Effects of *Matricaria chamomille* and *Cichorium intybus* powder on performance, Rumen microbial population and some blood parameters of Dallagh sheep

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Abstract:

BACKGROUND: Manipulation of rumen microbial population for improving animal performance is done by several methods including medicinal plants. *Matricaria chamomille* (chamomile) and *Cichorium intybus* (chicory) are two medicinal plants with antibacterial effect, however, little information exists about the effects of them on rumen microflora. **OBJECTIVES:** The present study was carried out to evaluate the effects of chamomile and chicory powder on performance, ruminal microbial population and some blood parameters of sheep. **METHODS:** 9 Dallagh sheep were used in a change over design experiment at three 21-d periods (14 days as adaptation and 7 days for sample collection). Treatments were: 1) control (without additive), 2) chamomile (contained 10% chamomile powder) and 3) chicory (contained %10 chicory powder). Rumen fluid was collected before, 4 h and 8 h after morning feeding and a blood sample was obtained 3 h after morning feeding at the last day of each period. **RESULTS:** Weight gain, dry matter intake, feed conversion ratio, total count of bacteria, acid lactic bacteria, rumen pH, blood glucose and total protein were not affected by treatments. Diet containing 100g chicory significantly decreased plasma triglyceride versus chamomile but the difference was not significant compared with control. Coliforms in rumen fluid at 4 h after morning feeding were lower in chamomile treatment than control ($p<0.05$). Lambs that received chamomile and chicory powder had significantly higher rumen protozoa number before morning feeding. **CONCLUSIONS:** Although *Matricaria chamomille* and *Cichorium intybus* dry powder had some significant effects on rumen microbial population it had no effect on performance, growth and blood metabolites.

Introduction

Manipulation of rumen microbial ecosystem for enhancing fibrous feed digestibility, reducing methane emission and nitrogen excretion by ruminants to improve their performance are some of the most important goals for animal nutritionists (Patra et al., 2006). Medicinal herbs or Plant extracts with high concentration of secondary me-

tabolites are good candidates for achieving one or more of these objectives (Wanapat et al., 2008).

Chamomile is a widely available herb with diverse therapeutic uses that has been used for centuries as a medicinal plant. The components of the essential oil extracted from chamomile flowers possess anti-inflammatory, anti-allergic, anti-spasmodic, anti-bacterial, anti-pyretic, ulcer-protective, anti-fungal, sedative, analgesic and anti-oxidant properties (Andishe et al., 2015). Several flavonoids and other phenolic compounds have been identified in various parts of the chamomile flower head. Apigenin (16.8%), quercetin (9.9%), patuletin (6.5%), luteolin (1.9%) and their glucosides are the major flavonoids present in the total flower (McKay and Blumberg, 2006). *Matricaria chamomille* extraction 5 ml Kg- body weight that were orally given to experimental infected lambs with *Ostertagia ostertagi* parasite showed favorable results in terms of antihelminthic in lambs (Bahrami et al., 2010).

Cichorium intybus commonly known as chicory, is an erect fairly woody perennial herb, around 1 m in height with a fleshy taproot of up to 75 cm in length and large basal leaves. Chicory is cultivated for numerous applications and can be divided into four main varieties or cultigroups according to their use: (1) "industrial" or "root" chicory produces the taproot as a coffee substitute or for inulin extraction; (2) "Brussels" or "witloof "chicory as industrial chicory for etiolated buds (chicons) by forcing; (3) "leaf "chicory is used as fresh or cooked vegetables; and (4) "forage"chicory, to intensify herbage obtainability in perennial pastures for livestock. Chicory is a medicinally important plant in Eurasia and in parts of Africa. The flowers of chicory contain saccharides, methoxy-coumarin cichorine, flavonoids, essential oils, and anthocyanins contributing to the blue color of the perianth. Chicory possess anti-inflammatory, anti-helminthic, anti-spasmodic, anti-bacterial, anti-oxidant, anti-diabetic, hepatoprotective and gastro protective properties (Street et al., 2013). In several studies the effect of chicory has been investigated on performance, carcass characteristics and gastrointestinal nematodes of lambs (Somasiri et al., 2015; Miller et al., 2011) and herbage intake and milk production of cows (Muir et al., 2015).

