IJAS Iranian Journal of Applied Animal Science

The Effects of a Medical Plant Mixture and a Probiotic on Performance, Antioxidant Activity and Weaning Age of Newborn Holstein Calves

Research Article

S. Seifzadeh[1], F. Mirzaei Aghjeh-Gheshlagh[1*], H. Abdi-Benemar[1], J. Seifdavati[1] and B. Navidshad[1]

[1] Department of Animal Science, Faculty of Agricultural Science, University of Mohaghegh Ardabili, Ardabil, Iran

Received on: 7 May 2015
Revised on: 25 Jul 2015
Accepted on: 15 Aug 2015
Online Published on: Jun 2016

*Correspondence E-mail: f_mirzaei@uma.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

The aim of this study was to investigate the effects of a medical plant mixture and a probiotic on performance, antioxidant activity and weaning age of suckling Holstein calves. For this experiment, 30 newborn calves (0-10 days of age) with an average birth weight of 42 ± 8 kg were used in a factorial design (3×2) with 6 treatments and 5 replicates. Treatments were: 1) basal diet including a calves starter feed and whole milk, 2) control diet + 2 g probiotic 3) control diet + 1.5% of medical plant, 4) control diet + 1.5% medical plant + 2 g probiotic, 5) control diet + 3% medical plant and 6) control diet + 3% medical plant + 2 g probiotic. The calves were offered experimental pelleted feeds *ad libitum* and after one month were supplied with imported hay. Results showed that the treatments had no significant effect on dry matter intake during the experiment. However, addition of the 1.5% medical plant increased dry matter intake ($P<0.05$). However, the 1.5% level of medical plant reduced calves weaning age ($P<0.05$). Calves receiving control diet + 1.5% of medical plant mixed and 1.5% medical plant mixed + 2 g probiotic showed a higher plasma antioxidant activity ($P<0.05$). This study suggested that 1.5% of medical plant in calves starter feed will improve performance and the immune system and will also reduce the weaning age of calves.

KEY WORDS antioxidant activity, calves, feed intake, medical plant, starter diet, weaning age.

INTRODUCTION

In livestock production systems, antibiotics are commonly fed to animals to prevent disease and metabolic disorders, as well as improve feed efficiency. However in recent years, public concern over routine use of antibiotics in livestock nutrition has increased due to the emergence of antibiotic resistant bacteria that may represent a risk to human health. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Plant extracts offer a unique opportunity in this regard (Wallace, 2004), as many plants produce secondary metabolites, such as saponins and tannins, which have antimicrobial properties. These compounds have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Wang *et al.* 2000; Hristov *et al.* 1999). Similarly, the well documented antimicrobial activity of essential oils (EO), and their active components, has prompted a number of scientists to examine the potential of these secondary metabolites to manipulate rumen microbial fermentation to improve production efficiency in ruminants. Contrary to their name, EO are not true oils (i.e., lipids) and are commonly derived from the components responsible for fragrance, or *Quinta essentia*, of plants. The antimicrobial properties of EO have been demonstrated against a wide range of microorganisms, including bacteria, protozoa and fungi (Chao *et al.* 2000).

Essential oils can be extracted from many parts of a plant, including the leaves, flowers, stem, seeds, roots and bark. However, the composition of the EO can vary among different parts of the same plant (Dorman and Deans, 2000).

This antimicrobial activity has been attributed to a number of terpenoid and phenolic compounds (Chao *et al*. 2000), as well as the chemical constituents and functional groups contained in the EO, the proportions in which they are present and the interactions between them (Dorman and Deans, 2000). In addition, antagonistic, and synergistic effects have been observed between components of EO (Burt, 2004). Thyme (*Thymus vulgaris*), mint (*Spearmint sativum)*, oregano (*Mentha pulegium*), cumin (*Cuminum cyminum*), camel thorn (*Alhagi persarum)*, garlic (*Allium sativum*) and Eucalyptus (*Eucalyptus*) are plants that because of their active ingredients are very important. Based on these effects, medical plants has been suggested as an alternative for antibiotics on livestock and especially calves (Soltan, 2009; Hosoda *et al*. 2006).

