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Possibilities of using humic acid in diets for Saanen goats

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Summary

This study was carried out to determine the effect of diets containing humic acid at different levels on some blood and milk components in Saanen goats. Saanen goats (2 years old, 52 kg live body weight) were fed three diets, containing 0 g kg^{-1} humic acid (T_1), 1 g kg^{-1} humic acid (T_2) and 3 g kg^{-1} humic acid (T_3) in a 3x3 Latin square design experiment. Compared to the control group, T_1 - treated group produced more milk/day ($P<0.05$; 2.45 vs. 2.11 kg d^{-1}) and this yield was similar to that of T_2 - treated group. The fat (3.55, 3.67 and 3.58 %), non-fat solids (SNF) (8.41, 8.30 and 8.58 %), protein (3.73, 3.60 and 3.92 %) and lactose percentages of milk (3.82, 3.83 and 3.77) were similar for T_1, T_2 and T_3 groups. Humic acid administration significantly reduced total cholesterol in blood ($P<0.05$; 124.1, 100.8 and 102.2 mg dL^{-1}) for T_1, T_2 and T_3, respectively. The LDL cholesterol levels in blood serum of T_1, T_2 and T_3 groups were 23.49, 15.14 and 16.19 mg dL^{-1}, respectively ($P<0.05$). Further investigation is required to elucidate the effects of humic acid on goat performance.

Key words: humic acid, goat, milk composition, blood metabolites

Introduction

Antimicrobial feed additives are worldwide used in animal husbandry to improve the economic and ecological aspects of animal production by increasing growth rate, decreasing feed conversion and diminishing the risk of disease (Hays 1981; Gropp et al., 1992). Unavoidable spread of bacterial resistance and cross-resistance to antibiotics used in veterinary and human therapy (Barton, 1998; Khachatourians, 1998) was increasingly considered as a hazardous; therefore the approval of antimicrobial growth promoters has been phased out by EU legislation by the end of 2005.

Among many alternatives humic acids (HA) are described. Humic acids or humate substances can be defined as the end product of the decomposition of organic matter by aerobic organisms. HA is not single molecule, it is a complex mixture of many different acids containing carboxyl and phenolate groups (Stevenson, 1982). The use of HA in feed rations is not based on providing energy, but on its health benefits. Stimulating effects on digestion, growth, and the immune system of the animals and absorbent and detoxifying capability are the most discussed (Trckova et al., 2005). Humic acids stabilize the intestinal microflora, ensuring an improved nutrient utilization and feed efficiency (Písaříková et al., 2010). The use of humic acid is preferable in ruminant nutrition because, as on the soil microbial population dynamics, it has beneficial effect on rumen microbial growth.

Effects of HA on dairy cow performance were mainly investigated. Thomassen et al. (2000) observed that humic acid significantly increased the milk yield. Another study reported that the use of HA in dairy cows has increased milk production and milk fat (Livestock R Us, 2003). Tunç and Yörük (2007) showed that diets supplemented with 0.0, 0.1, 0.2 and 0.4 % of humate (Bovifarm) significantly decreased the blood serum cholesterol and LDL levels in sheep. It is also difficult to compare the actual effects of HA preparations due to different

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Table 1. Basic information of examination goat

Groups	Number of goats	Live weight (kg)	Days in milk	Milk production (kg d^{-1})
Control T_1	6	51.10±3.298	60.33±4.177	2.90±0.209
Treatment T_2	6	54.15±4.059	65.50±6.339	2.96±0.242
Treatment T_3	6	49.20±3.769	69.00±1.291	2.98±0.243

sources and preparations as well as because of rearing animals in various regions of the world (Islam, 2005).

The objective of the present study was to investigate the different levels of natural humic acid in the diet on performance, milk yield and blood metabolites of Saanen goats.

Materials and methods

The study was conducted at a goat farm located in Karacabey. The humic acid material used for the study was purchased from the Natural feed Company. The humate product (Bovifarm, Bio Remedies) used for this study was dark black in colour. Certified composition of HA produced by Bovifarm, contained oxyhumolite - total humic acids 68 %, free humic acids 48 %, minerals 18 %. Goats were grouped primarily accordingto the milk yield prior to the start of the experiment (Table 1).

Eighteen Saanen goats (2-3 years old, 49-54 kg live weight) within 60-69 days of lactation were used in a replicated 3x3 Latin square design. Periods lasted for 30 days, in which the first 21 days were used for adaptation, with data for statistical analysis being collected in the final 7 days. Thus, total experimental period lasted for 90 days.