To the best of our knowledge, there is no study about the effect of chamomile and chicory as feed additive on rumen microflora and hematologic parameters in sheep. Thus the purpose of this study was to evaluate supplementation of these herbs on rumen microbial population, some hematologic parameters and performance of sheep.

Materials and Methods

Experimental design, diets and animals management: Nine adult male Dallagh sheep with an average initial BW of 35±3 kg were used in a change over design. The experiment consisted of three 21-day periods (14 days as adaptation and 7 days as sampling) and there were 3 treatments, each consisting of 9 replicates. At the start of the experiment sheep were weighted and randomly assigned to 1 of 3 treatments: 1) control (without additive), 2) chamomile (contained 10% chamomile powder) and 3) chicory (contained %10 chicory powder). Chamomile and chicory were collected from mountains around Minoodasht city, Golestan province, in the north of Iran.

These plants were shade-dried and ground through a 1 mm screen.

The basal diet was composed of 30% roughage and 70% concentrate, and the ingredients and nutrient composition of the basal diet (DM basis) is given in Table 1. Diets were fed as total mixed rations (TMR) and formulated according to NRC (1996) recommendations. The experimental diets were offered twice (08:00 and 18:00 h) daily for ad libitum intake (10% refusals) with one-half of the daily feed allotment offered at each feeding. Amounts of feed offered and refused were recorded daily for each animal throughout the trial. Body weight were measured at the beginning and the end of each period. The sheep were housed in individual stalls on mattresses bedded with straw in pens (2m× 1.5m) equipped with a water bowl. The pens were located in a covered barn. Each animal had free access to water at all times.

Measurements, sample collection and analysis: Average daily weight gain was calculated throughout the trial. Feed conversion ratio was calculated as the ratio between feed intake and weight gain.

Ruminal fluid was collected from animals at the last day of each period before morning feeding, and 4 h and 8 h after morning feeding via the esophagus using a 60 ml gavage syringe and a lubricated rubber tube. The first 20 ml of ruminal fluid content was discarded to ensure they were not polluted with saliva and then squeezed through four layers of cheesecloth. Rumen pH was measured immediately with a portable pH meter (Metrohm 691, Switzerland).

A 5-ml portion of the strained ruminal fluid was mixed with 5 ml of 0.2 N HCL. All samples were stored at -20 oC for subsequent analyses. The ruminal fluid concentration of NH3-N was determined using spectrophotometry by the phenol hypochlorite method of Broderick and Knag (1980). Serial 10-fold dilutions of strained ruminal fluid were prepared and used as inoculum on plate count agar (PCA), Modified Rogosa and Sharp Agar (MRSA) and Violent Red Bile Agar (VRBA) medium for total viable bacteria, acid lactic bacteria and coliforms count respectively (Ghoorchi et al., 2009).

Blood samples were collected at non-heparinized tube from the jugular vein at the end of each period (3 h after the morning feeding), samples were centrifuged (Denley BS400, England) at 10,000 rpm for 10 min and collected serum was frozen at -20^{o}C until analysis. Serum concentration of glucose, total protein and triglyceride were determined using the commercial kits of Darmanfaraz Company (Isfahan, Iran) by spectrophotometry.

A 1-ml portion of the strained ruminal fluid was preserved using 9 ml of MFS (methyl green- formaldehyde- saline) solution for enumeration of protozoa. Protozoa samples were stored at room temperature in the dark until counting. Protozoa were enumerated microscopically in a Neubauer cytometer at ×40 magnification. Each sample was counted twice and if the average of the duplicates differed by more than 10%, the counts were repeated (Cedrola et al., 2015).

Statistical analysis: All data were statistically analyzed according to a change over design using the general linear model (GLM) procedure of SPSS software (version 18). Significant differences between means of treatments were assessed by the LSD test, and the differences among treatments were declared significant at $p<0.05$. Initial weight of sheep were used as covariate for weight gain data analysis.

