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Influence of feeding synbiotic containing *Enterococcus faecium* and inulin on blood metabolites, nutrient digestibility and growth performance in sheep fed alfalfa-based diet

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The present study was conducted to investigate the effects of synbiotic (SYN) containing *Enterococcus faecium* and Inulin on blood metabolites, nutrients apparent digestibility and performance of sheep fed alfalfa based diet. The 21 Farahani sheep averaging body weight 33.9 kg were allocated in a completely randomized design with 7 replicates in each treatment. The basal diet was formulated based on NRC (1989) and three treatments were; T1 = un-supplemented treatment (control), T2 = 2 g/d/h SYN supplemented, and T3 = 10 g/d/h SYN supplemented treatments. The synbiotic was supplemented once a day at the time of morning meal. The experiment lasted 12 weeks which the first week was for adaptation period. Supplementation of SYN had no effect on dry matter intake (DMI), daily gain (DG) and feed conversion rate (FCR). Digestibility of DM, OM, and CP were not affected with SYN supplementation. However, digestibility of NDF improved significantly in supplemented treatments (NDF digestibility was 46.09, 47.11 and 49.54% for treatments 1, 2 and 3, respectively) (P < 0.05). Considering the blood metabolites, both non-esterifies fatty acids (NEFA) and total immunoglobulin (total IG) were affected with supplementation (blood total IG concentration was 1.91, 1.95 and 2.27 mg/dl for treatments 1, 2 and 3, respectively) (P < 0.05). The blood urea nitrogen (BUN), glucose and albumin concentrations did not differ among treatments. Based on the present study it can be concluded that although SYN supplementation had no effect on performance traits of sheep, it had significant effect on both fiber digestibility and immunoglobulin concentration in blood.

Key words: Synbiotic, sheep, digestibility, blood metabolites.

INTRODUCTION

Previous studies were extensively interested in testing the effects of natural products, as feed additives, in different animal species (Newbold et al., 1995; Benyacoub et al., 2003; Abd El-Ghani, 2004; Awad et al., 2009). The beneficial effect of these natural products is always based on their ability to modify the gut microflora (Fuller, 1992; Awad et al., 2009). The importance of the natural gut microflora for reducing

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Abbreviations: SYN, Synbiotic; **IG,** immunoglobulin; **NEFA,** non-esterifies fatty acids.

Table 1. Ingredients and chemical composition of the basal diet.

Item	%DM
Ingredients	
Alfalfa hay, Chopped	64.5
Wheat straw, Chopped	10.2
Cracked barley grain	19.9
Soybean meal	5.2
Salt	0.2
Chemical composition	
CP, %DM	14.2
ME (Mcal/kg)	2.3
NDF	39
EE	3.4

diseases in humans and animals has long been recognized and it is now apparent that the composition of the microflora plays a crucial role both in digestion and in resistance to diseases (Awad et al., 2009; Provenza and Villalba, 2010). Among these natural products, probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance (Fuller, 1992). Moreover they are mono-or mixed culture of living microorganisms, which induce beneficial effect on the host by improving the properties of microflora (Fuller, 1992; Gaggia et al., 2010). Although most of the studies on natural products are carried out on non-ruminant animal, some studies also showed that these products have positive effects on rumen microbes in sheep (Newbold et al., 1995). On the other hand, despite the positive results, meta-analysis carried out by Sales (2011) clarified that addition of saccharomyces cerevisiae to diets did not have any effect on growth, feed conversion, ruminal parameters or fiber digestibility in sheep. While the live microbes have been increasingly used as probiotics in animal nutrition and health, the macromolecules synthesized by some microorganisms are increasingly been used as prebiotics (Gaggia et al., 2010). Prebiotics are defined as food ingredients that stimulate selectively the growth and activity of beneficial microorganisms and thereby benefit health (Roberfroid, 1998). Synbiotic is considered as product which contains both probiotic and prebiotic together. Synbiotic products contain viable bacterial cultures that is found in gastrointestinal tract while the prebiotic present in them serve as a source of nutrient for the probiotics in addition to dietary sources. Some of these products have already penetrated the market and several researches have been carried out mainly in non-ruminant species (Roberfroid, 1998; Awad et al., 2009). Despite probiotic supplementation effects on different parameters were investigated in small ruminant nutrition (Jouany et al., 1998; Anandan et al., 1999; Abd El-Ghani, 2004; Kamel et al., 2004), the studies on synbiotic effects are very limited. The objective of this study is to evaluate the influence of synbiotic compound on blood metabolites, nutrients digestibility and performance of *Farahani* sheep which was fed on alfalfa-based diet.

