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Immune Responses and some Blood Metabolite Responses of Female Holstein Calves to Dietary Supplementation with Licorice Root (*Glycyrrhiza glabra*)

Research Article

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ABSTRACT

Different medicinal plants have been used in animal and poultry nutrition in last decade. It seems that there are some potential to these medicinal plants to replace with common antibiotics. Licorice root *(Glycyrrhiza glabra*) is a medicinal plant that extensively was used in different studies. In the present study, the effect of dietary addition of licorice root on performance and blood energy indicator concentrations (glucose, non-esterified fatty acid (NEFA), beta hydroxyl butyrate (BHB) and immune responses parameters (total immunoglobulin (IG), IgG and IgA) was evaluated on female Holstein calves. The fourteen female Holstein calves with average body weight of 85.5 kg were subjected to two different treatments (seven animals per each treatment) in a completely randomized design. The control treatment was considered as C and supplemented treatment with licorice was considered as L. The study lasted ten weeks. Of which the first week was considered as adaptation period. Feed intake was measured daily and blood sample were collected twice throughout the experiment. The results show that average dry matter intake (DMI) was 1790 and 1860 g/d for treatments C and L, respectively (P>0.05). The NEFA was different significantly between treatments (P<0.05). The IgA concentration was not differed between treatments; however both total Ig and IgG concentrations were positively increased by licorice supplementation treatments (P<0.05). Total Ig concentration was 105.1 and 138.2 mg/dL for treatments C and L, respectively which shows that licorice addition caused to 31% increase in immunity responses compared to control treatment. In conclusion the results show that although licorice supplementation did not affect performance of animal, but it has potential to improve energy status in dairy calves' nutrition and also increased immune response of animal.

KEY WORDS blood metabolites, Holstein calf, immune responses, licorice.

INTRODUCTION

Holstein calves in early stages of growth are highly susceptible to different infections and optimum immune responses in this period of time could be a useful indicator for better and healthier future in dairy production system (Pollock *et al*. 1994; Hidiroglou *et al.* 1995). Based on this, in livestock production, antibacterial feed additives are continuously used to promote daily body weight gain and prevent infection during the early stages of rearing (Pollock *et al.* 1994; Katamaya *et al.* 2011). However, on the other hand there is growing concern that the frequent use of antibiotics would increase the incidence of drug-resistant bacteria (Katamaya *et al*. 2011). These circumstances have prompted livestock scientists to look for alternatives to antibiotics in diets. As a potential effective solution to these problems, it could be expected that the reduction of antibiotics may be feasible through the addition of naturally derived dietary additives

that have enhancing effects on the endogenous immune function. Previously inclusion of different herbal medicines such as seaweed, fucodian and licorice were investigated in different animal species (Kim *et al*. 2006; Ahn *et al*. 2008; Suzuki *et al*. 2009). Licorice (*Glycyrrhiza Glabra*) is a perennial plant indigenous to southern Europe, the Middle East and northern China and it is cultivated in many parts of the world. Licorice is one of the plants that have many important nutritional components (Bernardi *et al*. 1994). Licorice extract has many phytochemical compounds (Craig, 1999). Licorice is known to have anti-stress effects, enhances detoxification in the liver, and suppresses inflammatory reactions (Kinoshita *et al*. 2005; Katamya *et al*. 2011). The effect of licorice on the immune function has been elucidated by various experiments, such as the effect on mouse macrophage induced by licorice extracts (Kim *et al*. 2006). Recent work also studied the licorice effects on immune responses (Kim *et al*. 2013; Okda *et al*. 2013). Licorice also contains the saponin that causes to the production of peripheral IgG1 and IgG2 of mouse (Sun and Pan, 2006). This finding has led us to hypothesis that the dietary addition of licorice might enhance the immune function of female calves, which may lead to a reduction in the use of antibiotics in feed. On the other hand licorice root contains glycyrrhizin, the source of most of the pharmacological effects of licorice root and rizome, which is about 50 times sweeter than sugar (Craig, 1999; Nassiri-Asl and Hosseinian, 2008). The present study investigates the effects of dietary supplementation with licorice root on performance, blood energy indicators concentrations (glucose, NEFA, BHB) and immune responses parameters (total IG, IgG and IgA) of female Holstein calves.

