Effects of supplemental Chinese herbs on growth performance, blood antioxidant function and immunity status in Holstein dairy heifers fed high fibre diet

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Abstract

Two experiments were carried out to investigate the effects of supplemental Chinese herbs, Fructus Ligustri Lucidi (PLL), Radix Astragali (RA) and Radix Codonopsis (RC) on growth performance, blood antioxidant and immune function in Holstein dairy heifers fed high fibre diet. Experiment 1 indicated that the supplementation of the three herbs had no effect on dry matter intake. PLL supplementation increased heifers average daily gain (ADG), final body weight and feed efficiency. Experiment 2 indicated that PLL supplementation improved the blood antioxidant function with higher concentration of superoxide dismutase (SOD) and lower concentration of malondialdehyde (MDA), and improved immune function with lower concentrations of prostaglandin E2 (PGE2) and immunoreactive fibronectin (IFN-γ). Addition of PLL increased apparent digestibility of diet’s dry matter and organic matter than the other groups. It was demonstrated that PLL supplementation improved nutrient digestion, feed efficiency, blood antioxidant function, immune and growth performance for Holstein dairy heifers.

Introduction

A goal of rearing dairy heifers is to obtain a economically relatively high body weight with a high-fibre diet, when they are at the age of first mating or calving. Various approaches such as use of antibiotics (monensin, etc.) or injection of bST (bovine Somatotropin), have been explored, but the use of antibiotics or bST has been increasingly facing reduction of social acceptance in animal production systems. Currently, use of antibiotics to promote dairy production has been banned in European Union and some other countries including People’s Republic of China. There is an increasing interest of some ruminant nutritionists to find substitutes for antibiotics and hormones. Some plants secondary metabolites such as tannins, saponins, and essential oils have been studied to investigate their effects on rumen fermentation, nutrient digestibility and productive performance in lamb and cattle (Benchaar et al., 2006, 2008; Busquet et al., 2006; Lila et al., 2003; Beauchemin et al., 2007). From their results, some positive effects, an increase of total volatile fatty acid concentration, an increase of total gas production in vitro, a decrease of ammonia-N concentration, a milk yield improvement, etc. were observed.

Chinese herbs or herbs extractions (compounds or purifications) containing large quantity of plant secondary metabolites have been used as drugs for sickness therapy or as products for health care for a very long time. Nowadays, some kinds of herbs or herbs extractions also are used as feed additives to promote feed intake, immunity status and antioxidant function for animals. The uses of Chinese herbs additives as substitutions for antibiotics in non-ruminants (chicks, hens, piglets) and ruminant (sheep, dairy cows) diets have been advocated; some positive observations, such as improvement of growth performance and blood antioxidant functions and immunity status were observed (Ma et al., 2004, 2005; Zhang et al., 2004, 2005; Chen et al., 2003; Yi et al., 2005a, 2005b; Liu et al., 2003; Qiao et al., 2011). Little is known on effect of supplemental Radix Astragali (RA) or Radix Codonopsis (RC) or Frutus Ligustri Lucidi (PLL) to diet on growth performance, digestion, blood antioxidant function, and immunity status in Holstein dairy heifers fed high fibre diet. The authors hypothesized that all the three Chinese herbs selected have favourable modulation effects on blood antioxidant and immunity status and rumen fermentation in Holstein dairy heifers, and will not adversely decrease nutrient digestibility, dry matter intake and average daily gain. Therefore, this present study was conducted to investigate the effect of Chinese herbs addition on dry matter intake, growth performance, digestion, antioxidant function, and immunity status in Holstein dairy heifers fed high fibre diet.

