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Full Length Research Paper

Effects of *Spirulina platensis* on performance, digestibility and serum biochemical parameters of Holstein calves

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This study was conducted to investigate the effects of different amounts of *Spirulina platensis* (SP) on the performance and digestibility of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), organic matter (OM), plasma cholesterol, LDL, HDL, BUN, albumin and globulin concentration of Holstein calves. Twenty four Holstein calves were randomly assigned to 4 treatments including 0, 2, 6 and 25 g per day of SP (designated as S0, S2, S6 and S25, respectively) each in 6 replicates for 57 days. Starter diet plus milk were used as basal diet for all treatments. Results showed that treatment effect was not significant on the final weight, daily gain, daily feed intake, feed efficiency and digestibility coefficient (P>0.05), while increase in the spirulina level up to 25 g, decreased digestibility of DM, CP, NDF and OM. Significant reduction in plasma cholesterol, LDL and HDL concentration were observed for S25 related to other groups (P<0.05). However, other blood parameters like BUN, albumin and globulin were not affected by spirulina (P>0.05).

Key words: Algae*, Spirulina platensis,* calves, performance, digestibility, serum biochemical parameters.

INTRODUCTION

Spirulina platensis (SP) is a blue-green microalga which has been considered as a suitable nutritional supplement because it is highly protein rich and highly abundant in vitamins and minerals (Kay, 1991; Belay, 2002). Application of SP in human ration has become popular after being successfully used as a food supplement by astronauts (Karkos et al., 2008). However, scarce studies have been undertaken on its usage in animal feeding (Grinstead et al., 2000; Qureshi et al., 1995). Chicks fed by SP had heavier spleen and thymus with lower microbial infection related to their compartments, which indicated its advocating effect on the immune system (Qureshi, 1995). Moreover, all growth parameters of chicks were similar in chicks which were fed diets with SP compared to control group (Habib et al., 2008). Replacing of skim milk with *Spirulina maxima* in pig diets showed similar performance (Yap et al., 1982). Swapping of soybean meal with SP in pig diets showed reduction in average daily gain (ADG) from days 0 to 14, while no significant difference was observed from days 14 to 28 or days 0 to 28 after weaning (Grinstead et al., 2000). Peiretti and Meineri (2008) reported that supplementation of 5, 10 and 15% SP had no significant effect on the final weight, average daily gain and feed efficiency in rabbits, while 10% SP increased the feed consumption (Peiretti and Meineri, 2008).

In a human study, it was noticed that supplementation of SP in the diet of ischemic heart disease patients led to significant reduction in blood cholesterol, triglyceride, LDL cholesterol and induction in HDL cholesterol (Karkos et al., 2008). Researches on mice had shown that addition SP to the diet for 14 successive days decreased LDL, HDL and cholesterol significantly compared to the control group (Belay, 2002; Nayaka, 1988). For dairy cows scenario, fishmeal, groundnut and soybean meal can be partially replaced by SP. However, there is insufficient information about these possible replacements. The only study in which SP was used for dairy cows showed that SP supplementation of 2 g/day per cow, significantly increased milk, fat, protein and lactose yields and decrease in somatic cell count in comparison with control group (Simkus et al., 2007). This experiment was aimed to assess the effects of SP supplementation on the

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Table 1. Ingredients of starter (%).

Case	%
Corn	50
Barley grain	9
Canola seeds	5
Soybean meal	27
Fish meal	2
Fat	3.5
Ferrous Sulfate	0.1
Dicalcium phosphate	0.8
Salt	0.8
Vitamin–mineral premix	1
Sodium Hydrogen Carbonate($NaHCO_3$)	0.6
Vit A	0.2

Table 2. Chemical composition of the starter (%).

Case	%
Dry matter	90.35
Ether extract	5
Crude protein	22.4
Crude fiber	5.5
Ash	4.5
Ca	0.462
P	0.543
Neutral detergent fiber	34.5
Acid detergent fiber	26

Table 3. Chemical composition of the spirulina.