Probiotics are another group of feed additives that are possible alternatives for antibiotics (Hume, 2011; Riddell *et al*. 2010). Probiotics can be defined as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" (Fuller, 1989).

Probiotics introduce beneficial microorganisms into the gut which act to maintain optimal conditions within the gastrointestinal tract and inhibit the growth of pathogenic or other undesirable bacteria. As far as we know, there are no studies on the effect of medical plants and probiotics as a combined treatment on performance and antioxidant activity in suckling calves. Therefore, this study was performed to investigate the effects of a medical plant mix and probiotic on performance, antioxidant activity and weaning age of Holstein calves.

MATERIALS AND METHODS

Animals and diets

Thirty Holstein male calves (average birth weight=42±8 kg) were selected from a commercial Dairy Herd of Moghan Agro Industrial and Animal Husbandry to determine the effect of a medical plant mix and probiotic in the starter diet on feed intake, antioxidant activity and weaning age of newborn Holstein calves. Treatments were: 1) basal diet including a calves starter feed and whole milk, 2) control diet with 2 g probiotic per head per day in milk, 3) control diet with 1.5% of a commercial a medical plant mix in starter feed (Sabzineh Co, Mashhad, Iran), 4) control diet with 1.5% of the medical plant mix with 2 g probiotic, 5) control diet with 3% of the medical plant mix and 6) control diet with 3% of the medical plant mix with 2 g probiotic.

The probiotics administration dosage was recommended about 0.5-2 g per day per head by the manufacturer. Also, the recommended dosage of the medical plant mix was 2% in the manufacturer's catalog. The probiotic supplement used in our study was protexin (Probiotics International Ltd., south Petherton, UK). It contains the following strains of probiotics and prebiotics: *Lactobacillus plantarum*, *Lactobacillus delbrueckii* ssp. *Bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus salivarius* ssp. *thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopesii*. The medical plant mix consisted of 9% thyme (*Thymus vulgaris*), 25% mint (*Spearmint sativum)*, 12% oregano (*Mentha pulegium*), 25% cumin (*Cuminum cyminum*), 10% camel thorn (*Alhagi persarum)*, 7% garlic (*Allium sativum*), and 12% Eucalyptus (*Eucalyptus*). The ingredients of the starter feed are shown in Table 1. The chemical composition of starter feed, hay and the medical plant mixed (% DM) are in Table 2.

The calves were housed in individual pens and fed with whole milk approximately at 10% of birth weight and they had free access to the feed starter and water. Milk was offered in two equal meals daily at 09:00 and 17:00. The medical plant was mixed with the starter feed and provided to the animals. Feed intake were recorded daily and the criterion for weaning of calves was at least 1500 g of starter feed per day for three days.

Sampling and analysis

Feed samples for chemical analysis were air-dried at 60 ˚C and ground with a 1 mm sieve prior to analysis for crude protein, ether extract, crude ash (AOAC, 2000), neutral detergent fiber and acid detergent fiber (Van Soest *et al*. 1991).

Blood samples were collected from the jugular vein using tubes with heparin or EDTA just before morning feeding, and the tubes were immediately placed in ice. The collected samples were centrifuged at 2500 × g for 15 min at 4 ˚C, and thereafter plasma samples were stored at -20 ˚C until analysis.

Plasma antioxidant activity was measured by a commercially available kit according to the manufacturer's protocol (Total Antioxidant Status, Randox Laboratories, Co. Antrim, UK). Briefly, the standard and sample were mixed with chromogenic reagent and were incubated with H_2O_2 for 3 min at 37 ˚C.

Thereafter, the optical density was measured for its absorbance at 600 nm using a spectrophotometer (U-2001, Hitachi). Plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured by enzymatic methods (pars azmon kit, co. Iranian).

Table 1 The ingredient composition of the starter feed

Ingredient	% of dry matter (DM)
Barley	40.5
Soybean meal	22.1
Molasses	28.8
Salt	5.6
Shell	2.0
Mineral premix[1]	1.0
Vitamin premix[2]	0.04

[1] Vitamin premix provided per kg of diet: vitamin A: 200000 IU; vitamin D_3: 300000 IU; vitamin E: 10000 IU; vitamin K: 2 mg and Anti-oxidant: 1000 mg/kg.
[2] Mineral premix provided per kg of diet: Cu: 3300 mg/kg; Fe: 100 mg; Zn: 16500 mg/kg; Mn: 9000 mg; I: 120 mg/kg; Co: 90 mg/kg and Se: 90 mg/kg.