Materials and methods

Experiment 1

Experimental design, heifers and management

Experiment 1 was conducted at the Lanzhou dairy farm in Gansu province during summer. Randomized complete block design (RCBD) was used in this study. A total of thirty two Holstein heifers (body weight of 231.33 kg, SD=16.44 kg and days of 254.55, SD=18.99) were selected. Heifers with close body weight and age were blocked into one group. Each group had eight heifers. Two heifers of each group were randomly selected and assigned to each treatment. The treatments were addition of Fructus Ligustri Lucidi (PLL), Radix Astragali (RA), Radix Codonopsis (RC) and control (without herb addition) at doses of 10 grams per heifer per day. Dairy heifers were fed ad libitum twice daily at 06:00 and 17:30. Fresh water was available at any time throughout the day. Each heifer was individually kept in a tie stall pen in barn and allowed 1-h free movement twice daily after morning feeding and afternoon feeding. All the heifers were treated against parasites using Eprinomectin in spring (Kanglong Co. Ltd, Wuhan, China). A total of 28 weeks was involved in this growth performance trial. Heifers were allowed two weeks for adaptation to Chinese herbs supple-
mentation. Then the experimental period started and lasted for 26 weeks. Body weight of each heifer at beginning of the 3rd week and the end of 28th week were recorded and treated as initial body weight and final body weight, respectively. Feed intake of each heifer was daily recorded by weighting the feed offered and refused by the heifers, but refusals were not sampled. Mixed diet was weekly sampled and stored in a refrigerator at 20°C. Composited samples were pooled by heifer and put into an air forced oven at 65°C for 72 h. Dry matter (DM) contents were determined according to the AOAC (1990) method.

**Diet and herbs**

Diet formula is shown in Table 1. All the chemical components were determined in laboratory with the exceptions of total digestible nutrient (TDN) and metabolizable energy (ME) that was estimated according to NRC (2001). Forage (sole corn stover silage without kernels) and concentrates were totally mixed using a diet mixer (RC500, Keyang Co, Shandong, China) and fed to heifers. Chinese herbs were collected from Anyue city of Sichuan province in China. RA (root) and RC (root) and FLL (seed) were washed under tap water until they were clean. Then they were put into an air forced oven at 55°C for 72 h. Dried herbs were ground through a 1-mm screen and collected for further use. A dose of 10 g of each herb powder was firstly mixed with a small part of concentrate and fed to heifers. In all cases, all dosages of herbs supplemented were completely consumed by heifers. Based on the previous study in sheep of our research group (Qiao et al., 2011) and combining current daily average dry matter intake of dairy heifers, a dose of 10 g of each Chinese herb powder per day per heifer was used in this present study.

**Experiment 2**

**Apparent total tract nutrient digestibility and blood sampling**

Experiment 2 was started when Experiment 1 was completed. Heifers' management was in accordance with Experiment 1, but heifers were kept in barns without movements. Digestibility trial consisted of 15 days. Apparent total tract digestibility of diet's nutrients was determined by using Cr2O3 as an external marker contained in concentrates. Markers were firstly mixed with a small part of concentrate and fed to each heifer in each group to ensure all doses of markers be consumed by heifers. A total dose of 10 g of chromium (Cr) (250 grams of Cr2O3) was consumed by each heifer every day, and lasted for 15 consecutive days. The 5 consecutive days' faecal and feed collections were started at 11th day. Faecal samples (200 g of wet weight) were collected twice daily at 08:00 and 18.00 h from the rectum of each heifer (Benchaar et al., 2006). The faecal samples were composited and sprayed with 6 N HCl by heifer and put into refrigerator at -20°C. The pooled samples of feed and faeces subsequently were dried at 65°C for 48 h in an air force oven. Samples were analyzed for contents of DM, total nitrogen (N), organic matter (OM) and acid detergent fibre (ADF) using method of AOAC (1990), and Cr. Content of neutral detergent fibre (NDF) was determined according to the method of Van Soest et al. (1991) with heat stable α-amylase and without sodium sulfate. Content of Cr in faeces was analyzed using atom absorption chromatography (Contra 300). The apparent digestibility of DM, OM, NDF, ADF and N was calculated as per the following equation:

\[
\text{digestibility} = (1 - [\frac{\text{Cr (mg/d)}}{\text{DMI (kg/d)}}] \times \text{Cr in faeces (mg/kg of DM)}) \times 100
\]

where dry matter intake (DMI) represents DMI consumed during the 5 days' faecal collection period. Blood samples were removed once daily from jugular vein into a 10 mL vacutainer (Rongsheng Biotech, Shanghai, China) on 13th d to 15th d. Efforts were made to return to laboratory immediately. Blood samples were kept for 2 h at room temperature, and then the blood samples were put in a refrigerator at 4°C for 3 h for serum separation. Finally, blood samples were centrifuged at 3000x g at 4°C for 15 min. Supernatants were collected and stored in a refrigerator at -40°C for further analysis.

