



RESEARCH ARTICLE

Effect of Administration of Humic Acid on Somatic Cell Count and Total Bacteria in Saanen Goats

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ABSTRACT

This study was carried out to determine the effect of diets containing humic acid on composition and quality traits of milk (selected) in 18 Saanen goats. The animals (2 years old, 52 kg live body weight) were in 2nd lactation and fed three diets containing 0 g kg⁻¹ humic acid (T₁), 1 g kg⁻¹ humic acid (T₂) and 3 g kg⁻¹ humic acid (T₃) in a 3x3 Latin square design experiment. Each period consisted of a 21d -adaptation phase and a 7 d- sample collection phase. Milk samples were collected at the end of the sample collection periods. Total DM intake values were found to be 1.73, 1.74 and 1.79 kg d⁻¹ for T₁, T₂ and T₃, respectively. 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 T₁, T₂ and T₃ groups. The administration of humic acid did not increase hygienic quality of milk. Somatic Cell Count of milk were determined as 257, 212 and 189 SCC (x 10³) for T₁, T₂ and T₃, respectively (P>0.05). Total Bacteria in milk were also determined as 239.7, 154.9 and 159.7 TB (x10³). In conclusion, administration of humic acid had a minor effect on somatic cell count and total bacteria.

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INTRODUCTION

The nutritional quality of raw milk is influenced by many factors. Milk somatic cell count (SCC) constitutes a useful tool to measure milk quality, health status of the mammary gland and the changes in milk composition. Penetration of pathogenic microorganisms in the teat canal irritates and invades the delicate mammary tissue causing an inflammatory response and consequent changes occur in the milk. Increased SCC is associated with reduction in milk yield, changes in milk quality and composition. Increased cost also results from cow treatment, discarded milk and premature culling.

Bad milk quality has an impact on the economics of milk production and technological properties (Pridalova *et al.*, 2009). The determination of the somatic cell count (SCC) is used worldwide in dairy practice to describe the hygienic control of the milk (Urech *et al.*, 1999; Ma *et al.*, 2000; Wellnitz *et al.*, 2009). Milk processors strive for reduced SCC because somatic cells cause a disagreeable taste and decrease the shelf life of milk. Low somatic cell count is important, and some dairy firms pay a premium price for milk with low SCC (Revilla *et al.*, 2007).

SCC of milk also is an important factor in relation to both the processing properties and stability of dairy products. In cheese production, high cell count milk leads to lower cheese yields, longer coagulation and ripening times, weaker curds (Chen *et al.*, 2010).

Besides, high SCC is directly associated with adverse effects on human health including poor farm hygiene, antibiotic residues and the presence of pathogenic bacteria and toxins in milk.

Humic substances have been known to exhibit antimicrobial properties. Species for which natural humic substances have been shown to be inhibitory include *C. albican*, *Ent. Cloacac*, *Prot. Vulgaris*, *Ps. Aeruginosa*, *S. typhimurium*, *St. aureus*, *St. epidermidis*, and *Str. pyogenes* Riede *et al.* (1991). It seems that within the body, humates stimulate the "good" microbes while suppressing the "bad" microbes. Although numerous studies evaluated use of HA fermentation products, there is no research data on the evaluation of its use in goat milk production. The aim of this work was to evaluate the effects of humic acid on performance of dairy goats in early lactation, and on milk composition, total bacteria and somatic cell counts.

MATERIALS AND METHODS

This research was conducted in goat farm located of Karacabey. The humic acid (HA) material used for this study was purchased from the Natural Feed Company. The humate product (Bovifarm, Bio Remedies) used for this study 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%). Lactating goats aged 2-3 years were grouped primarily according to the milk yield and live body weight prior to the start of the experiment (Table 1).

Eighteen Saanen goats at 60-69 days of their lactation were selected in a replicated 3×3 Latin square design. The experimental period lasted for 4 weeks, in which the first three weeks were used for adaptation and data for statistical analysis were collected in the fourth week. Thus, the total experimental period lasted for 90 days.