Table1. Ingredient and nutrient composition of experimental diets (DM basis). [1] Contained: 21 g/kg Mg, 0.3 g/kg Zn, 2.2 g/kg Mn, 3 g/kg Fe, 0.3 g/kg Cu, 0.001 g/kg Se, 0.1 g/kg Co, 0.12 g/kg I, 195 g/Kg Ca, 80 g/Kg P, 600 IU/g of vitamin A, 200 IU/g of vitamin D, and 2.5 IU/g of vitamin E. *Treatments include: Control: without additive; Chamomile: contained 10% *Matricaria chamomile* powder; Chicory: contained 10% *Cichorium intybus* powder.

	Treatments*		
	Control	Chamomile	Chicory
Ingredient (%)			
Barley grain, ground	35	35	35
Alfa alfa	25	15	15
Chamomile dry power	-	10	-
Chicory dry powder	-	-	10
Wheat straw	18.5	18.5	18.5
Wheat bran	19	19	19
salt	0.5	0.5	0.5
Mineral-vitamin premix[1]	1	1	1
Pearl powder	1	1	1
Composition			
CP (%)	12	12.15	12.1
ME (Mcal/kg)	2.3	2.25	2.22
NDF (%)	42	43	43
Ash (%)	6.8	6.7	6.7
Ca (%)	0.80	0.83	0.82
P (%)	0.45	0.48	0.49

Table 2. Effect of *Matricaria chamomille* and *Cichorium intybus* powder on performance of sheep. *Treatments include: Control: without additive; Chamomile: contained 10% *Matricaria chamomile* powder; Chicory: contained 10% *Cichorium intybus* powder. *Standard error of means.

	Treatments*			
Item	Control	Chamomile	Chicory	SEM*
Initial weight (kg)	35.42	35.92	36.13	1.65
Final weight(kg)	38.62	38.05	38.37	1.74
Final weight gain (Kg)	3.18	2.13	2.24	0.50
Daily weight gain (g)	151.85	101.58	106.87	23.94
Daily dry matter intake (g)	857.53	830.95	825.42	93.71
Feed conversion ratio	6.58	7.46	9.24	2.08

Results

Dry matter intake and performance data are presented in Table 2. Initial and final BW did not differ among the experimental treatments and consequently, daily weight gain was not affected by treatments. Similarly, feed conversion ratio, expressed as feed: gain ratio (DMI to average daily gain ratio), was similar among treatments.

Mean of blood metabolites is shown in Table 3. Concentrations of serum glucose and total protein were not affected by additive treatments. Triglyceride level in chamomile treatment compared to chicory increased significantly but the differences were not significant compared with control.

Table 4 represents the effect of experimental treatments on rumen fluid pH, NH3-N and protozoa. Rumen fluid pH was not affected by treatments at before morning feeding, 4 h and 8 h after morning feeding. There was no significant difference in ruminal NH3-N concentration among treat-

Table 3. Effect of *Matricaria chamomille* and *Cichorium intybus* powder on some blood parameters of sheep. *Treatments include: Control: without additive; Chamomile: contained 10% *Matricaria chamomile* powder; Chicory: contained 10% *Cichorium intybus* powder. a-bMeans within the same row without common letters differ significantly ($p<0.05$). *Standard error of means.

	Treatments*				
Item	Control	Chamomile	Chicory	SEM*	p-value
Total protein (mg/dl)	5.58	5.27	5.18	0.48	0.83
Glucose (mg/dl)	70.60	67.65	87.90	7.47	0.14
Triglycerides (mg/dl)	21.27^{ab}	28.38^{a}	15.49^{b}	2.82	0.01

Table 4. Effect of experimental treatments on pH, NH3-N and protozoa of rumen fluid. *Treatments include: Control: without additive; Chamomile: contained 10% *Matricaria chamomile* powder; Chicory: contained 10% *Cichorium intybus* powder. * a-bMeans within the same row without common letters differ significantly ($p<0.05$). *Standard error of means.