**Measurement of serum antibodies by ELISA**

Serum concentrations for immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) levels were determined by using kits of cow ELISA (enzyme linked immunosorbent assay, Montgomery, TX, USA). Protocols for determination of these parameters strictly followed the instructions of kits. ELISA kit for prostaglandin E2 (PGE2) determination was purchased from Tongzheng Biotech (Lanzhou, Gansu province, China). The concentrations of IgA, IgG, IgM and PGE2 were expressed as µg/mL, mg/mL, mg/mL and pg/mL respectively.

**Determination of cytokines in the serum**

Serum concentrations of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10) and immunoreactive fibronectin-γ (IFN-γ) were analyzed by using the cow ELISA kits (Groundwork Biotechnology Diagnostics, San Diego, CA, USA). Procedures for determination were strictly followed the instructions of kits. Concentrations of IL-2, IL-4, IL-6, IL-10 and IFN-γ were constantly expressed as pg/mL, respectively.

**Statistical analysis**

Randomized complete block design (RCBD) was used in this study. Model sums of squares were separated into blocks, treatments and random residual. The statistical model was described in SAS 8.1. It is as follows:

\[
Y_{ijk} = \mu + a_i + b_j + e_{ijk}
\]

where \( \mu \) is the general mean; \( a_i \) is the fixed effect of herbs; \( b_j \) is the fixed effect of block; \( e_{ijk} \) is the random residual effect; \( e_{ijk} \sim N(0, \sigma_e) \).

All the data obtained in Experiment 1 and Experiment 2 was subjected to a one-way ANOVA using GLM procedure of SAS. The differences between groups were established by means of Duncan’s multiple range tests at a significant level of P<0.05 or P<0.01.

**Results**

**Feed efficiency and growth performance**

As presented in Table 2, addition of RA, RC, and FLL to the diet had no effect on DMI and initial body weight of dairy heifers (P>0.05). Diet supplemented with FLL significantly increased final body weight, total gain, average daily gain, and feed efficiency in dairy heifers

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**Table 1.** Diet formula for dairy heifers. All the data obtained in Experiment 1 and Experiment 2 was subjected to a one-way ANOVA using GLM procedure of SAS. The differences between groups were established by means of Duncan’s multiple range tests at a significant level of P<0.05 or P<0.01.
compared with the other groups (P<0.05). Diet supplemented with RA significantly decreased body weight gain and average daily gain in dairy heifers compared with the other three groups (P>0.05).

Dry matter intake and nutrient digestibility
As presented in Table 3, diets supplemented with RA or RC or FLL had no effect on diet’s apparent digestibility of NDF, ADF and N in heifers compared with control group (P>0.05). However, diet supplemented with FLL increased apparent digestibility of diet’s DM and OM compared with control group (P<0.05).

Antioxidant function and immunity
As presented in Table 4, diets supplemented with RA, RC, and FLL did not affect serum IgA, IgM and IgG concentrations respectively (P>0.05). Cytokine concentration of IFN-γ in the serum was decreased by the diet supplemented with FLL relative to the other groups (P>0.05). No differences were observed for cytokine concentrations of IL-2, IL-6, IL-4 and IL-10 among all the four treatments respectively (P>0.05). Concentration of PGE2 was increased in heifers fed diet supplemented with FLL compared with control group (P<0.05). Blood antioxidant enzymes concentrations of SOD and MDA were significantly increased and decreased by FLL supplementation compared with the other groups respectively (P<0.05). All the three Chinese herbs supplementation had no effect on GSH concentration in the serum compared with the control group (P>0.05).