In each of the three periods, the goats were randomly assigned to one of three dietary treatments (dry matter (DM) basis). T₁ diet with no humic acid (HA); T₂ diet with 1.0 g HA kg⁻¹; or a T₃ diet with 3.0 g HA kg⁻¹ were used for the study. Diet also consisted of 40% barley, 34% wheat, 14% soya-bean meal, 10% sunflower meal, 1.4% marble powder, 0.5% salt and 0.1 Vitamin+mineral mix (Table 2). The dosages of HA added to diet were determined after reviewing multiple studies from Thomassen *et al.* (2000) and by Tunc and Yoruk (2007). Saanen goats were fed on ad libitum pasture, corn silage (1 kg d⁻¹), alfalfa (500 g) and 0.50 kg of the experimental diet (per 1.0 kg of milk per day). Goat ration was formulated for 2.90 kg d⁻¹ of milk production with 3.5% fat and 3.5% protein in the 2nd 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 the study. The dry matter, organic matter, crude protein, crude fat and ash contents in the diets were analysed according to AOAC methods (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) values were determined using the methods outlined by Robertson and Van Soest (1981). The metabolizable energy and (net energy lactation (NET)) contents were also estimated (NRC, 1975). The somatic cell count (SCC) was determined with a somacount 150 (Bentley Instruments, chaska, USA). The total bacteria count was determined the number of bacteria in a sample that can grow and form countable colonies on *Standard Methods Agar* after being held at 32°C (90°F) for 48 hours (Anonymous, 1992). The non-fat solids (SNF), fat, protein and lactose contents of milk were analysed using a Milcosan FT - 120 device.

Data for various milk parameters were analysed using the general linear models procedure in 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

The chemical compositions of the diets, alfalfa hay and corn silage are presented in Table 2. Dry matter (DM), crude protein (CP) and ME values were similar for diets T₁, T₂ and T₃; however, the crude ash content in diet T₁ was lower than those in diets T₂ and T₃. Dry matter (DM), crude protein (CP), Ether extract (EE) and ME values were similar for diets T₁, T₂ and T₃.

The effects of the treatments on DMI, milk composition, SCC and total bacteria are presented in Table 3. Silage, alfalfa and total DMI were similar for all goats.

The effects of diets with 0, 1.0 and 3.0 g HA kg⁻¹ on goat live body weight were tested. No significant changes in live body weight or DMI were noted. These results were consistent with a previous report (Livestock, 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, Chirase *et al.* (2000) demonstrated a decrease in DMI during the first 28 days of lactation for dairy cows 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, respectively). Similarly, McMurphy *et al.* (2011) reported that DMI in Holstein steers decreased for 5.0 and 10.0 g kg⁻¹ HA and increased for 15.0 g kg⁻¹ HA compared to controls. Degirmencioglu (2014) concluded that the administration of HA improved milk yield by 0.34 kg d⁻¹. HA had no significant effect on the percentages of fat, SNF, protein or lactose in milk. Contrary to these observations, some reports have shown that HA treatment improved the milk composition (Thomassen *et al.*, 2000; Livestock, 2003). The observed response could be due to lactation length, ruminant species (dairy cows vs goat) and the source of the HA product.

Mastitis is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue of the udder (Sharma, 2007). It is also defined as inflammation of mammary gland parenchyma, which is caused by bacteria and its toxins (Sharma *et al.*, 2006). The milk SCC is the basis for abnormal milk control program for cows, goats and sheep. In USA the legal limit established by the Food and Drug Administration for cows is 750 000 cells ml⁻¹, and for goats and sheep it is 1 000 000 cells ml⁻¹. In The European Union (EU) the legal limit for cows is 400 000 cells ml⁻¹ and there is no legal limit for goats and sheep (Paape *et al.*, 2007). Paape *et al.*, 2007 also reported SCCs were lowest at first parity averaging approximately 200 000 cells ml⁻¹ at 15 days of lactation and reached maximum counts of around 500 000 cells ml⁻¹ at 285 days of lactation for goats. By the fifth parity, the counts averaged approximately 250 000 cells ml⁻¹ at 15 days and increased to a maximum of 1 150 000 cells ml⁻¹ at 285 days of lactation. Somatic cell counts for uninfected mammary glands have been reported to increase with stage of lactation and parity (luengo *et al.*, 2004). SCC

