo it has an important role in antibody production and class switching. IL-6 via increasing the production of IL-21 boost the B-cell helper capabilities of CD4 (+) T cells and consequently increases antibody production. In IL-6-deficient mice, the levels of antigen-specific IgG1, IgG2a and IgG3 were reduced (Dienz et al., 2009). Originally, IL-6 was recognized as a B-cell differentiation factor which induce terminal B-cell differentiation and involve in IgG production (Maeda et al., 2010).

A significantly higher IgM levels were reported in fish (*Labeo rohita*) receiving yellow banana peel flour for 30 days and this was attributed to immunostimulants (Giri et al., 2016). An immunostimulatory effect was reported for banana peel which was attributed to cell-mediated and humoral-antibody-mediated activation of T and B cells (Singhal & Ratra, 2013). In general, the mineral content of peels is higher than that of pulps (except for magnesium). The effects of zinc on antibody production have been proved and its amounts in bananas vary from 0.1430 to 2.7360 μg/g (Islam et al., 2012). B-lymphocyte mitogenic and cytokine response to lipopolysaccharide require zinc and B-cell lymphopoiesis and antibody-mediated immune defence especially the production of IgG are compromised in zinc deficiency (Iñigo-Fig ueroa et al., 2013; Shankar & Prasad, 1998). Increased total immunoglobulin concentrations were reported in weaned calves received organic zinc supplementation (Dresler et al., 2016). Besides these direct effects on antibody production, zinc is one of the two metals which banana pulp lectin requires. Therefore, it affects antibody production in this way (Kobayashi et al., 2014). The other mineral which affects antibody production is potassium which is more abundant in peel (1,062.1 ± 33.5 to1387.5 ± 22.5 mg/100 g fresh weight) than pulp (295.7 ± 6.6 to 463.6 ± 5.7 mg/100 g fresh weight). In the study conducted by Sato et al. (1989) on human–human hybridoma cells (HB4C5), it was revealed that potassium phosphate stimulated the monoclonal antibody production. Similarly, it was reported that potassium acetate increased antibody production on a per cell basis (Fong et al., 1997). In another study, it was showed that potassium supplementation in broiler chicken results in elevation of lymphocyte percentage (Zarrin-Kavyani et al., 2018). These reports are in agreement with the results of the present study which shows higher values of IgG in calves supplemented with both extracts compared with the calves of groups 1 and 3. This can be attributed to the synergistic effect of the extracts on each other in IgG production.

No significant differences were obtained in total weight gain and mean body weight in the present study. Treatment also did not have any significant effects on the number of lymphocytes and granulocytes and the values of measured cytokines. In broiler chickens supplemented with green bananas, daily weight gain was significantly elevated. Giri et al. (2016) reported that in Labeo rohita supplemented with yellow banana peel flour for 60 days, the final weight gain, gene expression of IL-1β and TNF-α were significantly higher (Giri et al., 2016). Prawns supplemented with green banana peel extract for 120 days resulted in a significant increase in granular cell number (Rattanavichai et al., 2015). Iwasawa and Yamazaki (2009) orally administered two different varieties of banana pulp juice at different stages of maturity to mice for 2 weeks. The amounts of TNF-α in the supernatant of cultured peritoneal exudate cells were evaluated. They concluded that in comparison with the control group, in the banana supplementation group, the amounts of TNF-α were significantly increased. Matsuda et al. (2006) administered banana to 1-month-old F1 hybrid calves for 5 days and examined the leukocytes subset. They founded an increase in the number of T-lymphocytes subsets in the treatment group.

5 | CONCLUSION

In conclusion, banana supplementation in neonatal calves had beneficial effects on the values of RBC, MCV, MCH, bilirubin, IgG and average daily weight gain. But according to low number of calves in each group and resulted low power of statistical test further studies will be need for evaluating the effects of supplementation on variables without significant difference between groups and also to identify the optimal dosage and duration of banana supplementation in dairy calves.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTION

Nafiseh Keyvanirad: Conceptualization; Investigation; Writing-original draft; Writing-review & editing. **Mehrdad Mohri:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. **Hesam A Seifi:** Data curation; Methodology; Validation; Visualization; Writing-review & editing. **Alireza Haghparast:** Methodology; Writing-review & editing.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has

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