Table 2 The chemical composition of concentrate, hay, and the mixed herbs (% DM)

Ingredient	Concentrate	Hay	Mixed herbs
Dry matter	90.48	89	96.38
Energy (kcal/kg)	-	-	3781
Crude protein	22.10	15	10.33
Crude fat	2.28	1.9	-
Acid detergent fiber (ADF)	11.00	50	-
Neutral detergent fiber (NDF)	24.00	37	-
Calcium	0.62	1.5	1.04
Phosphorus	0.42	0.21	0.47

Mallon di aldehyde (MDA) content in the blood samples was measured based on the method reported by Moore and Robert (1998).

Statistical analysis

Data were analyzed by the GLM procedure of SAS (SAS, 1998) with factorial arrangement of the treatments using following model:

$$Y_{ijk} = \mu + A_i + \beta_j + A\beta_{ij} + \varepsilon_{ijk}$$

Where:
μ: total average.
A_i: effect of herbal additive.
B_j: effect of probiotic.
AB_{ij}: effect of the interaction of between herbal additive and probiotic.
e_{ijk}: random error.

The level of statistical significance was preset at ($P<0.05$).

RESULTS AND DISCUSSION

Intake and gain performance

Intake and gain performance of calves were presented in Table 3. Probiotics supplementation had no effect on intake, whereas medical plant significantly increased starter intake ($P<0.05$).

Calves on starter feed with 1.5% medical plant consumed more concentrate in comparison to the other other groups. Feeding of 250 mg day of EO from oregano plants to sheep (Wang *et al.* 2009), 2 g of juniper berry EO (containing 35% α-pinene) to cows (Yang *et al.* 2007), 0.75 or 2 g of EO mixture to dairy cattle (Benchaar *et al.* 2007) did not influence feed intake. Ababakri *et al.* (2012) reported that addition 0.05% essence sprayed on of starter feed increased alfalfa intake in suckling Holstein calves. Busques *et al.* (2003) observed a 12% reduction in concentrate dry matter intake (DMI) in dairy cattle fed 0.6 g of cinnamaldehyde per kg of dry matter. Also Cardozo *et al.* (2006) reported that the cinnamaldehyde and eugenol mixture decreased dry matter and concentrate intakes compared with controls in growing heifers. The different results about the effects of medical plant additives, that have been reported by previous studies can be attributed to the route of administration, the form of medical plant additives and the dosage of EO. For the present study, the medical plant additives was mixed with starter feed to ensuring proper mixing and the better intake in calves fed 1.5% medical plant can explained bythe better daily and final weight gain in this group. The reduced intake in 3% medical plant additive may be was due to the effects of high levels of EO on feed intake, impaired gastro-intestinal microflora and could lead to compounds accumulating in animal tissues and products (Lambert *et al.* 2001). Generally, improvement in animal performance due to the addition of herbal additives could be attributed to the presence of different chemical compounds in these plants that can affect the digestive tract, feed efficiency and feed intake and eliminate bad microorganisms in digestive tract and feed (Ababakri *et al.* 2012). Riddell *et al.* (2010) observed that the DMI was not significantly different in calves fed bacterial probiotic in milk or starter in comparison whith a control group. But other studies showed that adding probiotic significantly increased feed intake (Chaudhary *et al.* 2008; Donovan *et al.* 2002). In terms of nutrition and toxicology, a low level of essential oils is important.

The effects of experimental treatments on performance are shown in Table 3. Daily weight gain was significantly increased by the medical plant additive ($P<0.05$) whereas no effect of probiotics was observed. The highest body weight gains were recorded in calves fed starter with 1.5% medical plant.

Riddell *et al.* (2010) reported that use of probiotic in calf starter had no significant effect on body weight gain. Inclusion of probiotics in the diet of young calves has been shown to improve performance characteristics including body weight gain and feed conversion as well as average daily gain in the first two weeks of life (Timmerman *et al.* 2005).