Case	Value
Dry matter (%)	96.5
Ether extract (%)	5.5
Crude protein (%)	62.5
Crude fiber (%)	9
Ash (%)	7.5
Ca (mg/100 g)	400
P (mg/100 g)	900
Fe (mg/100 g)	70
K (mg/100 g)	1475
ME (M cal/kg)	3.15

digestion, performance and some blood parameters in Holstein calves.

MATERIALS AND METHODS

In this experiment, the effects of adding 0, 2, 6 and 25 g of SP, designated as SP0, SP2, SP6 and SP25) on calf performance and digestibility of DM, CP, NDF, OM, plasma cholesterol, LDL, HDL, BUN, albumin and globulin concentration in calves were investigated. Twenty four calves, 18±7 days old and 42.2±8.6 kg weight, were assigned to each mentioned group with six replicates in a completely randomized design. Each calf was environmentally controlled in separate boxes, fed with its allotted diet for 57 successive days. The basal diet was formulated based on starter and milk plus 0, 2, 6 and 25 g per day of SP. SP intake was in the form of powder, prepared from Qheshm SinaRizJolbak Company, Tehran, Iran. The chemical composition of the starter and algae are presented in Tables 1 and 2, respectively. The calves were fed with starter and milk. SP powder was added to their milk. To investigate the calves' performance, subsequent weightings were conducted once every two weeks after they were kept hungry 12 to 14 h before the morning feedings. Feed intakes were also measured weekly.

The apparent digestibilities of the four diets were calculated within the last week of the study period. The faeces were collected from three calves pertaining to common treatment over a period of seven days. Each pooled faecal sample was taken and placed in tow-layer plastic bag to prevent moisture loss and immediately frozen at -20°C. The samples were individually mixed and homogenized followed by weighting and drying in an oven at 80°C and stored for the next chemical analyses. The proximate composition of the starter, algae and faeces was determined according to the AOAC method (Association of Official Analytical Chemists, 1990). The samples were analyzed to estimate DM, ash by ignition to 550°C and then ether extract (EE), crude fiber, using Soxhlet method and Weende's method, respectively. To assess NDF without sodium sulfite and α-amylase and acid detergent fiber (ADF), the described procedure by Van Soest et al. (1991) was applied. The acid insoluble ash content of the diets and faeces was calculated according to the method of Van Keulen and Young (1977). The digestibility coefficients were calculated based on the indirect digestibility method (Furuichi and Takahashi, 1981) using acid insoluble ash (AIA) as an inert marker. Calculation of the DM digestibility was as follows:

$$\text{DM digestibility (\%)} = (1-A/B) \times 100,$$

where XA and XB are the concentrations of X in the feed where A and B are the acid insoluble ash concentrations in the feed and faeces, respectively. The digestibility of the other nutrients (X) was calculated as follows:

$$\text{Digestibility (X in \%)} = (1- A/B \times XB/XA) \times 100,$$

where XA and XB are the concentrations of X in the feed and faeces, respectively.

Blood samples were collected from the jugular vein in days 1, 21 and 57 of the experiment, immediately sent to the laboratory to separate the serum and assign the cholesterol, LDL, HDL, BUN, albumin and globulin by Auto Analyzer system (model 2002). Statistical analysis was performed using the SAS (9.0) package. Variance analysis was used to evaluate the effects of different concentrations of SP on growth performance, digestibility of the four different diets and serum biochemistry parameters in calves. Mean comparisons were performed using Tukey's post-hoc test and considering $P<0.05$ as level of significance.

RESULTS

Performance traits including initial weight, live weight, daily gain, daily feed intake and feed efficiency in four consecutive periods (each period was taken in two weeks) are shown in Table 3. There was no significant

Table 4. The effect of spirulina on performance traits in different experimental periods.