MATERIALS AND METHODS

Animals, treatments and management

This study was conducted in Dehlagh dairy farm which is located 30 km from Arak city in Markazi province, Iran. The fourteen female Holstein calves averaging BW 85.5 ± 2.4 kg were assigned to a completely randomized design with two different treatments (seven animals per each). Basal diet for both treatments was included milk plus starter which was contained corn grain, barley grain, soybean meal, wheat bran, canola meal, calcium carbonate and sodium bicarbonate. The concentrate ingredients were ground well and mixed and offered fresh daily to avoid aflatoxine toxicity. All the animals were fed similar basal diet which its formulae was mentioned before and the addition of licorice was considered as variable between two treatments. The licorice free treatment was considered as control treatment or C and 10 g/d added licorice in diet was considered as L. Ground licorice root was added to daily feed and mixed well and then offered to the experimental animals.

The animals were kept in individual stanchions and had free access to water. The study lasted ten weeks and the first week was considered as an adaptation period for the animals to experimental conditions that the licorice is added to diet but the data was not used in statistical analysis. Orts were collected and weights recorded once daily at 07:30 h and the starter feeding rate were adjusted daily to yield orts of about 5-10% intake.

Experimental procedures and chemical analyses

Dry matter (DM) was determined in composites of feed by drying at 60 ˚C for 48 h (AOAC, 2000). Intake of DM was computed based on the 60 ˚C DM determinations for total mixed ration (TMR) and orts. The calves were weighed three times on weeks 1, 5 and 10 of the experiment. Blood was sampled at 0 (just before feeding) and 4 h after feeding from the jugular vein of each calf on d 30 and d 60 of study. Blood samples were heparinized and stored at 2 ˚C for about 6 h; plasma was then prepared and centrifuged (3000 ×g 4 ˚C, 15 min) and stored at -20 ˚C. Later, plasma was analyzed for glucose, NEFA and immunoglobulin's A and G (Ig A and Ig G) and total immunoglobulin (Ig).

Statistical analysis

Data were analyzed using Proc Mixed of SAS (2000). The following model was used for variables which there were repeated measurements over time (blood metabolites):

$$Y_{ijk} = \mu + C_i + T_j + Z_k + ZT_{jk} + e_{ijk}$$

Where:
Y_{ijk}: the dependent variable.
μ: the overall mean.
C_i: the effect of calf i.
T_j: the effect of treatment j.
Z_k: the effect of sampling time k.
ZT_{jk}: the interaction between time k and treatment j.
e_{ijk}: the residual error.

Differences between least square means were considered significant at $P < 0.05$ and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$ using PDIFF in the LSMEANS statement.

RESULTS AND DISCUSSION

Intake, weight gain and feed conversion rate

The animal performance data are given in Table 1. The results show that intake and average daily gain of animal did not differ between two treatments. The results show that licorice root supplementation in animal diet did not have any statistically significant effect on the performance of the animals. Because intake is the most important factor which

could affect daily gain (Allen, 2000) therefore similar amounts of intake between two treatments caused no difference in feed conversion rate or daily gain between two treatments in the present study. In livestock production, antibacterial feed additives are continuously used to promote daily body weight gain. Katamaya *et al*. (2011) stated that addition of licorice in pig diet for 76 consecutive days did not affect average daily gain or average gain throughout the whole study length that is consistent with the acquired data in the present study. To our knowledge there is no data regarding the licorice effect on intake, gain and efficiency in animal nutrition. Furtherer research is needed to evaluate the licorice supplementation effects on different animal species.

Table 1 Least square means for blood DMI and performance in animal fed experimental diets

Item	Treatments*			
	C	L	SE	P-value
DMI (g/d)	1790	1860	26.23	NS
ADG (g/d)	557	572	7.9	NS
FCR (intake/weight gain)	3.21	3.25	1.04	NS

* Treatments were; C: control treatment and L: supplemented with 10 g/d licorice. NS: non significant.
SE: standard error and FCR: feed conversion ratio.