Item	Time	Treatment *			SEM *	p-value
		Control	Chamomile	Chicory		
pH	Before feeding	6.91	6.79	6.82	0.07	0. 50
	4 hours after feeding	6.53	6.57	6.53	0.06	0.89
	8 hours after feeding	6.57	6.54	6.56	0.07	0.95
NH3-N	Before feeding	2.67	2.52	2.61	0.27	0.92
(mg dl^{-1})	4 hours after feeding	2.49	4.19	1.99	1.24	0.43
	8 hours after feeding	2.25	2.20	2.01	0.17	0.60
Protozoa	Before feeding	4.96^{a}	7.65^{b}	7.65^{b}	0.90	0.04
($\times 10^5$ ml^{-1})	4 hours after feeding	6.91	8.98	8.51	1.16	0.29
	8 hours after feeding	6.53	9.40	9.03	1.13	0.48

Table 5. Effect of *Matricaria chamomille* and *Cichorium intybus* powder on rumen bacteria ($Log^{cfu/ml}$) of sheep. * Treatments include: Control: without additive; Chamomile: contained 10% *Matricaria chamomile* powder; Chicory: contained 10% *Cichorium intybus* powder * a-bMeans within the same row without common letters differ significantly ($p<0.05$). * Standard error of means.

Item	Time	Treatments*			SEM*	p-value
		Control	Chamomile	Chicory		
Total bacteria	Before feed	9.91	10.08	10.16	0.13	0.64
	4 hours after feed	9.95	9.86	9.99	0.14	0.82
	8 hours after feed	10.02	9.82	9.93	0.12	0.55
Coliform bacteria	Before feed	3.58	3.33	3.59	0.09	0.10
	4 hours after feed	4.08^{a}	3.55^{b}	3.93^{ac}	0.11	0.01
	8 hours after feed	3.74	3.91	4.04	0.15	0.40
Lactic acid bacteria	Before feed	5.46	5.46	5.62	0.61	0.71
	4 hours after feed	5.22	5.21	5.51	0.12	0.15
	8 hours after feed	5.41	5.27	5.29	0.15	0.79

ments. The rumen protozoan was significantly lower in control compared to chamomile and chicory treatment before morning feeding but the differences was not significant among three treatments at 4 and 8 h after the morning feeding.

Table 5 summarize data obtained on the effects of experimental diets on rumen microbial population. Total viable bacterial count and Acid lactic bacteria had no significant difference before feeding, 4h and 8 h after feeding. Coliforms count was influenced by treatments. Coliforms count was significantly lower in chamomile treatment

compared with control and chicory treatment 4 h after morning feeding. But Before morning feeding and at 8 h after feeding there was no significant difference among treatments.

Discussion

Feed intake and performance: There is little information on the effects of chamomile and Chicory 10% on DMI and performance in sheep so we must compare the results of this study with other medicinal plants and species. Somasiri et al. (2015) found that both Plantain mix and Chicory mix lambs had greater live weight gains and were heavier at slaughter and displayed greater carcass weights and dressing-out percentages compared to the Pasture mix lambs. In a study lambs grazing chicory had increased performance and average daily gain and reduced gastrointestinal infection compared to those grazing Cynodon dactylon (Miller et al., 2011). Torabi Goudarzi et al., (2010) reported that mixture of chicory and anise significantly increased rate of appetite, frequency and strength of ruminal contractions in cattle. Payvastegan et al. (2015) showed that 10 and 20 g/d *Satureja hortensis* dry powder had no significant effect on dry matter intake, average daily gain, and feed conversion of kids. The findings of present study for DMI were in line with previous researches in growing lambs (Chaves et al., 2008a), sheep (Distel et al., 2007; Nolte and Provenza, 1992) and cattle (Benchaar et al., 2007; Beauchemin and Mcginn, 2006) who found no effect of essential oil (EO) on DMI. In contrast with our results, Demir et al. (2003) reported that pennyroyal EO increased nutrient absorption, growing and weight gain. Ghahari et al. (2016) showed that inclusion of *Mentha spicata* and *Mentha pulegium* calves diet significantly decreased dry matter intake. Our result confirmed the report which showed that *Leucaena leucocephalaon* and *Salix babylonica* had no effect on weight gain in growing lamb (Salem et al., 2011). The effects of EO on DMI might vary with EO source, type of diet, diet interaction or adaptation of rumen microbial population to EO (Yang et al., 2010a; b; Geraci et al., 2012). Furthermore, it has been reported that it can be affected by a number of dietary or management factors such as body weight, animal growth stage, and specific physical and chemical characteristics of diet or rumen fermentation metabolites (Allen, 1997; Yang et al., 2010b).