Discussion
Dry matter intake and growth
The result of unaffected dry matter intake in Experiment 1 indicated that three Chinese herbs selected had no effect on palatability of feeds. This result agreed well with the previous study in sheep conducted by our research group, and found that these three Chinese herbs had no effect on palatability of feeds in sheep (Qiao et al., 2011). This result also agreed with observation in chickens (Ma et al., 2004, 2005) and dairy cattle (Zhang et al., 2004, 2005; Benchaar et al., 2006). Whereas negative effects of herbs or herbs extractions as presented in Table 4, diets supplemented with Frutus Ligustri Lucidi (root); RC, Radix Astragali (root); Radix Codonopsis (root); FLL, Fructus Ligustri Lucidi (seed). Each herb was supplemented for 10 weeks at dose of 10 g per day per heifer. DMI, dry matter intake; ADG, average daily gain. *kg of ADG/ kg of DMI. **Values in the same rows with different superscripts differ (P<0.05). *P<0.05.

Table 1. Diet formula and chemical components.

| Diet°                                      | Ingredients, % DM |
|-------------------------------------------|-------------------|
|                                           | Corn stover silage| 80.00 |
|                                           | Cracked corn      | 5.74  |
|                                           | Soybean meal (sol)| 11.61 |
|                                           | Sodium bicarbonate| 0.40  |
|                                           | Premixx           | 2.25  |

Chemical components
DM, % 46.47
NDF, % DM 44.13
ADF, % DM 25.35
NFC°, % DM 37.91
CP, % DM 11.36
TDN^, % 69.83
ME1, MJ/kg DM 10.58
Calcium, % 0.35
Phosphorus, % 0.31

°Chemical components listed in table were determined in laboratory, while total digestible nutrients and metabolizable energy were estimated. *Contained: vitamin E, 7.0%; vitamins A-D, 5.0%; dry distillers corn with soluble vitamin D3, 25.0%; salt, 14.1%; limestone, 33.5%; magnesium oxide, 5.8%; trace mineral premix, 5.8%; selenium premix, 3.0%. DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; NFC, non-fibre carbohydrate; CP, crude protein; TDN, total digestible nutrients; ME, metabolizable energy. 1Analyzed by AOAC according to the equation of NFC=100-(NDF+NDCP)+CP+EE+Ash. 2Calculated according to the equation TDN=TDN×0.036+tdCPf+tdFA+tdNDF (NRC, 2001). 3Estimated ME=TDN×0.036 (NRC, 2001).

Table 2. Effect of Chinese herbs on growth performance, feed efficiency and average daily gain in heifers.

| Treatments | Control | RA | RC | FLL | SEM | Treatment | Block |
|------------|---------|----|----|-----|-----|-----------|-------|
| Initial body weight, kg | 217.83 | 224.69 | 235.63 | 228.15 | 3.85 | 0.28 | 0.77 |
| Final body weight, kg | 363.95 | 364.24 | 381.17 | 379.91 | 1.79 | * | 0.38 |
| DMI, kg/d | 5.06 | 5.13 | 5.11 | 5.47 | 0.31 | 0.25 | 1.07 |
| Total gain, kg | 146.01 | 139.55 | 145.54 | 151.76 | 1.70 | * | 0.41 |
| ADG,° kg/d | 0.80 | 0.77 | 0.80 | 0.83 | 0.02 | * | 0.27 |
| Feed efficiency# | 0.16 | 0.15 | 0.15 | 0.16 | 0.01 | * | 0.21 |

°Chemical components listed in table were determined in laboratory, while total digestible nutrients and metabolizable energy were estimated. *Contained: vitamin E, 7.0%; vitamins A-D, 5.0%; dry distillers corn with soluble vitamin D3, 25.0%; salt, 14.1%; limestone, 33.5%; magnesium oxide, 5.8%; trace mineral premix, 5.8%; selenium premix, 3.0%. DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; NFC, non-fibre carbohydrate; CP, crude protein; TDN, total digestible nutrients; ME, metabolizable energy. 1Analyzed by AOAC according to the equation of NFC=100-(NDF+NDCP)+CP+EE+Ash. 2Calculated according to the equation TDN=TDN×0.036+tdCPf+tdFA+tdNDF (NRC, 2001). 3Estimated ME=TDN×0.036 (NRC, 2001).