Blood metabolites and immune responses

The data for blood metabolites and immune responses are presented in Table 2. Among the blood metabolites glucose, and BHB did not differ between two treatments (P>0.05) and among the immune responses parameters, IgA concentration did not differed between C and L treatments. However, NEFA as an energy level indicator (P=0.03) and total IG (P=0.008) and IgM (P=0.002) concentrations as immune responses parameters were significantly affected by supplementing licorice root in animal diet.

Table 2 Least square means for blood metabolites and immune responses in animal fed experimental diets

Item	Treatments*			
	C	L	SE	P-value
Glucose (mg/dl)	47.59	48.13	1.23	0.44
NEFA (mmol/L)	0.23^{a}	0.16^{b}	0.05	0.03
BHB (mmol/L)	0.36	0.35	0.12	0.79
Total IG (mg/dL)	105.1^{b}	138.2^{a}	4.9	0.008
IgA (mg/dl)	15.57	16.11	1.04	NS
IgM (mg/dl)	79.42^{b}	94.23^{a}	5.16	0.002

* Treatments were; C: control treatment and L: supplemented with 10 g/d licorice. The means within the same row with at least one common letter, do not have significant difference (P>0.05).
NS: non significant.
SE: standard error.

Comparing two treatments acquired data in the present study revealed that total Ig concentration increased with 31% and IgM concentration also increased by 18% in supplemented treatment compared by control treatment. Glycyrrhizin is the primary identified active principle in licorice which has a chemical structure similar to that of steroid molecules (Latif *et al*. 1990; Baker, 1994). Its similarity to steroids may account for its anti-inflammatory action, one of licorice's important effects on immune response (Latif *et al*. 1990). Rather than demonstrating steroidal, cortisone-like effects on inflammation-reduction of tissue swelling from histamine, increased blood flow and leukocyte infiltration of damaged or infected tissues-licorice root has been shown *in vitro* studies to effectively reduce inflammation by mopping (Nara, 1984; Shinada *et al*. 1986). Super oxide, hydrogen peroxide and hydroxyl radicals are released during inflammation to disable targeted bacteria and viruses, a beneficial effect that is often overdone. Similar results were obtained in a study in which licorice root reduced the number of free radicals liberated by macrophages (Akamatsu *et al*. 1991). Researchers found that licorice appears to promote proliferation of B (from the bone) and T cells and stimulate production of interleukin-19, which stimulates T cells (Hatano *et al*. 1988; Ultee, 2000). Licorice also appears to stimulate the production of gamma-interferon by lymphocytes (Chavali *et al*. 1987) and the differentiation of T3, T4 and T8 cells, specific kinds of activated lymphocytes. (Nara, 1984). Licorice may also act as anti-oxidant agents (Utsunomiya *et al*. 1997) or antiviral and antitumor effects (Kim *et al*. 2013). It is known that supplementation of anti-oxidant agent (such as vitamin E) would protect and stabilize cell membrane and increase immune responses (Brzezinska-Slebodzinsk *et al*. 1998). The activity of licorice on the immune system has been described as "nonspecific" by most investigators (Ultee *et al*. 2000; Nassiri-Asl and Hosseinzadeh, 2008). This means licorice stimulates, activates or promotes an immune response in multiple ways. This mechanism showed to have anti-viral and anti-tumor effects in biology (Kim *et al*. 2013; Okda *et al*. 2013). There is a possibility that the feed additives, which enhance the immune capacity of animal, would be effective to reduce the use of the antibiotics in feed. If naturally derived additives such as licorice are proven to be effective in enhancing the immune function, they would be beneficial as a substitute for antibiotic additives and a possible solution for the public concern about food safety. Future studies should clear the specific role of licorice in both *in vitro* and *in vivo* studies in the cell and organ level and the comparisons of this additive with different antibiotics could be an important investigation area.

CONCLUSION

The results show that dietary supplementation of licorice root (*Glycyrrhiza glabra*) in female Holstein calves had no effect on performance. However, decreased concentration of NEFA in the blood of supplemented animals shows that

licorice root has the potential to improve the energy status of animals. Significant increase in total Ig and IgM concentrations clarified that this medicinal plant has also potential to use as immune intensifier in calf production system in this critical period of time of rearing. Further research is recommended to evaluate the effectives of licorice root as a replacement to antibiotic use in calf rearing systems.

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