Blood metabolites: Payvastegan et al. (2015) showed that 10 and 20 g/d *Satureja hortensis* dry powder had no significant effect on glucose, total protein, triglyceride, cholesterol and HDL in kids. Our results are in agreement with some studies that declared peppermint and dill oil feeding at 0.05% DM had no effect on blood glucose in Kordi breed growing lamb (Tohidi, 2014) and peppermint EO feeding did not affect blood metabolites significantly (Hosoda et al., 2006). In our study serum triglyceride was increased by chamomile compared to chicory supplementation which was in contrast to some researches (Tohidi, 2014; Hosoda et al., 2006). Ghahari et al. (2016) showed that supplementation of 20 g/d *Mentha spicata* and *Mentha pulegium* dry powder had no significant effect on plasma glucose, triglyceride and total protein in calves. The result of total serum protein in this study was in agreement with Ahmadi Naghadehi et al. (2014) who reported that 100 and 200 mg peppermint EO did not significantly change

total serum protein of sheep. It has been reported that concentration of some blood metabolites such as triglyceride can be influenced by EO supplementation via changing of feed intake (Yang et al., 2010b) and no change in blood metabolites in present study may have contributed to lack of DMI alteration by dry powder.

Rumen pH, NH3-N and protozoa: Rumen pH is a resultant of produced volatile fatty acids (acetate, propionate and lactate), ammonia, rumen buffers and saliva (Vansoet, 1994) and an index for fermentation. Payvastegan et al. (2015) found that 20 g/d dry powder and 200 mg/d essential oil of *Satureja hortensis* decreased rumen pH. 100 and 200 mg peppermint EO feeding did not affect rumen pH in sheep (Ahmadi Naghdahi et al., 2014). In agreement with our result peppermint feeding at 5% DM (Hodosa et al., 2006) and peppermint EO feeding at 0.025% DM (Tohidi, 2014) had no effect on rumen pH in steers and sheep respectively. In contrast with our study, peppermint feeding (200 g/day) decreased rumen pH and protozoa count in steers (Ando et al., 2003). Variable effects of EO on rumen pH can result from different diets and levels of EO in studies as when EO was added to diets with higher roughage/concentrate ratio, rumen pH increased (Meyer et al., 2009).

Similarly, supplementation with 0.2g/kg (DM basis) of carvacrol and cinnamaldehyde did not alter ruminal ammonia in growing lamb (Chaves et al., 2008b). In contrast, in a study it was reported that free thymol and sustained release thymol decreased plasma urea concentration and rumen ammonia at 5, 7 h after feeding (Zamani et al., 2015). Taherinia et al., (2015) showed that supplementation of garlic powder at 2% DM had no effect on blood urea nitrogen and rumen ammonia. Hosoda et al. (2006) found that peppermint feeding at 5% of the diet (DM basis) significantly increased rumen ammonia in Holstein steers. Also, this is in disagreement with the in vitro results of Newbold et al. (2004), who observed a decrease in ruminal ammonia when rumen contents of cows or sheep supplemented with EO and acid hydrolyzed protein were incubated for 24-48 h in strained ruminal fluid (1 or 110 mg/d, respectively).

Wanapat et al. (2013) reported that lemongrass supplementation at 100g/d alone or plus peppermint powder at 10 g/d with or without garlic powder 40g/d decreased rumen protozoa. Busquet et al. (2005) showed in vivo that 200 g peppermint powder decreased rumen protozoa. In a study it was reported that peppermint decreased rumen protozoa and ammonia nitrogen (Bach et al., 2005). Our result was in agreement with some researchers (Newbold et al., 2004; Benchaar et al., 2007) who reported that EO feeding had no effect on rumen protozoa in sheep. Similarly, Djouvino et al. (1997) reported that 450 g/d peppermint did not influence protozoa count in sheep.