Table 3 The effects of a medicinal herb mix and probiotics on feed intake and growth rate of calves (g/day)

Items	Treatments						SEM	Interaction		
	1	2	3	4	5	6		H	P	H × P
Starter intake (g/d)	1089.3^{C}	1280.2^{a}	1273.4^{ab}	1121.3^{bc}	1086.6^{c}	1046.6^{c}	52.3	*	NS	**
Hay intake (g/d)	168.0	215.1	225.8	191.7	203.1	178.4	22.4	NS	NS	NS
Dry matter intake (g/d)	1144.9^{c}	1361.6^{a}	1356.1^{ab}	1163.4^{c}	1173.9^{c}	1115.4^{c}	56.3	NS	NS	NS
Body weight (kg)	103.6^{b}	106.3^{ab}	111.4^{a}	100.2^{b}	103.0^{b}	100.5^{b}	2.4	NS	NS	*
Daily weight gain (kg)	0.844^{bc}	0.924^{ab}	0.951^{a}	0.838^{bc}	0.748^{c}	0.768^{c}	0.03	**	NS	*

Treatments included: 1) control group (diet without herbal drugs mixture an probiotic), 2) 1.5% of medical plant mixture based on dry matter without probiotic, 3) 1.5% medical plant mixture + 2 g probiotic, 4) control diet + 3% medical plant mixture and 5) 3% medical plant mixture + 2 g probiotic and 6) 2 g probiotic without medical plant.
H: herbal anf P: probiotic.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
NS: non significant.

Other research has shown no benefit from feeding probiotics to calves (Winds-Chitl *et al*. 2008). Soltan (2009) reported that essential oils (*mint and eucalyptus*) given at different levels in milk replacer for calves had no effect on daily body weight gain when compared with controls. Cardozo *et al*. (2006) observed that the essential oil had no significant effect on average daily gain during the experiment. Fathi *et al*. (2009) reported that addition of vanilla in a starter diet, increased dairy body weight in the Holstein calves. Generally, body weight gain depends on feed intake and nutrient digestibility and animal health (Ababakri *et al*. 2012). The higher body weight gain in calves fed 1.5% medical plants may be due to the higher starter intake and healthier calves in this group.

Antioxidant activity

The effects of medical plant mixture on blood total antioxidant activity and mallon di aldehyde (MDA) content was significant (P<0.05) (Table 4). Blood antioxidant activity of calves fed 1.5% medical plant with 2 g probiotics was significantly higher than the other groups. No difference was observed in the AST and ALP between the other groups. Vakili *et al*. (2013) reported that vanilla addition in starter had no effect on AST and ALP in blood of calves. The lowest blood MDA content was measured in calves fed 3% medical plant mixed with 2 g probiotic. Concentrations of MDA, a degradation product of lipid peroxidation (Halliwell and Chirico, 1993), were used as a proxy measure for oxidative damage. Malondialdehyde is formed under oxidative stress conditions (Nielsen *et al*. 1997) and offers the advantage that it can be measured in blood as well as in liver tissue and in milk.

The influence of probiotics on enhancing immune system were related to its attachment to intestinal epithelium and mucosa which is the key factor in host's safety. Also, they can improve the immune system by assessment of the establishment of beneficial bacteria (Sami *et al*. 2001). Immune response and immunity status can be affected by the ingestion of antioxidant substances and oxidative stress (Miyazaki *et al*. 2001).

Stabel *et al*. (1989) demonstrated that the administration of selenium as an antioxidant source to calves inoculated with *Pasteurella hemolytica* resulted in a decrease in anti *Pasteurella hemolytica* a titers.

Chatterjee *et al*. (2003) reported that vitamin E supplementation as an antioxidant source increased both the antioxidant activity and IgG concentration in plasma in periparturient cows. The dietary supplementations of antioxidants such as selenium or vitamin E have been reported to decrease the risks of retained placenta, metritis and clinical mastitis after delivery in cattle (Erskine *et al*. 1997).

These previous studies suggest that antioxidant ingestion should bring health benefits in cattle. Use of plants with anti-oxidant characteristics results in free radicals being annihilated so they will improve animal health. Polyphenolic compoundsare diverse antioxidants which inhibit these free radicals.

