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Using humic acid in diets for dairy goats

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The effects of diets containing different levels of humic acid (HA) were determined on several blood and milk parameters in dairy goats. Eighteen Saanen goats (2 years old, 52 kg body weight) were fed three diets containing: 0 g HA/kg body weight (T_1), 1 g HA/kg – (T_2) or 3 g HA/kg – (T_3) in a 3x3 latin square experimental design. Each period consisted of 21 days preliminary period and 7 days collection phase. Blood and milk samples were collected at the end of the sample collection periods. The total DM intake values were 1.73, 1.74 and 1.79 (kg/d) for goats fed T_1, T_2 and T_3 respectively. Goats fed the T_3 diet had higher ($P<0.05$) milk yields than goats fed the T_1 diet (milk yield=2.45 and 2.11 kg/d, respectively), and this yield was similar to that of diet T_2 (2.37 kg/d). However, the addition of HA did not improve the milk fat, solids non-fat, protein, or lactose content of milk. HA administration to goats significantly reduced the total cholesterol of blood ($P<0.05$; 3.21, 2.61 and 2.64 mmol l^{-1} for T_1, T_2 and T_3, respectively), and the LDL cholesterol levels of blood serum that were determined as 0.60, 0.39 and 0.42 mmol l^{-1}, respectively ($P<0.05$). Further investigation will be required to elucidate the effects of humic acid on goat performance.

KEYWORDS: blood metabolites / goat / humic acid / milk composition

The unavoidable spread of bacterial resistance and cross-resistance to antibiotics used in veterinary and human therapy [Barton 1998, Khachatourians 1998] is increasingly considered a hazard; therefore, the approved routine use of antimicrobial growth promoters was phased out by EU legislation by the end of 2005. The present work concerns the interesting issue of the influence of natural additives in diets for goats on animal health and production traits. Such types of products are obtained

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during the biodegradation processes of organic substances by oxygen microorganisms. These products contain, among others, humic acid which exerts the beneficial effect on the composition of microorganisms in the digestive tract of ruminants. Thus, the humic acid influences positively the productivity of ruminants and the quality of their products. Natural organic additives to animal diets can be an interesting alternative for feed antibiotics.

Humic acid (HA) is a long-chain molecule high in molecular weight, dark brown and soluble in an alkali solution [Stevenson, 1982]. HA is used in feeding rations not only because it provides energy but because for its other health benefits, namely stimulating effects on digestion, growth, and the immune system. Moreover, HA has absorptive and detoxifying properties [Trckova *et al*. 2005]. Humic acids stabilise the intestinal microflora, ensuring improved nutrient utilisation and feed efficiency [Písaříková *et al.* 2010].

The milk yield-increasing effect of humic acid has been investigated mainly in dairy cows. Positive effects for humic acid on milk production, milk fat and milk protein in dairy cows were reported by Thomassen *et al.* [2000]. Another study showed that the use of HA as an animal feed supplement leads to increased milk production and increased butterfat percentage in dairy cows. Furthermore, weaning weights increased and more rapid weight gain was observed in dairy cattle, while problems with scours were greatly reduced [Livestock R Us, 2003]. Tunç and Yörük [2007] showed that diets supplemented with 0.0, 0.1, 0.2 and 0.4% humate (BOVIFARM) significantly decreased the serum cholesterol and the LDL levels in the serum of sheep. However, it is difficult to compare the effects of HA across studies due to the different sources and preparations of HA used, as well as because animals reared in various regions of the world are exposed to different climates and environmental conditions [Islam 2005].

The objective of the present study was to investigate the effects of natural humic acid on performance, milk yield and blood metabolites in dairy goats fed diets containing different levels of humic acid.

Material and methods

The study was conducted at a specialised goat farm located in Karacabey, Turkey. The humic acid material used was purchased from the Natural Feed Company. The humate product (BOVIFARM, BIO REMEDIES) used was dark black in colour. The certified composition of HA produced by BOVIFARM contained oxyhumolite (total humic acids 68%, free humic acids 48%, minerals 18%). Goats were grouped primarily by milk yield prior to the start of treatment (Tab. 1).

Eighteen Saanen goats (2-3 years old, 49-54 kg body weight) at 60-69 day of lactation were used in a replicated 3×3 latin square design. Periods lasted 30 days, in which the first three weeks were preliminary for adaptation and data for statistical analysis were collected in week 4. So, the total experiment lasted 90 days.