Rumen bacteria: Rumen microbiome plays a key role in rumen feed fermentation, methane production and, nitrogen utilization. Its activity directly affects ruminant performance, health, and welfare, but it is still poorly understood. Rumen microbial populations are highly dynamic and are able to adapt to a wide range of nutritional strategies or host physiological status so it is often difficult to understand the interactions between dietary components and rumen microbial populations (Cobellis et al., 2016). Considering the large number of different groups of chemical compounds present in essential oils, it is most likely

that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell (Brenes and Roura, 2010). In one study a mixed EO did not change rumen microbial population (Benchaar et al., 2007). Also, it was reported that EO inhibited rumen bacteria (Mcintosh et al., 2003). Factors such as EO type, diet property, species, and geographical situation affect variability and bacterial count including lactic acid bacteria of rumen (Dehority et al., 2003). In our study chamomile decreased coliforms which was in disagreement with a study that showed clove and garlic EO did not affected rumen coliforms (Ghoorchi et al., 2009).

Conclusions: The results of this study showed that although chamomile 10% decreased coliform bacteria 4 h after feeding but chamomile and chicory 10% had no effect on performance, blood metabolites, rumen pH, NH3-N and protozoa count. More in vivo studies are required to investigate the effects of different supplementations levels on rumen microbial fermentation and blood metabolites to improve nutrient utilization and growth performance in sheep.

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اثر پودر گیاهان بابونه و کاسنی بر عملکرد، جمعیت میکروبی شکمبه و برخی فراسنجه‌های خونی گوسفند

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چکیده

زمینه مطالعه: تغییر جمعیت باکتریایی شکمبه به منظور افزایش عملکرد با چندین روش از جمله گیاهان دارویی انجام می شود. بابونه و کاسنی دو گیاه دارویی با اثرات ضد میکروبی هستند که اطلاعات اندکی در مورد اثر آنها بر میکروفلور شکمبه وجود دارد **هدف:** این مطالعه به‌منظور بررسی اثرات پودر بابونه و کاسنی بر عملکرد، جمعیت میکروبی شکمبه و برخی فراسنجه‌های خونی گوسفند انجام شد. **روش کار:** از ۹ رأس گوسفند نر نژاد دالاق در قالب طرح چرخشی در ۳ دوره ۲۱ روزه شامل ۱۴ روز به‌عنوان دوره عادت‌پذیری و ۷ روز نمونه‌برداری استفاده شد. تیمارهای آزمایشی شامل تیمار۱ (شاهد): بدون افزودنی، تیمار۲ (بابونه): جیره حاوی ۱۰٪ پودر بابونه و تیمار ۳ (کاسنی): جیره حاوی ۱۰٪ پودر کاسنی بودند و گوسفندان در قفس‌های انفرادی بطور آزاد به آب و غذا دسترسی داشتند. به‌منظور تعیین جمعیت میکروبی، pH و ازت آمونیاکی، نمونه‌های مایع شکمبه قبل از خوراک‌دهی صبح، ۴ و ۸ ساعت بعد از خوراک‌دهی صبح جمع‌آوری شدند. خون‌گیری در پایان هر دوره از سیاهرگ گردنی صورت گرفت. **نتایج:** افزودن پودر خشک شده گیاه بابونه و کاسنی تأثیر معنی‌داری بر افزایش وزن روزانه، مصرف غذا، ضریب تبدیل، pH، تعداد کل باکتری‌ها و باکتری‌های اسید لاکتیکی نداشت. تعداد کلی‌فرم‌ها در تیمار بابونه نسبت به تیمار شاهد ۴ساعت بعد از خوراک‌دهی صبح کاهش معنی‌داری داشت. جمعیت پروتوزوآها در تیمارهای بابونه و کاسنی نسبت به گروه شاهد، قبل از خوراک‌دهی صبح به‌طور معنی‌داری بالاتر بود. تیمارهای بابونه و کاسنی تأثیر معنی‌دار بر گلوکز و پروتئین کل سرم خون نداشتند ولی غلظت تری گلیسرید بین تیمار کاسنی و بابونه اختلاف معنی‌داری داشت. **نتیجه‌گیری نهایی:** بطور کلی نتایج آزمایش نشان داد که پودر خشک شده بابونه و کاسنی هر چند بر جمعیت میکروبی شکمبه موثر بود اما تأثیری بر عملکرد، رشد و فراسنجه‌های خونی نداشت.

واژه های کلیدی: متابولیت‌های خونی، پودر کاسنی، جمعیت میکروبی، پودر بابونه، گوسفند

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