In each of the three periods, goats were randomly assigned to one of the three dietary treatments (DM basis; Table 2): T1 diet without humic acid (HA); T_2 diet with 1.0 g HA kg^{-1}; T_3 diet with 3.0 g HA kg^{-1}. The applied dosages of HA were determined after reviewing multiple references of Thomassen et al. (2000), Tunç and Yörük (2007).

During the treatment period all goats received *ad libitum* pasture, corn silage (1 kg d^{-1}), alfalfa (500 g) and 0.50 kg of experimental diets (per 1.0 kg of milk day). Goat ration was formulated to 2.90 kg/d of milk production with 3.5 % fat and 3.5 % protein in 2nd lactation according to the NRC recommendations. The animals were milked twice daily at 6:30 a.m. and 7:30 p.m. Milk production for each goat was measured daily. All the **samples** were stored at 5±1 °C before analysis or shipment.

The individual consumption of roughage was not determined as a reason of applying of group feeding in study. Dry matter intake was measured at the end of sample collection period by weighing the offered diet and refusals from the previous day. The content of dry matter, organic matters, crude protein, crude fat and ash in the diets were analysed by methods of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fibers (ADF) values were determined using methods outlined by Robertson and Van Soest (1981). The metabolizable energy and net energy lactation contents were also estimated (NRC, 1975). Solids - not-fat content (SNF), fat, protein and lactose contents of milk were analysed using a Milcosan FT - 120 device. The blood samples were obtained from jugular vein of each experimental subject 2 h post feeding at the end of each phase. Tubes were centrifuged at 2800 rpm and serum was carefully harvested and analysed within two hours. The total protein, glucose, triacylglyerol, total cholesterol, LDL cholesterol and HDL cholesterol were analysed in the blood serum using a Siemens dimension biochemistry device.

Data for yield and blood parameters were analysed by the general linear models procedure of Minitab (1998) using the following model described by Cochran and Cox (1957):

$$Y_{ijkl} = \mu + T_i + P_j + C_k + E_{ijkl}$$

Y_{ijkl}=observation, μ = population mean, T_i = treatment (i = 1, 2 or 3), P_j = period (j = 1, 2 or 3), C_k = 1, 2, 3, ……16, 17 or 18) and E_{ijkl} = residual error. Means were separated by Duncan's multiple range test.

Results and discussion

Chemical composition of the diets and alfalfa hay and corn silage is presented in Table 2. Dry mat-

ter (DM), crude protein (CP) and ME values were similar for diets T_1, T_2 and T_3, however, crude ash content in diet T_1 was lower than those of diets T_2 and T_3. The effects of the treatments on the dry matter intake (DMI), milk yield and composition and blood metabolites are presented in Table 3.

The effects of the diets with 0, 1.0 and 3.0 g HA kg^{-1} on live weight for goats were tested. No significant changes were noted for live body weight and DMI of goats. These result are in agreement with Livestock (Livestock, 2003) who found that HA did not affect the feed intake of dairy cows and Vucskits et al. (2010) who reported that low or high doses of HA did not affect the DMI and live weight in rats. However, McMurphy et al. (2011) reported that greater DMI in Holstein steers was observed in HA group than in the control group. Similarly, Chirase et al. (2000) demonstrated a decrease in intake during the first 28 days of lactation for cattle fed a lower HA concentration (7.8 g kg^{-1}) *vs*. a control and increased concentrations (15.6 and 31.2 g HA kg^{-1}).

In the present experiment, daily milk yields for the T_1, T_2 and T_3 diets were 2.90, 2.96 and 2.98 kg day^{-1}, respectively, at the beginning of the study (initial period) ($P>0.05$). The milk yield of groups appeared to be reduced as a result of the late weaning period 60. The milk production of goats increased as the HA dosage of diet increased (Table 3). In this study, daily milk yields were higher for goats fed the T_3 diet than for goats fed the T_2 and T_1 diets (3.26 % and 13.87 % ($P<0.05$)). The goats fed T_3 diet had the highest daily milk yield ($P<0.05$) in trial. The differences between the means of diet T_1 and diets T_2 and T_3 were significant ($P<0.05$). Significant increases in milk production associated with HA, have previously been reported in dairy cows (Thomassen et al., 2000; Livestock, 2003). Milk response to feeding HA usually ranges between 2 and 6 % by Thomassen et al. (2000). The positive effect of humic substances can be explained by enhancement of the metabolic