In each of the three periods, the goats were randomly assigned to one of three

Table 1. Basic information of examined goats

Group	Number of goats	Live weight (kg)	Days in milk	Milk production (kg d-1)
Control T1	6	51.10±3.298	60.33±4.177	2.90±0.209
Treatment T2	6	54.15±4.059	65.50±6.339	2.96±0.242
Treatment T3	6	49.20±3.769	69.00±1.291	2.98±0.243

dietary treatments (DM basis; Tab. 2). T1 diet contained no humic acid (HA); T_2 diet contained 1.0 g HA/kg, and T_3 diet contained 3.0 g HA/kg body weight. The applied dosages of HA were chosen based on those used by Thomassen *et al.* [2000] and by Tunç and Yörük [2007]. The dosage also accounted for the diet, which included a DM content of 90 %. During the treatment period, all goats received *ad libitum* pasture, corn silage (1 kg/d), alfalfa hay (500 g) and 0.50 kg of the experimental diet (per 1.0 kg of milk per day) (T_1, T_2 and T_3: 225, 224 and 220 g CP/kg DM, and 10.956, 11.011 and 10.940 ME (MJ/kg DM). Goat ration was formulated for 2.90 kg/day of milk production with 3.5% fat and 3.5% protein in the 2^{nd} lactation according to the NRC recommendations. The animals were milked twice daily at 6:30 a.m. and 7:30 p.m. The milk production of each goat was measured daily. All the samples were stored at 5±1°C before analysis or shipment. Dry matter intake was measured at the end of sample collection period by weighing the offered diet and refusals from the previous day. The individual roughage consumption was not determined because a group feeding protocol was used in this study. The dry matter, organic matter, crude protein, crude fat and ash contents of the diets were estimated according to AOAC [1990]. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the methods outlined by Robertson and Van Soest [1981]. The metabolisable energy and net energy of lactation were also estimated [NRC 1975]. Solids non-fat (SNF), fat, protein and lactose contents of milk were determined using a Milcosan FT-120 device. Blood samples were withdrawn from the jugular vein of each experimental goat 2 h post-feeding at the end of each phase. Samples were centrifuged to obtain serum (2800 rpm × time (minutes)) after time (10 min) of rest to allow coagulation. The total protein, glucose, triacylglycerol, total cholesterol, LDL cholesterol and HDL cholesterol of the blood serum were analysed using a Siemens dimension biochemistry device. Data for milk yield and blood parameters were analysed with the general linear models procedure [Minitab 1998] using the following model described by Cochran and Cox [1957]:

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

where: 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; E_{ijkl} – residual error.

Means were separated by Duncan's multiple range test.

Results and discussion

Chemical composition of diets and alfalfa hay and corn silage are presented in Table 2. Dry matter (DM), crude protein (CP) and ME values were similar for diets T_1, T_2 and T_3; however, the crude ash content of diet T_1 was lower than those of T_2 and T_3. There was little difference in the chemical compositions of all diets. The effects of the treatments on DMI, milk yield and composition and blood metabolites are given in Table 3. Silage, alfalfa and total DM intake were similar in all goats. Milk yields (kg/day) were higher (P<0.05) of goats fed the T_3 diet than in goats fed the T_1 and T_2 diets. The percentages of fat, SNF, protein and lactose of milk occurred not affected by the dietary treatments.

Humic acid administration significantly reduced total cholesterol (P<0.05; 3.21, 2.61 and 2.64 mmol l^{-1} for T_1, T_2 and T_3, respectively). The LDL cholesterol levels of the blood serum were 0.60, 0.39 and 0.42 mmol l^{-1}, respectively (P<0.05). There was no significant difference identified in serum total protein, glucose, triacylglycerol or HDL cholesterol levels (mmol l^{-1}).

The effects of diets were tested with 0, 1.0 and 3.0 g HA/kg of body weight. No significant changes in live body weight or DMI were noted. These results are in accordance to a previous report [Livestock R. Us, 2003], which stated that HA did not affect the feed intake of dairy cows at the end of the trial; it is also consistent with the findings of Vucskits *et al.* [2010] who reported that low or high doses of HA did not affect the DMI and body weight in rats. However, McMurphy *et al.* [2011] reported that DMI in Holstein steers was reduced for 5.0 and 10.0 g HA/kg body weight and increased for 15.0 g/kg compared to controls. Similarly, Chirase *et al.* [2000] reported a decrease in intake during the first 28 days for cattle fed a lower HA concentration (7.8 g/kg) *vs*. a control and increased concentrations (15.6 and 31.2 g HA/kg). In the present experiment, the daily milk yields for goats fed the T_1, T_2 and T_3 diets were 2.90, 2.96 and 2.98 kg/day, respectively, at the beginning of the study (initial period) (P>0.05). The milk yield of groups occurred to be reduced as a result of the late weaning period. The milk yield of goats increased as the HA dosage of diet increased (Tab. 3) the daily milk yields were higher in goats fed the T_3 diet than in goats fed the T_2 and T_1 diets (3.26 % and 13.87 %; P<0.05). The differences between the mean milk production values 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 [Livestock R. Us, Thomassen *et al.* 2000]. Milk response to feeding HA usually ranges between 2 and 6 % Thomassen *et al.* [2000]. The positive effect of humic substances can be explained by an enhancement of the metabolic activity of cell membranes by acceleration of oxidative processes due to increased nutrient uptake, which stimulates vital functions [Islam *et al*. 2005]. However, HA had no significant effect on the percentages of fat, SNF, protein or lactose of milk. Contrary to these observations, some reports have shown that HA treatment improved the milk composition [Thomassen *et al.* 2000; Livestock R. Us, 2003]. The observed response of variation may be related to several factors, such as forage type, forage-to-