Case*	Spirulina level(g)				Standard error of measurement(SE)
	0	2	6	25	
Primary weight(kg)	42.58	43.5	42.25	40.5	9.15
Second week					
Live weight(kg)	48.6	50.5	52.17	46.82	11.78
Daily gain(kg)	0.43	0.5	0.71	0.45	0.28
Daily feed intake(kg)	0.45	0.53	0.78	0.39	0.46
Feed efficiency	1.03	0.91	1.11	0.94	0.49
Fourth week					
Live weight(kg)	57.42	59.25	62.5	56.17	14.78
Daily gain(kg)	0.63	0.63	0.74	0.67	0.26
Daily feed intake(kg)	0.81	0.86	1.14	0.75	0.61
Feed efficiency	1.3	1.24	1.4	1.12	0.48
Sixth week					
Live weight(kg)	68.92	72.17	73.67	69.25	17.42
Daily gain(kg)	0.82	0.92	0.8	0.93	0.23
Daily feed intake(kg)	1.15	1.24	1.37	1.09	0.59
Feed efficiency	1.37	1.26	1.67	1.16	0.44
Eighth week					
Daily gain(kg)	0.85	0.92	0.97	0.94	0.2
Daily feed intake(kg)	1.48	1.77	1.69	1.56	0.68
Feed efficiency	1.72	1.84	1.74	1.64	0.49
Final weight(kg)	80.75	85.08	87.25	82.42	19.25

* For all performance traits, the differences among treatment means were not statistically significant (P>0.05).

difference among calves' initial weights in four groups. Application of S6 during all periods maximized the live weight. Average daily gain of calves attributed to S6 group was greater than other groups in all periods, except in the third period, in which S2 got the first rank. Average daily feed intake of S6 during the first three successive periods was higher than other groups. Ascending usage of SP did not increase daily gain, while improvement in feed efficiency was achieved when 25 g of SP was supplemented. Even though, numerical discrepancy existed among different groups for all performance traits, these differences were not statistically significant (P>0.05). Estimated apparent digestibility coefficients are presented in Table 4. The rate of the digestibility among treatments were not significantly different (P>0.05). Digestibility of DM, CP and OM in the control diet was higher than those of SP diets. It was interesting that increased in the SP level led to decrease in digestibility of all mentioned nutrient materials. The only exception in this scenario was observed for NDF, which SP6 had slightly higher digestibility than SP2 group. On the other side, supplementation of 25 g SP, directed the lowest digestibility for all measured cases.

Finally, we measured blood parameters to see if there is any significant change following inclusion of different SP levels (Table 6). Increasing the SP level from 0 to 25 g reduced blood cholesterol, LDL and HDL. The amounts of cholesterol, LDL and HDL via S2 and S6 treatments were not significantly different from the control treatment. However, supplementation of 25 g SP led to significant decrease in Cholesterol, LDL and HDL parameters compared to the control group (P<0.05). Also, no significant difference was observed among S2, S6 and S25 groups for cholesterol and HDL traits (P>0.05). The blood LDL concentration was not significantly different between S2 and S6 treatments, while there was a significant difference between S25 and S2 treatments (P<0.05). Assessment of nitrogen related parameters like BUN, Albumin, Globulin and Albumin/Globulin ratio are presented in Table 5. There was not a systematic trend in changing of these parameters when ascending amount of SP was supplemented into Holstein calves diet. Mean comparisons for these parameters could not reveal any significant differences (P>0.05). We could not detect association of SP level with these nitrogen related parameters.

Table 5. The apparent digestibility coefficients (means) of treatments (%).

Case*	Spirulina level(g)				Standard error of measurement(SE)
	0	2	6	25	
Dry matter	80	75.67	75	73.33	0.0633
Crude protein	74.48	73.29	72.6	67.95	0.06
Neutral detergent fiber	58.42	48.43	49.73	44.94	0.13
Organic matter	79.18	73.44	73.23	70.95	0.055

* Estimated digestibility parameters did not significantly different among treatment groups (P>0.05).

Table 6. Mean comparisons of cholesterol, LDL, HDL, BUN, albumin, globulin (g/dl) and albumin/globulin between treatments in different periods of the experiments.