Carvacrol and thymol are the most important antioxidant components of thyme that have powerful antioxidant characteristics.

Thymol has OH group and uses it as H transporter for peroxidation and reduces hydroxyl peroxide free radicals (H_2O_2) (Lee *et al*. 2007). Hosoda *et al*. (2006) reported that between three herbs (mint, cloves and lemongrass), clove significantly increased total antioxidant activity of the serum.

Higher antioxidant levels in this plant is the reason for this increase that is in agreement with results of the present study. These antioxidant effects in the current plants are not temporary and can guaranty the health of calves.

Weaning age

Weaning of calves was when a fixed starter intake of at least 1500 g/day was achieved for three consecutive days. Based on this criterion, calves fed the starter with 1.5% of a medical plant mix were weaned earlier compared with other groups (Figure 1). Fathi *et al*. (2009) observed that using a flavored starter met weaning criteria at a younger age and resulted in a shorter preweaning period and increasing DMI.

Table 4 The effect of a medicinal plant mix and probiotics on the Antioxidant activity in the Holstein calves

Items	Treatments						SEM	Interaction		
	1	2	3	4	5	6		H	P	H × P
Antioxidant activity (µmol/L)	0.640^{b}	0.647^{b}	0.746^{ab}	0.766^{a}	0.665^{b}	0.716^{ab}	0.03	*	NS	NS
Malone di aldehyde (µmol/L)	1.74^{a}	1.72^{ab}	1.55^{bc}	1.73^{abc}	1.66abc	1.41^{d}	0.06	*	NS	**
AST (U/L)	50.72	53.00	52.90	52.88	50.36	53.00	1.02	NS	NS	NS
ALT (U/L)	11.75	12.98	13.32	11.64	11.69	12.79	0.60	NS	NS	NS

Treatments included: 1) control group (diet without herbal drugs mixture an probiotic), 2) 1.5% of medical plant mixture based on dry matter without probiotic, 3) 1.5% medical plant mixture + 2 g probiotic, 4) control diet + 3% medical plant mixture and 5) 3% medical plant mixture + 2 g probiotic and 6) 2 g probiotic without medical plant.

H: herbal; P: probiotic; AST: aspartate aminotransferase and ALT: alanine aminotransferase.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

NS: non significant.

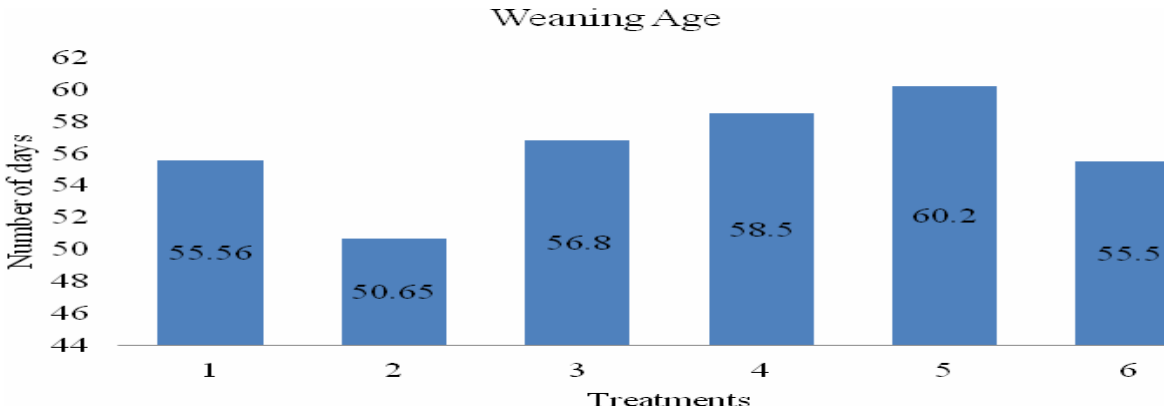

Figure 1 Treatments included: 1) control group (diet without herbal drugs mixture), 2) 1.5% of medical plant mixture based on dry matter without probiotic, 3) 1.5% medical plant mixture + 2 g probiotic, 4) control diet + 3% medical plant mixture, 5) 3% medical plant mixture + 2 g probiotic and 6) 2 g probiotic without medical plant

Age of weaning was significantly decreased when peppermint oil was added to the diets, so calves receiving peppermint oil were weaned 8 and 2 days earlier at 0.05% and 0.025% inclusion levels, respectively, (Ababakri *et al.* 2012).