Table 3. Effect of Chinese herbs supplementation on dry matter intake, apparent total tract digestibility in dairy heifers.

| Treatments | Control | RA | RC | FLL | SEM | Treatment | Block |
|------------|---------|----|----|-----|-----|-----------|-------|
| DMI, kg/d | 5.16 | 5.31 | 5.27 | 5.21 | 0.61 | 0.13 | 0.45 |
| Apparent total tract digestibility | | | | | | | |
| DM, % | 72.25 | 72.39 | 73.11 | 75.28 | 0.79 | * | 0.35 |
| OM, % | 69.17 | 69.55 | 69.74 | 73.28 | 0.71 | * | 0.49 |
| NDF, % | 55.34 | 53.19 | 53.08 | 54.26 | 1.74 | 0.53 | 0.28 |
| ADF, % | 41.82 | 40.23 | 42.80 | 39.94 | 1.35 | 0.35 | 0.41 |
| CP, % | 66.68 | 68.93 | 67.65 | 66.34 | 1.43 | 0.31 | 0.27 |

°Chemical components listed in table were determined in laboratory, while total digestible nutrients and metabolizable energy were estimated. *Contained: vitamin E, 7.0%; vitamins A-D, 5.0%; dry distillers corn with soluble vitamin D3, 25.0%; salt, 14.1%; limestone, 33.5%; magnesium oxide, 5.8%; trace mineral premix, 5.8%; selenium premix, 3.0%. DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein. **Values in the same rows with different letters differ (P<0.05). *P<0.05.
on dry matter intake was observed in deer (Oh et al., 1968) and beef cattle (Cardozo et al., 2006), and this kind of effect was found due to the organoleptic effect of *Pseudostuga menziesii*, limonene and pinene. In our study, unchanged dry matter intake of heifers fed diets supplemented with Chinese herbs demonstrated that the three herbs selected have no organoleptic effect. It was reported that active components of FLL are saponins (Qiao et al., 2011). Diet supplemented with FLL increased final body weight of heifers compared with the other groups. This result agreed well with observation in lamb (Bosler et al., 1997) where it was found that saponin increased slaughtered body weight of lamb. In the present study, the increase in final body weight may be due to the high content of saponins in FLL (13.47%).

### Nutrient digestibility

Many studies were conducted in recent years to investigate the effects of herbs or herbs extractions with three main plant secondary metabolites (tannins, saponins and essential oils) on diet’s nutrient digestibility in non-ruminant livestock (chicks and piglets), beef cattle, and dairy cattle. The responses of animals’ digestion to these promising materials have been varied. Benchaar et al. (2006) demonstrated that, in *in vivo* studies using cattle, no effects of three main plant secondary metabolites on apparent nutrient digestibility were detected respectively, and the reason was found due to the lower dosages used. Subsequently, higher dosages were used, and found that rumen degradation dynamics of diet’s N was changed, and ammonia-N was decreased, and total tract digestibility of diet’s N, ADF, NDF, DM, and OM was not affected (Benchaar et al., 2008). Lila et al. (2003) reported that purified sarsaponin decreased dry matter degradability in *in vitro* study. In the present study, addition of RA to diet decreased apparent total tract digestibility of diet’s OM, NDF, and ADF, but it did not reach the significant level (Table 3). Studies concerning the doses of herbs or herbs extractions added into the diets have been performed in recent years. As mentioned, the doses of several Chinese herbs supplemented in this current study were based on previous study in sheep and combining average daily dry matter intake of heifers in present study. When expressed as grams per head per day, 10 g of herbs powder was used. Further studies to determine the effects of these herbs addition on rumen fermentation and degradation dynamics of individual nutrient in dairy heifers are needed. FLL supplementation did not affect apparent digestibility of diet’s N, NDF and ADF, but increased DM and OM digestibility compared with the control group. This result agreed with our previous study in sheep, in which total tract digestibility of diet’s DM and OM was also increased by addition of FLL to diet (Qiao et al., 2011). The reason for this result was found due to the stimulating role of FLL to amylase degrading bacteria in the rumen of sheep. Present results of increased digestibility of DM and OM demonstrated that FLL also can stimulate the amylase activity of starch degrading bacteria in the rumen of dairy heifers.