Table 2. Composition of feed mixtures and roughages fed to experimental Saanen

Compound (g kg^{-1})	Diet			Roughages	
	0 Humic acid	1 Humic acid	3 Humic acid	Alfa hay	Corn silage
Barley	400.0	399.0	397.0		
Wheat	340.0	340.0	340.0		
Soya-bean meal	140.0	140.0	140.0		
Sunflower meal	100.0	100.0	100.0		
Humic acid	-	1.0	3.0		
Marble powder	14.0	14.0	14.0		
Salt	5.0	5.0	5.0		
Vitamin+minerals[1]	1.0	1.0	1.0		
Total	1000	1000	1000		
Nutrient composition (g kg^{-1})					
DM[2]	903.0	900.9	901.0	890.0	368.2
OM	821.8	814.5	809.2	778.6	350
CP	225.3	224.7	220.1	195.7	29.8
EE	35.2	34.6	33.3	28.5	13.7
CELl.	106.8	101.4	101.8	247.2	76.7
CA	81.2	86.4	91.8	111.4	18.2
Nitrogen free ext.	454.5	453.8	454.0	307.2	229.8
Starch	169.2	168.2	167		
NDF	301.5	303.6	300.8	353.6	150.5
ADF	139.1	140.1	138.79	294.3	91.8
ADL	38.9	38.7	37.0	70.5	17.7
ME (Kcal kg^{-1})[3]	2617.0	2630.0	2613.0	1864.0	912.0
NEL (Kcal kg^{-1})	1531.0	1544.0	1527.0	1135.0	525.0

[1]Trace minerals and vitamins (per kg): 150 mg of ZnSO47H2O, 80 mg of MnSO4H2O, 200 mg of MgO.
5 mg of CuSO47H2O, 1 mg of KIO3, 5000 IU Vitamin A, 1000 IU Vitamin D and 20 IU Vitamin E.
[2]DM, dry matter; OM, organic materials; CP, crude protein; EE, Ether extract ; Cell, cellulose ADF, acid detergent-fibre; NDF, neutral detergent fibre; CA, crude ash. [3]Obtained by calculation (NRC, 1975)

Table 3. The effects of humic acid containing diets on live weight, DM intake, milk yield and composition and blood metabolites of goats

Humic acid (g kg^{-1} DM)	Diet				
	Control (T_1)	1 (T_2)	3 (T_3)	S.E.M[3]	P-value
Live body weight (kg)	52.53	52.18	52.26	0.52	
Silage DM intake (kg d^{-1})[1]	0.387	0.394	0.405	-	
Alfa hay DM intake (kg d^{-1})	0.449	0.448	0.490	-	
Concentrate DM intake	0.903	0.900	0.901	-	
Total DM intake (kg d^{-1})[1]	1.739	1.742	1.796	-	
Milk parameters					
Milk yield (kg d^{-1})	2.11^{b}	2.37^{ab}	2.45^{a}	0.02	*
Fat (%)	3.55	3.67	3.58	0.07	NS
SNF (%)[2]	8.41	8.30	8.58	0.03	NS
Protein (%)	3.73	3.60	3.92	0.04	NS
Lactose (%)	3.82	3.83	3.77	0.03	NS
Blood parameters					
Total protein (g dl^{-1})	7.86	7.82	7.59	0.020	NS
Glucose (mg dl^{-1})	40.50	40.22	35.94	0.491	NS
Triacylglycerol (mg dl^{-1})	33.61	33.40	36.52	0.560	NS
Total cholesterol (mg dl^{-1})	124.1^{a}	100.8^{b}	102.2^{b}	1.454	*
LDL cholesterol (mg dl^{-1})	23.49^{a}	15.14^{b}	16.19^{b}	0.529	*
HDL cholesterol (mg dl^{-1})	79.83	77.56	86.44	0.876	NS

[1]Total DM intake values for buffalo cows were not added to pasture consumption
[2]DMI, dry matter intake; [2]SNF, solids-not-fat;
[3]SEM=Standard error mean, ab Significant differences *(P-value<0.05), NS, Not significant

activity of cell membranes by acceleration of oxidative processes that due to increased nutrient uptake stimulate the vital functions (Islam et al., 2005).

Percentages of fat, SNF, protein and lactose in milk were not affected by dietary treatment. Contrary to these observations, some reports exist to show that HA treatment improved the milk composition (Thomassen et al., 2000; Livestock, 2003). The observed response variance may be related to several factors, such as forage type, forage-to-concentrate ratio, feeding strategy, animal differences, the lactation length tested and the source of the HA product.