It is common practice within the dairy cattle industry to restrict the consumption of milk or milk replacer to stimulate an earlier consumption of calf starter, as this decreases the age at which calves can be completely weaned from milk.

CONCLUSION

Inclusion of a medical plant additive at 1.5% of starter feed resulted in higher starter intake and daily weight gain. Based on these results, no effect of probiotic supplementation was observed. The results showed that a medical plant mixe improved calves gain performance and immune parameters, so it can be considered as a proper feed additive in calves rearing programs.

ACKNOWLEDGEMENT

The authors thank the Manager and Staff of the Dairy cow Station of Agro-Industrial Complex of Moghan, special to Mr. A. Basiri and Mr. M. Ramezani, for their assistance in providing farm and experimental animals and for their other kindly helps.

REFERENCES

AOAC. (2000). Official Methods of Analysis. Vol. I. 17th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Ababakri R., Riasi A., Fathi M.H., Naeimipur H. and Khorshidi D. (2012). Effect of peppermint oil added to the initial concentration on ruminal fermentation, weaning age and performance Holstein dairy calves. *J. Appl. Anim. Sci*. **22,** 141-154.

Benchaar C., Calsamiglia B., Chaves A., Fraser G., Colombatto D., McAllister T. and Beauchemin K. (2007). A review of plant-derived essential oils in ruminant nutrition and production. *Anim. Feed Sci. Technol*. **255,** 409-447.

Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods: a review. *Int. J. Food Microb*. **94,** 223-253.

Busques M., Greathhead H., Calsamigilia S., Ferret A. and Kamel C. (2003). Effect Del extra to deajoyel cinemaldehid sobrela production, composition residues enlech end vacas de altar production. *ITEA*. **24,** 756-758.

Cardozo P.W., Calsamiglia S., Ferret A. and Kamel C. (2006). Effects of alfalfa, extract, anise, capsicum and a mixture of cinamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. *J. Anim. Sci*. **84,** 2801-2808.

Chao S., Young D. and Oberg C. (2000). Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *J. Essent. Oil. Res*. **12,** 639-649.

Chatterjee P., Kaur H. and Panda N. (2003). Effect of vitamin E supplementation on plasma antioxidant vitamins and immunity status of crossbred cows. *Asian-Australas J. Anim. Sci*. **16,** 1614-1618.

Chaudhary L., Sahoo N., Agrawal D., Kamra and Pathak N. (2008). Effect of direct fed microbial on nutrient utilization, rumen fermentation, immune and growth response in crossbred cattle calves. *Indian Anim. Sci*. **78,** 515-521.

Donovan D., Franklin S., Chase C. and Hippen A. (2002). Growth and health of Holstein calves fed replacer supplemented with antibiotics or Enteroguard. *J. Dairy Sci*. **85,** 947-950.

Dorman H. and Deans S. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbial*. **88,** 308-316.

Erskine R., Bartlett J., Herdt T. and Gaston P. (1997). Effects of parenteral administration of vitamin E on health of periparturient dairy cows. *J. Am. Vet. Med. Assoc*. **211,** 466-469.

Fathi M., Riasi A. and Allahresani A. (2009). The effect of vanilla flavored calf starter on performance of Holstein calves. *J. Anim. Feed Sci*. **18,** 412-419.

Fuller R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol*. **66,** 365-378.

Halliwell B. and Chirico S. (1993). Lipid peroxidation: its mechanism, measurement and significance. *Am. J. Clin. Nutr*. **57,** 715-724.

Hosoda K., Kuramoto K., Eruden B., Nishida T. and Shioya S. (2006). The effects of three herbs as feed supplements on blood metabolites hormones, antioxidant activity, IgG concentration and ruminal fermentation in holstein steers. *Anim. Sci*. **19,** 35-41.

Hristov A., McAllister T., Van Herk F., Cheng K., Newbold C. and Cheeke P. (1999). Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. *J. Anim. Sci*. **77,** 2554-2563.