### Blood antioxidant function

Responses of animal productive performance to blood antioxidant parameters (SOD, GSH and MDA) have been comprehensively studied in recent years. It has been regarded that blood SOD and/or GSH, and MDA (end-product of membrane lipid oxidation) are positive and negative indicators to evaluate animal antioxidant status respectively. Results from the published literature indicated that when animal were under poor blood antioxidant status, lower concentrations of SOD or GSH, and higher concentration of MDA were always observed, and productive performance were significantly decreased (Ma et al., 2004, 2005). The decrease of animal productive performance was found due to the oxidation of unsaturated fatty acids in cell membrane, which led to disrupt ion of normal membrane structure and function. Our present study did agree with this demonstration. FLL improved blood antioxidant status with higher concentrations of SOD and lower concentration of MDA. Therefore, the increased of final body weight of dairy heifers could be due to the improvement of blood antioxidant function by the FLL supplementation to diet.

Table 4. Effect of Chinese herbs on levels of antibodies, blood antioxidant enzymes and cytokines in the serum of dairy heifers.

| Antibodies concentrations | Treatments | Control | RA | RC | FLL | SEM | Treatment | Block |
|---------------------------|------------|---------|----|----|-----|-----|-----------|-------|
| IgM, mg/mL                |            | 3.25    | 3.35| 3.06| 3.03| 0.71| 0.53      | 0.76  |
| IgA, g/mL                 |            | 62.91   | 74.01| 65.80| 70.52| 4.17| 0.77      | 0.59  |
| IgG, mg/mL                |            | 4.06    | 4.35| 3.99| 4.15| 0.22| 0.23      | 0.62  |
| PGE₂, pg/mL               |            | 59.13b  | 56.64a| 55.38ₚ| 45.85ₚ| 1.19| *         | 0.15  |

| Cytokines concentrations  | Treatments | Control | RA | RC | FLL | SEM | Treatment | Block |
|--------------------------|------------|---------|----|----|-----|-----|-----------|-------|
| IL-2, pg/mL              |            | 46.19   | 48.99| 48.85| 44.79| 3.01| 0.24      | 0.39  |
| IL-4, pg/mL              |            | 25.56   | 22.94| 35.01| 27.17| 3.39| 0.13      | 0.41  |
| IL-8, pg/mL              |            | 73.14   | 74.42| 77.98| 68.03| 6.31| 0.91      | 0.97  |
| IL-6, pg/mL              |            | 80.05   | 77.12| 68.09| 66.64| 6.04| 0.36      | 0.68  |
| IFN-γ, pg/mL             |            | 55.13b  | 46.71ₚ| 43.95ₚ| 44.36ₚ| 0.92| *         | 0.29  |

| Blood antioxidant enzymes concentrations | Treatments | Control | RA | RC | FLL | SEM | Treatment | Block |
|-----------------------------------------|------------|---------|----|----|-----|-----|-----------|-------|
| SOD, U/mL                                |            | 120.29ₚ| 120.60ₚ| 124.33ₚ| 141.65ₚ| 0.52| *         | 0.47  |
| GSH, mg/L                                |            | 145.36  | 143.64| 142.96| 144.88| 1.39| 0.44      | 0.51  |
| MDA, mol/mL                              |            | 7.01ₚ   | 5.91ₚ| 6.63ₚ| 3.44ₚ| 0.36| *         | 0.25  |