Case*	Spirulina level(g)				Standard error of measurement(SE)
	0	2	6	25	
Cholesterol	148.3[a]	120.67[ab]	120.67[ab]	99 [b]	19.27
LDL	34.6[a]	33.67 [a]	29.67[ab]	26[b]	3.61
HDL	114.3[a]	98.67[ab]	98.67[ab]	83.67[b]	14.63
BUN	11.44	14.96	13.45	14.38	2.08
Albumin	3	3.33	3.33	3.23	0.27
Globulin	3.43	3.43	3.7	3.23	0.47
Albumin/Globulin	0.88	1.03	0.9	1.00	0.18

ab, Means in each column letters are different but these difference were not numerically significant. a, b the means in each column with different letters are significant (P<0.05).

DISCUSSION

In this study, we supplemented 3 levels of SP on Holstein calves to see how it acts on their performance and digestibility along with blood lipid and protein factors. Since, the method of algae preparation was considered to be largely effective on its protein content utilization (Peiretti and Meineri, 2008; Grinstead et al., 2000), we mixed SPs with appropriate amount of wheat powder and made pellet forms for calves usages. Use of 6 g SP led to the highest final weight, daily feed intake and daily gain in most of the growth periods. However, the best feed efficiency pertained to S25 group. In terms of digestibility parameters, increase in SP level caused decrease in digestibility of NDF, CP, DM and OM. Digestibility of DM, OM, CP, CF, EE, NDF and ADF was reported to be higher in the control diet in comparison with SP diet (Peiretti and Meineri, 2008). In rabbit's diet, use 5, 10 and 15% SP showed no significant difference on the final weight and daily gain among different treatments. This could be due to the fact that reduction in digestibility due to applying SP in the diets had been compensated by the increase in feed intake (Peiretti and Meineri, 2008).

Spirulina is a “micro vegetable” that can provide some of the antioxidants needed. Many studies have also revealed that antioxidants like the carotenoids in fruits, vegetables, and *Spirulina* have a synergistic effect (Belay, 2002). *Spirulina* also contains phycocyanin and polysaccharides, both known to have antioxidant properties. In addition, antioxidants that have a direct effect on reactive oxygen species, *Spirulina* contains an important enzyme, superoxide dismutase (1,700 units/g), that acts indirectly by slowing down the rate of oxygen radical generating reactions (Belay, 2002). We also measured albumin, globulin and their assigned ratio. Results showed that SP supplementation on calves 'diets did not play a significant effect on fluctuation of albumin and globulin levels among treatment groups. As diet’s SP level increased, the amount of total cholesterol, LDL and HDL decreased, which was statistically noticeable for 25 g level compared to the control group. However, the difference was not big enough among S2, S6 and S25 groups for cholesterol and HDL traits.

The blood LDL concentration in S2 group was signify-cantly greater than S25 treatment. Fat concentration of SP is only 5%, which is very low compared to other protein sources. About 10 g of SP has only 1.3 mg cholesterol and 36 calorie energy, whereas an egg yolk includes about 300 mg cholesterol and 80 calorie energy, which is the same protein level as SP (Scircus, 2005). So, the mechanism in which SP plays a significant role in lipid metabolism should not be directly due to its lipid contents *per se*. Inclusion of 2 g SP in diabetic diets led to significant decrease in total cholesterol, total lipids, LDL-cholesterol and VDL-cholesterol and blood glucose (Kamalpreet et al., 2008). Decrease in total cholesterol and LDL was also reported when SP was added into human diet (Lee et al., 2008). This cholesterol serum reduction has been stated as the effect of SP on lipoproteins metabolism and the increase of the lipoprotein enzyme activity levels (Iwata et al., 1990; Nayaka, 1988; Ramamoorthy et al., 1996; Karkos et al., 2008).

Conclusion

Results of the present investigation indicated that the effect of *S. platensis* on growth parameters were not statistically different. Inclusion of SP decreased digestibility of NDF, CP, DM, OM, concentration of total cholesterol, LDL and HDL. So, further experiments with implementation of different levels between 6 and 25 g with higher replications are required to assess the SP effect more accurately and precisely.

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