Hume M. (2011). Food safety symposium: potential impact of reduced antibiotic use and the roles of prebiotics, probiotics, and other alternatives in antibiotic-free broiler production. Poult. Sci. **90,** 2663-2669.

Lambert R., Skandamis P., Coote P. and Nychas G. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, listeria monocytogenes and *Salmonella enteric*. *J. Food Protec*. **65,** 1545-1560.

Lee M., Park Y.B., Moon S.S., Bok S.H., Kim D.J., Ha T.Y., Jeong T.S., Jeong K.S. and Choi M.S. (2007). Hypocholesterolemic and antioxidant properties of 3-(4-hydroxyl) propanoic acid derivatives in high-cholesterol fed rats. *Chem. Biol. Int*. **170,** 9-19.

Miyazaki Y., Yamasaki H., Mishima K., Mansho H. and Yamada H. (2001). Oxidative stress by visible light irradiation suppresses immunoglobulin production in mouse spleen lymphocytes. *Biosci. Biotechnol. Biochem*. **65,** 593-598.

Moore K. and Robert L. (1998). Measurement of lipid peroxidation. *Free. Radic. Res*. **28,** 659-671.

Nielsen F., Mikkelsen B., Nielsen J., Andersen H. and Grandjean P. (1997). Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. *Clin. Chem*. **43,** 1209-1214.

Riddell J., Gallegos A., Harmon D. and Mcleol K. (2010). Addition of a *Bacillus* based probiotic to the diet of pre ruminant calves: influence on growth, health and blood parameters. *Int. J. Appl. Res. Vet. Med*. **8,** 78-85.

Sami N., Salminen S., Bylund G. and Ouwehand A. (2001). Characterization of properties of human and dairy-derived probiotic for prevention of infectious disease in fish. *Appl. Environ. Microbial*. **67,** 2430-2435.

SAS Institute. (1998). SAS®/STAT Software, Release 8.2. SAS Institute, Inc., Cary, NC. USA.

Soltan M. (2009). Effect of essential oils supplementation on growth performance, nutrient digestibility, health condition of Holstein male calves during pre- and post- weaning periods. *Pakistan J. Nutr*. **8,** 642-652.

Stabel J., Spears W., Brown T. and Brake J. (1989). Selenium effects on glutathione peroxidase and the immune response of stressed calves challenged with *Pasteurella hemolytica*. *J. Anim. Sci*. **67,** 557-564.

Timmerman H., Koning C., Mulder L., Rombouts F. and Beynen A. (2005). Monostrain, multistrain and multispecies probiotics-a comparison of functionality and efficacy. *Int. J. Food Microbiol*. **96,** 219-233.

Vakili A., Khorrami B., Mesgaran M. and Parand E. (2013). The Effects of thyme and cinnamon essential oils on performance, rumen fermentation and blood metabolites in Holstein calves consuming high concentrate diet. *Asian-Australas J. Anim. Sci*. **26,** 935-944.

Van Soest P., Robertson J. and Lewis B. (1991). Methods for dietary fiber, neutral detergent fiber, and no starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. **74,** 3583-3597.

Wallace R. (2004). Antimicrobial properties of plant secondary metabolites. *Proc. Nutr. Soc*. **63,** 621-629.

Wang Y., McAllister T., Yanke L., Xu Z., Cheeke P. and Cheng K. (2000). *In vitro* effects of steroidal saponins from *Yucca schidigera* extract on rumen microbial protein synthesis and ruminal fermentation. *J. Sci. Food Agric*. **80,** 2114-2122.

Wang C., Wang S. and Zhou H. (2009). Fnfhomycin, ropadiar and seponim on nutrient digestibility, roman fermentation and methane emission from sheep. *Anim. Feed Sci. Technol*. **148,**

157-166.

Winds-Chitl P., Randall K. and Brainard D. (2008). Growth and performance of Holstein dairy calves supplemented with a probiotic. *Res. Prog. Rep*. **22,** 1991-2022.

Yang W., Benchaarc B., Chaves H. and Mcalliste T. (2007). Effect of garlic and juniper berry essential oils on ruminal fermentation and on the site and extend of digestion raminal fermentation and on the site and extend of digestion in lactating cows. *J. Dairy Sci*. **12,** 5671-5681.