MATERIALS AND METHODS

Animals, treatments and management

This study was carried out in Arak University Farm, Iran. The vaccination and deworming process was done based on the routine protocol of farm. The 21 Farahani male sheep averaging BW 33.9 ±2.5 kg were allocated to a completely randomized design. The experiment contained three different treatments with 7 animals per each treatment. The experimental diet and composition are shown in Table 1. Three different treatments were as follow; T1= control; T2= 2 g/d/h supplemented SYN and T3= 10 g/d/h supplemented SYN. The commercial synbiotic feed additive which was evaluated in this study (Biomin IMBO; Biomin GmbH, Herzogenburg, Austria) was a combination of the probiotic strain *Enterococcus faecium* (DSM 3530), a prebiotic fructooligosaccharid (derived from chicory) and immunemodulating substances (derived from sea algae). Animal were confined for 84 days in individual stanchions and were fed twice daily at 0800 and 1600 h. The study lasted 12 weeks and the first week was considered to adaptation of the animals to experimental conditions. The animals had free access to water. Orts were collected and weights recorded once daily at 0730 h and the feeding rate were adjusted daily to yield orts of about 5 to 10% intake.

Sample procedure, chemical analysis and calculations

The 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. After drying, ingredients and TMR were ground through a 1 mm screen (Wiley mill). The samples were analyzed for total nitrogen, dry matter, ash and organic matter was estimated based on DM and ash contents of samples (AOAC, 2000). Sequentially the samples were analyzed for neutral detergent fiber (Van Soest et al., 1991). The sheep were weighed three times on weeks 2, 6 and 12 of study. Feed conversion rate (FCR) was calculated by dividing average intake to average daily gain of animal. Fecal samples were collected throughout the last week of study for five consecutive days (two samples per day and totally 10 samples per animal). Samples were composite per sheep and then oven-dried at 55 for 72 h and then ground through a 1 mm sieve. After analyzing the fecal samples for nutrients, total tract apparent digestibility of nutrients was determined by using acid insoluble ash as an internal marker which was acid insoluble ash (AIA) (Van Keulen and Young, 1977). Blood was sampled at 0 (just before morning feeding) and 4 after feeding from the jugular vein of each sheep on weeks 3 and 12 of study. Blood samples were heparinized and stored at 2°C for about 6 h; plasma was then prepared, centrifuged (3000 × g 4°C, 15 min) and stored at -20°C. Later, plasma was analyzed for glucose, non esterifed fatty acids (NEFA), blood urea nitrogen (BUN), albumin and total immunoglobulin (IG).

Statistical analysis

Data were analyzed using Proc Mixed in SAS (version 8.1; SAS Institute Inc., Cary, NC 1998). The following model was fitted to variables that did not have repeated measurements over time; $Y_{ij} = \mu + S_i + T_j + \varepsilon_{ij}$; Where Y_{i_j} is the dependent variable,

Table 2. Least square means for intake, weight gain and feed conversion rate in animal fed different experimental diets (n=21).

Item	Treatments[1]			SE	P
	1	2	3		
Total weight gain (kg)	12.67	12.94	13.11	0.84	N.S.
ADG (g/day)	165	168	170	3.64	N.S.
DMI (g/day)	1363	1382	1415	11.16	0.09
Total DMI (kg)	104.9	106.4	108.9	3.47	0.09
Feed to gain	8.41	8.26	8.32	0.87	N.S.