RA, Radix Astragali (root); RC, Radix Codonopsis (root); FLL, Frutus Ligustri Lucidi (seed). Each herb was supplemented at dose of 10 g per day per heifer. *Determined antibodies included immunoglobulin A (IgA), IgG, IgM and prostaglandin E₂ (PGE₂). *Determined cytokines included interleukin-2 (IL-2), IL-4, IL-10 and immunoreactive fibronectin-γ (IFN-γ). SOD, superoxide dismutase; GSH, glutathione peroxidase; MDA, malondialdehyde. *Means within same rows with different superscripts differ (P<0.05). *P<0.05.
Immunity

Responses of animal productive performance to immunity status have been well documented in the past decade. Observations in chicks (Ma et al., 2005; Chen et al., 2003; Yi et al., 2005a), piglets (Liu et al., 2003; Yi et al., 2005b) and dairy cows (Sun et al., 2010; Zhang et al., 2004, 2005) from the published literature indicated that when animals were in good immunity status, they showed a relatively higher productive performance. Some clinical therapies in persons also demonstrated that some Chinese herbs including RA, RC, and FLL have capability to improve immunity status (Tang et al., 2003). Based on these observations and perspective substitution to antibiotics, many kinds of Chinese herbs or extractions are being further investigated for improvements of animal productive performance. Our present results of an increase in growth performance in Holstein dairy heifers did agree with those observations mentioned above. Lower concentrations of PGE2 and IFN-γ were observed in dairy heifers that were fed diet supplemented with FLL was better than the other three groups. From the results of the published articles, it could be concluded that PGE2 is very important to regulate the development and roles of immune cells and to synthesis immunity regulatory cytokines (Calder, 1999; Yi et al., 2005a, 2005b; Sun et al., 2010; Chen et al., 2003; Liu et al., 2003). It was reported that when animals suffered from acute or chronic inflammation or pathogens challenge, relatively higher concentrations of PGE2 and/or IFN-γ will be secreted into blood (Kinsella et al., 1990; carter and Dutton, 1996; Desmedt et al., 1998). In our present study, decreases of serum concentrations of PGE2 and IFN-γ were observed when heifers were fed diet supplemented with FLL, which indicated that FLL modulated immunity status for heifers to a better physiological status. Almost no variations were observed for cytokines concentrations of IL-2, IL-4, IL-6, and IL-10 in the serum of heifers received addition of several Chinese herbs, respectively. This observation did not agree with observations by Liu et al. (2003) who evaluated effect of herbs polysaccharides (extracted from RC) on series of interleukins in chicks, and found that IL-2 was significantly increased. Possible explanation may be the larger extent of degradation of RC by the microorganisms in the rumen. The underlying mechanism of this phenomenon needs further studies. IFN-γ is an important indicator cytokine secreted by Th1 lymphocytes that initiates cell-mediated immune responses (Enke et al., 2008). According to the report of Blok et al. (1996) who conducted several clinical studies in healthy persons, when the body was exposed pathogens or suffering from inflammation, the yield capability of T cell-derived cytokines was suppressed and relatively higher concentration of IFN-γ was secreted into blood. Investigations concerning Chinese herb (FLL) supplementation in diets of chicks, or hens, or piglets, or dairy cows showed that addition of FLL reduced circulating IFN-γ and IFN-γ mRNA (Ma et al., 2004, 2005; Chen et al., 2003; Zhang et al., 2004, 2005). These results were in agreement with our present study in which a decrease of serum IFN-γ concentration was also detected. The determination of cytokines in the serum of dairy heifers showed that supplementing FLL to diet decreased IFN-γ concentration level. This result demonstrated that addition of FLL has the capability to favourably modify immunity status for dairy heifers.

Conclusions

The addition of FLL at dosage of 10 g per head per day to diet increased apparent total tract digestibility of diet’s dry matter and organic matter, and increased average daily gain and feed efficiency in dairy heifers. Diet supplemented with FLL improved blood antioxidant status and immunity status for dairy heifers. In Holstein dairy heifers, reasons for the increase of growth performance may be due to FLL supplementation increased digestion, or improved of antioxidant status, or improved immunity status, or all of them.

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