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

Compound (g kg-1)	Diet			Roughages	
	0g HA/kg body weight (T1)	1g HA/kg body weight (T2)	3g HA/kg body weight (T3)	alfa hay	corn silage
Barley	400.0	399.0	397.0	not determined	
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 (MJ/kg DM)3	10.956	11.011	10.940	7.804	3.818
NEL	6.409	6.464	6.393	4.752	2.198

[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 mattter; CP – crude protein; EE – ether extract ; Cell – cellulose; ADF – acid detergent-fibre; NDF – neutral detergent fibre; CA – crude ash. 3Obtained by calculation (NRC, 1975).

concentrate ratio, feeding strategy, differences in individual animals, lactation length and the source of the HA product.

Cholesterol, among other substances, participates actively in the synthesis of cortisol, a very important steroidal hormone, responsible for an activation of glucose and potassium metabolism. Tandem glucose-kalium influences the activity of the whole organism. A low concentration of cholesterol in blood reduces the activity of cells and the immunological system of organism, destabilizes the proteins, hormones, electrolites and minerals metabolism. High cholesterol is more common in sick animals and requires careful consideration of potentially related nonhepatic disorders including hypothyroidism, diabetes mellitus, pancreatitis, nephrotic syndrome, idiopathic dyslipidemias, and rarely a postprandial effect. (www.merckvetmanual.com). In the current study, the total cholesterol parameters in the serum were

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

Item	Diet				P-value
	Control (T1)	1 (T2)	3 (T3)	SEM[3]	
Body weight (kg)	52.53	52.18	52.26		
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 (mmol l-1)	0.44	0.43	0.42	0.020	ns
glucose (mmol l-1)	2.24	2.23	1.99	0.491	ns
triacylglycerol (mmol l-1)	0.38	0.37	0.41	0.560	ns
total cholesterol (mmol l-1)	3.21^{a}	2.61^{b}	2.64^{b}	1.454	*
LDL cholesterol (mmol l-1)	0.60^{a}	0.39^{b}	0.42^{b}	0.529	*
HDL cholesterol (mmol l-1)	2.06	2.00	2.23	0.876	ns

[1]Total DM intake values for goats were not added to pasture consumption.
[2]SNF – solids-not-fat.
[3]SEM – standard error mean.
[ab]Significant differences; *P<0.05; ns – not significant.

significantly reduced by HA supplementation (P<0.05). These reductions were 0.60% and 0.57% in the T_2 and T_3 groups, respectively. 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 animals fed diets containing 1.0, 2.0 and 4.0 g HA/kg exhibited decreases of approximately 1.73%, 1.14% and 1.19% in total cholesterol values. Broilers fed diets containing natural humic compounds and sodium humate also showed lower concentrations of cholesterol [Šamudovská and Demeterová 2010]. These reported values were higher than those found here. A similar trend was also recorded for LDL cholesterol level. The LDL cholesterol levels in goats of both tested groups occured lower than those of the control group. Significant differences were observed between the HA group and the control group ($T_{2,}$ T_3 diets *vs*. T_1 diet; P<0.05). Tunç and Yörük [2007] reported that animals fed diets containing 1.0, 2.0 and 4.0 g HA/kg body weight exhibited decreases of approximately 1.77%, 0.81% and 1.23% in LDL cholesterol level. Miśta *et al*. [2012] observed that the total and LDL cholesterol in rabbits decreased linearly with increasing concentrations of supplementary humic fatty acid. Stepchenco *et al.* [1991] reported that diets with HA decreased the total lipid content of liver. Liver dysfunction can increase the cholesterol level in the blood serum. It is also clear that the HA plays a role in liver function and is somewhat protective against liver disease [Lotosh 1991]. The reduction in LDL and total cholesterol levels of blood serum may be due to the ability of HA to reduce and release iron from ferritin storage as well as to promote lipid peroxidation. However, the precise modes of action involved are not yet clear and require further investigation. We observed no significant differences in the mean blood serum glucose, total protein and triacylglycerol levels ($P>0.05$; Tab. 3). The total protein parameters for the T_1, T_2 and T_3 diets were similar, and reached 0.44, 0.43 and 0.42 mmol l^{-1}, respectively. These results are similar to those of Tunç and Yörük [2007]. However, the results of this study may vary in comparison to others performed elsewhere; performance differences due to humate supplementation might be modulated by compositional differences among the commercially available humate products [Kocabađli *et al.* 2002].

In conclusion, our study showed that the addition of natural humic substances to dairy goat diets improved performance of animals. The addition of humic acid to the diet affected milk yield but not milk composition, and reduced the level of cholesterol in blood serum. Therefore, diet T_3 with 3.0 g HA/kg body weight can be offered as alternative method of enhancing milk production because its feeding did not affect the health of Saanen dairy goats.

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