*Treatments were; 1- un-supplemented treatment; 2- supplemented with 2 g/day/h SYN; and 3- supplemented with 10 g/day/h SYN. N.S.: non-significant.

Table 3. Least square means for blood metabolites concentrations in animal fed different experimental diets.

Item	Treatments[1]			SE	P
	1	2	3		
Glucose (mg/dl)	60.93	61.87	61.23	1.09	N.S.
NEFA (mmol/L)	0.48^{a}	0.39^{ab}	0.36^{b}	0.03	0.03
Albumin (mg/dl)	3.31	3.38	3.25	0.24	N.S.
Total IG (mg/dl)	1.91^{b}	1.95^{b}	2.27^{a}	0.08	0.003
Blood urea nitrogen (mg/dl)	17.16	16.78	16.74	0.69	N.S.

*Treatments were: 1- un-supplemented treatment; 2- supplemented with 2 g/d/h SYN; and 3- supplemented with 10 g/day/h SYN. a, b, c Least squares means within the same row without a common superscript differ (P < 0.05). N.S.: non-significant.

μ is the overall mean, S_i is the effect of sheep i, T_j is the effect of treatment j, and ε_{ij} is the residual error. The following model was used for variables which there were repeated measurements over time: $Y_{ijk} = \mu + S_i + T_j + Z_k + ZT_{jk} + \varepsilon_{ijk}$; Where Y_{ijk} is the dependent variable, μ is the overall mean, S_i is the effect of sheep i, T_j is the effect of treatment j, Z_k is the effect of sampling time k, ZT_{jk} is the interaction between time k and treatment j and ε_{ijk} is the residual error. All terms were considered fixed except for ε_{ijk} which was considered random. 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

Performance parameters

The data for animal performance are shown in Table 2. The average daily intake was tended to be significant (P = 0.09). The BW changes of animal was not affect by SYN supplementation (the ADG were 165, 168 and 170 g/d for treatments 1, 2, 3 and respectively). The FCR also did not differ among treatments.

Blood metabolites

The data for blood metabolites are presented in Table 3. The glucose, albumin and BUN concentrations did not differ among treatments. However, the NEFA (P = 0.03) and total IG (P = 0.003) significantly differed by SYN inclusion in sheep diet. Previous studies indicated that glucose could be as energy indicator in animal nutrition and is function of feeding level (Vanhatalo et al., 2003). The NEFA concentration was decreased by using the SYN in diet. This depression was about 25% (NEFA concentration was 0.48 and 0.36 for control and T3, respectively).

Nutrients digestibility

The apparent nutrients digestibility data are shown in Table 4. The DM and CP digestibility did not show any differences among treatments. The OM digestibility was

Table 4. Least square means for apparent nutrients digestibility (%) in animal fed different experimental diets.

Item	Treatments[1]			SE	P
	1	2	3		
Dry matter	66.30	66.81	68.19	1.23	N.S.
Organic matter	76.10	77.39	79.63	0.88	0.07
Crude protein	68.18	69.52	69.36	0.95	N.S.
Neutral detergent fiber	46.09[b]	47.11[ab]	49.54[a]	0.57	0.001

*Treatments were: 1- un-supplemented treatment; 2- supplemented with 2 g/day/h SYN; and 3-supplemented with 10 g/day/h SYN. a, b, c Least squares means within the same row without a common superscript differ (P < 0.05). N.S.: non-significant.

tended to be greater in supplemented treatment (T3) compared to control treatment (P = 0.07) and NDF digestibility was increased in SYN supplemented diets (P = 0.001).