Total cholesterol levels were lower when humic acids were added to the diet (P<0.05; 100.8 and 102.2 vs. 124.1 mg dL^{-1} for T_2 and T_3 vs. control, respectively).

In the present study, such reductions were determined as 23.11 % and 21.42 % in treatment groups (T_2 and T_3). This result was similar to that of Banaszkiewicz et al. (1994) who found that diets with HA decreased the total cholesterol levels in rats. Similarly Tunç and Yörük (2007) reported that total cholesterol values were 60.48 %, 33.06 % and 34.81 % in treatment groups. Broilers fed with diets containing natural humic compounds and sodium humate also showed a lower concentration of cholesterol (Šamudovská and Demeterová, 2010). These reported values were higher than findings in this study. Similar trend was also recorded for LDL cholesterol level. The LDL cholesterol levels of goats in both tested groups were lower compared to the control group. Significant differences were observed between the HA group and the control group (T_2, T_3 diets and T_1 diet; (P<0.05). Tunç and Yörük (2007) reported that diets with HA decreased the LDL cholesterol level about 74.75 %, 24.37 % and 42.22 % with treatment of three different HA (1.0, 2.0 and 4.0 g HA kg^{-1}). Mišta et al. (2012) ob-

served that the total and LDL cholesterol in rabbits decreased linearly with increasing concentrations of supplemental humic-fatty acid. Stepchenco et al. (1991) reported that diets with HA decreased total lipid content in liver. The disorder of the liver can increase the cholesterol level in blood serum. It is also clear that the HA plays a role in the liver function and protects somewhat from disease (Lotosh, 1991). The reduction of LDL and cholesterol levels of blood serum may be due to HA that has the ability to reduce and release iron from ferritin storage as well as to promote lipid peroxidation. But the precise modes of action involved are not yet clear and need further investigation.

There were no significant differences for means of glucose, total protein and triacyglycerol of blood serum in present study ($P>0.05$; Table 3). Total protein parameters for the T_1, T_2 and T_3 diets were similar to 7.86, 7.82 and 7.59 g dL^{-1}, respectively, these result could be considered as similar to the results of Tunç and Yörük (2007). Comparing results of studies by many researches worldwide, performance differences due to humate supplementation might result from the compositional differences among the commercially available humate products (Kocabağli et al., 2002).

Conclusion

Humic acid based diets affected the milk yield, but the treatment did not affect milk composition; however, it reduced the level of cholesterol in blood serum. Therefore, diet T_3 with 3.0 g HA kg^{-1} can be offered as an alternative method for milk production as its feeding did not affect the health of Saanen goats.

Mogućnosti korištenja huminske kiseline u hranidbi sanskih koza

Sažetak

Istraživanje je provedeno kako bi se utvrdio utjecaj hranidbe s različitim sadržajem huminske kiseline na neke komponente krvi i mlijeka pasmine sanska koza. Sanske koze (2 godine starosti, 52 kg tjelesne težine) hranjene su obrocima koji su sadržavali: 0 g kg^{-1} huminske kiseline (T1), 1 g kg^{-1} huminske kiseline (T2) i 3 g kg^{-1} huminske kiseline (T3) u latinskom 3x3 kvadratnom dizajnu. U usporedbi s kontrolnom, T1 - tretirana skupina proizvela je više mlijeka na dan ($P<0,05$; 2,45 vs 2,11 kg d^{-1}), i ta je količina bila slična onoj T2-tretirane skupine. Udjeli masti (3,55, 3,67 i 3,58%), bezmasne tvari (SNF) (8,41, 8,30 i 8,58%), proteina (3,73, 3,60 i 3,92%) i laktoze (3,82, 3,83 i 3,77) bili su slični za T1, T2 i T3 skupine. Prisutnost huminske kiseline značajno smanjuje ukupni kolesterol u krvi ($P<0,05$; 124,1, 100,8 i 102,2 mg dL^{-1}) za T1, T2 i T3, respektivno. Razina LDL kolesterola u krvnom serumu T1, T2 i T3 skupine bile su 23,49, 15,14 i 16,19 mg dL^{-1}, respektivno ($P<0,05$). Potrebno je provesti daljnja istraživanja radi utvrđivanja djelovanja huminske kiseline na proizvodne rezultate koza.

Ključne riječi: huminska kiselina, koza, sastav mlijeka, krvni metaboliti

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