Table 1: Basic information of examined saanen goat

Groups	Number of goats	Body 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

Table 2: Chemical composition of diet and alfalfa and corn silage (g kg⁻¹)

Nutrients	Diet			Roughages	
	0 Humic acid	1 Humic ac.	3 Humic acid	Alfa hay	Corn silage
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

¹Trace minerals and vitamins (per kg): 150 mg of ZnSO₄·7H₂O, 80 mg of MnSO₄·H₂O, 200 mg of MgO, 5 mg of CuSO₄·7H₂O, 1 mg of KIO₃, 5000 IU Vitamin A, 1000 IU Vitamin D and 20 IU Vitamin E; ²DM, dry matter; OM, organic materials; CP, crude protein; EE, Ether extract; Cell, cellulose ADF, acid detergent-fibre; NDF, neutral detergent fibre; CA, crude ash. ³Obtained by calculation (NRC, 1975).

Table 3: The effects of Humic acid containing diets on live weight, DM intake, Somatic cell count and Total bacteria for goats

Humic acid (g kg ⁻¹ DM)	Diet			S.E.M ³	P-value
	Control (T ₁)	1 (T ₂)	3 (T ₃)		
Live body weight (kg)	52.53	52.18	52.26	0.52	NS
Silage DMI (kg d ⁻¹) ¹	0.387	0.394	0.405	-	
Alfa hay DMI (kg d ⁻¹)	0.449	0.448	0.490	-	
Concentrate DMI	0.903	0.900	0.901	-	
Total DMI (kg d ⁻¹) ¹	1.739	1.742	1.796	-	
Milk parameters					
Fat (%)	3.55	3.67	3.58	0.07	NS
SNF (%)	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
SCC (x 10 ³) ²	257	212	189	11.20	NS
TB (x 10 ³) ²	239.7	154.9	159.7	9.801	NS

¹Total DM intake values for goats were not added to pasture consumption; ²DMI, dry matter intake; ³SEM, somatic Cell Count; TB, Total Bacteria; ³SEM=Standard error of the mean, NS, Not significant

parameters for the T₁, T₂ and T₃ diets were 257, 212 and 189 (x10³), respectively. There was no significant differences in the mean values of SCC in present study (P>0.05; Table 3). However, some studies have reported that HA supplementation significantly decreases the SCC in milk. Griban *et al.* (1988) reported that lower SCC in dairy cows was observed in HA group than in the control group. Thomassen *et al.* (2000) reported that diet with 3 g HA kg⁻¹ decreased about 50% the SCC level of milk dairy. Similarly, Xiaowang *et al.* (2010) reported that lower SCC was observed (by 40.09%) in the fulvic acid group compared to the control group. Bacterial invasion occurs mostly during the dry period, particularly during late gestation, and leads to glandular damage in parenchymatous tissue. The glandular tissue damage leads to increased SCC and reduced milk production. The cellular presence in milk is one of the important protective mechanisms of the mammary gland (Sharma *et al.*, 2011). According to Dabovich *et al.*, 2003 HA have nutraceutical properties in that it stimulates neutrophil activity which may protect against bacterial pathogens and reduce mortality during acute bacterial infection.

Comparing results of studies conducted by many researchers worldwide, performance differences due to HA supplementation might result from the compositional differences among the commercially available humate products (Kocabagli *et al.*, 2002).

Total bacteria load in milk was decreased by HA supplementation but this decreasing in total bacteria did not non significant. In the present study, such reductions for total bacteria were determined as 84.8, 80.0 (x 10³) in treatment groups (T₂ and T₃ groups).

Conclusions

It has been determined that, there were no significant effects of diets containing humic acid on DM intake, SCC and total bacteria. In conclusion, administration of humic acid had a minor effect on somatic cell count and total bacteria. The authors wish to thank the American Journal Experts for English corrections.

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