DISCUSSION

Previous studies clarified that intake of lambs supplemented with lactobacillus inoculants was greater compared to control treatment (Umberger and Notter, 1989) or also FCR was improved in *Awassi* lambs fed with probiotics (Abdelrahman and Hunaiti, 2008). The reason which was suggested by these authors was greater digestibility of nutrients which consequently caused higher daily intake of animals. Considering the inclusion of SYN in broiler diet revealed that FCR positively affected and daily gain was improved by 5.9% in supplemented treatment compared to control treatment (Awad et al., 2009). In the present study although the intake in T3 was numerically higher than that of the control treatment (about 50 g/day), no statistical difference was observed and the difference just had trend to be significant among treatments (P = 0.09). The results clarify that although the SYN might have negligible effect on intake, daily gain and FCR did not significantly respond to SYN addition in sheep diet. Considering the blood metabolites responses to probiotic and prebiotic clarified this supplement could decrease TG and cholesterol concentrations in blood (Fukushima and Nakano, 1995). The findings suggested that natural product supplements could decrease lipid absorption via gastrointestinal tract and therefore the lipid metabolites in blood negatively were affected by this supplementation (Fukushima and Nakano, 1995). Because NEFA concentration in blood is directly related with lipid metabolism and in the present study supplementing the SYN decreased NEFA concentration, it might be conceivable that SYN supplementation have potential to decrease lipid metabolites concentrations in blood. It seems that manifestation of this phenomenon in ruminant gastrointestinal needs to carry more studies and increase the knowledge about natural product effects in ruminant nutrition. The IG concentration positively affected by SYN supplementation in this study. The positive effects of both probiotic and prebiotic on immune system in different animal strains have been confirmed (Newman, 1994; Benyacoub et al., 2003; Provenza and Villalba, 2010). The natural product could improve immune system via different mechanisms such as microbial antagonism or immune modulation (Provenza et al., 2010). The results revealed that blood IG concentration increased by 15% in 10 g/day SYN inclusion in diet compared to control treatment. The data show that the difference between T1 (control) and T2 (2 g/day) was not meaningful (total IG was 1.91 and 1.95 mg/dl for T1 and T2, respectively) but the difference between T1 and T3 (10 g/day) was huge (1.91 vs. 2.27 mg/dl for T1 and T3 respectively). This shows that greater level of SYN is more useful to improve IG concentration in sheep and lower level of SYN might not be an additive to increase this parameter. Nutrients digestibility were also considered in different previous studies which used natural products. Abd El-Ghani (2004) also reported improvement in nutrient digestibility in goat by using probiotic. The nutrients digestibility is affected by different factors such as feeding level, feed ingredients quality and supplements (Vanhatalo et al., 2003). The SYN supplement in this study was shown to have considerable effect in fiber digestibility improvement. This improvement was about 3.5 unit increases in NDF digestibility which was gained in T3 compared to control treatment (46.09 vs. 49.54% for treatments T1 and T3, respectively). It has been shown that a favorable environmental condition for gastrointestinal microbes caused improvement in nutrients digestibility and probiotic and prebiotic are shown to prepare these conditions in animal (Gibson and Roberfroid, 1995; Gibson and Fuller, 2000). It has been clarified previously that probiotic and prebiotic had potential to increase short chain fatty acids production in gastrointestinal tract (Santoso et al., 1995). From the other side, studies in ruminants reported that greater short chain fatty acids in their gastrointestinal caused improvement in nutrient digestibility (Yang, 2002; Reynal

et al., 2007) and the reason might be related to direct or indirect effects of these organic acids on bacteria in rumen (Yang, 2002). Other possible reason for increased fiber digestibility may be probiotics positive effect on ruminal pH, leading to improved fiber degradation (Umberger and Notter, 1989). Considering the digestibility, in the present study the results show that SYN supplementation significantly increased fiber digestibility that possibly is related to end-product modifications which could affect on bacteria responses.

Conclusion

The results clarify that using synbiotic in sheep diet did not affect on performance traits such as DMI, DG and FCR. However it seems that this supplementation could improve fiber digestibility and also had positive effect on blood total IG concentration. It could be concluded that this product might be used accompanied with low quality forage diet to improve digestion or also it could be used to improve immune function. This study results confirm that greater level of SYN could have meaningful effect on immune responses.

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