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Effects of Fresh Alfalfa on Performance and the Composition of Fatty Acids in Milk of Dairy Cows

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Abstract: According to the principle of similar milk yield, parturition numbers, lactation day and body weight, 15 early- and 15 mid-lactation Holstein cows were chosen and randomly allocated to three groups in pairs. The dairy cows in group 1 (control group) were fed basic diet, group 2 and 3 were fed fresh alfalfa 2.5 and 5 kg (DM basis), respectively in addition to the basic diet. The results showed that by supplementing fresh alfalfa DMI of dairy cows could be increased; fresh alfalfa had the trend of increasing higher milk density, milk fat, milk protein and SNF. The milk density of group 2 (26.53±0.62%) was higher ($p<0.05$) than group 3 (24.95±1.50%) in the early lactation and that of group 1 (25.91±1.08%) and group 2 (26.22±0.52%) were higher ($p<0.05$) than group 3 (24.96±0.98%) in the middle lactation; fresh alfalfa had no effect on the proportions of C12:0-C16:0 ($p>0.05$) but could enhance the level of UFA, especially the proportion of UFA in milk. For the dairy cows in either early or middle lactation, C18:2 and C18:3 in group 2 or 3 was higher than that of group 1 ($p>0.05$). It was also found that the better supplementary level of fresh alfalfa was 2.5 kg/head/day. The high level of fresh alfalfa could increase the level of BUN significantly ($p<0.05$).

Key words: Fresh alfalfa, dairy cows, performance, milk fatty acids, serum biochemical parameter, China

INTROCUCTION

Alfalfa has high protein, energy, vitamins, minerals and effective fiber. Its composition of amino acids is balanced. Thus, it was applied as feed by the form of alfalfa fed as fresh, alfalfa hay, alfalfa silage and grazing. Most studies were focused on the latter three form. During the dairy cow production fresh alfalfa was a kind of routine feeding form but dry matter and crude protein degradability in the rumen and utilization rate in the small intestine of the fresh alfalfa changed very much along with growing stage (Gonzalez *et al*., 2001). So, the report of alfalfa fed as fresh was less. Compare with forage, fresh forage had higher Polyunstatured Fatty Acids (PUFA) including linolenic acid (18:3) and linoleic acid (18:2). And the composition of fatty acids in the milk was affected by that in the diet (Harfoot, 1978). It was showed that the milk containing the rich PUFA maked for decreasing the cholesterin content in the blood and cardiovascular diseases in the human (McGuire *et al*., 1997). This experiment was conducted in order to study the effect of the fresh alfalfa on the performance, the metabolism of the nutriment and the composition of fatty acids in the milk of the dairy cows in the different lactation, fed with the different level of fresh alfalfa.

MATERIALS AND METHODS

Dairy cows: According to the principle of similar milk yield, parturition numbers, lactation day and body weight, 15 early- and 15 mid-lactation Holstein cows were chosen. Average days in milk of early-lactation cow was 54.5 days and their milk yield was 19.11 kg day^{-1}. And days in milk of mid-lactation Holstein cows was 134.1 days and their milk yield was 14.93 kg day^{-1}. All the chosen cows were randomly allocated to 3 groups in pairs. And there were 10 cows in every group including cows in early- and mid-lactation half and half.

Fresh alfalfa: Fresh alfalfa in the experiment was obtained in the same field. The whole field was disparted into 10 plots and alfalfa was reaped one by one in order to ensure that all the alfalfa was obtained at the original florescence at the third stubble. During the experiment, fresh alfalfa was reaped by the cropper every day and stubble height was about 5 cm. Alfalfa was sent to cowhouse by the quadricycle for the use.

Diets: The basic diet was formulated with NRC and based on DM% and was contained 3.20 MJ kg^{-1} net energy for lactation, 19.6% CP, 4.94% crude fiber, 1.92% calcium and

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0.95% phosphorus and comprised with wheat straw, whole-plant corn silage, beer residue and mixed concentrate. The composition of concentrate (DM basis) was as follows; 500 g kg^{-1} corn grain, 160 g kg^{-1} soybean meal, 80 g kg^{-1} wheat bran, 60 g kg^{-1} cottonseed meal, 130 g kg^{-1} sesame cake, 30 g kg^{-1} bone meal, 20 g kg^{-1} calcium carbonate, 10 g kg^{-1} salt and 10 g kg^{-1} additive.

Experimental design

Group 1: Ten cows were fed basic diet (control).

Group 2: Ten cows were fed basic diet plus 2.5 kg day^{-1} fresh alfalfa.

Group 3: Ten cows were fed basic diet plus 5 kg day^{-1} fresh alfalfa.

The quality of concentrate and beer residue that the three group received was same and the basic forage was wheat straw and whole-plant corn silage (4:1) (DM basis). The experiment included adaptation period of 7 days and formal experimental period of 60 days.

Feeding managements: In the present study, all the cows were fed and milked two times, respectively daily had free access to water and was tie fed. The milking employed 2×10 abreast pipeline milking machine. The procedure was feeding first the fresh alfalfa then wheat straw and whole-plant corn silage and at the last beer residue and mixed concentrate.

Feed intake and cow's performances: Feed intake was determined once every week to calculate average intake daily. Milk yields were recorded daily while milk samples were taken once every week. They were then analyzed for fat, protein, SNF and density by Milk analyzer (MILKYWAY-1).

Analysis of fatty acid by Gas Chromatography (GC): The fatty acid profiles of milk were determined by gas chromatography. Lipids were extracted with benzene-petroleum aether (5:1 vol/vol). Methyl esters of fatty acids from milk were prepared by the potassium hydroxide solution in methanol. The methyl esters of fatty acids were injected into an gas chromatography (Agilent 4890) fitted with a flame-ionization detector (Agilent technologies). The capillary column(DB-WAX 30 m× 0.53 mm×0.50 μm, Agilent technologies) was used to separate fatty acid methyl esters. The conditions of gas chromatography were as follows: The injection volume was 1 μL. The ultrapure hydrogen was the carrier gas and the injector and detector temperatures were 250 and 270°C, respectively. The contents of the fatty acids were determined by using the normalization method.

Blood samples and analysis: Approximately, 10 mL blood from *V. jugularies* was collected pre-feeding from each animal for at the begin, middle and end of the experiment. Serum was drawn from blood and stored at -20°C for analysis. Serum Total Protein (TP), Alkaline Phophatase (ALP), Carbon Dioxide Combining Power (CO_2CP) and Blood Urea Nitrogen (BUN) were analyzed by an Automatic Biochemistry analyzer (Monarch 1000). Triiodothyronine (T3), Thyroxine (T4) and Insulin (INS) were determined using Radioimmunoassay kit (Beijing North Institute of Biological Technology, China) by Gamma radioimmunoassay counter (GC-911).

Statistical analysis: Data were analyzed by ANOVA with the General Linear Model (GLM) procedure of SPSS 11.5 software and multiple comparison used Duncan method.

RESULTS

Feed intake of cows: Dry matter intake of cows in group 2 was highest then group 3 and 1 was lowest whether in the early lactation or the middle lactation (Table 1).

Milk yield and milk composition: The effect of the fresh alfalfa on milk yield and milk composition was shown in Table 2. Milk yield was showed as follows: Group 2> group 3>group 1 but there was no significant difference in them ($p>0.05$). Milk density of group 2 was higher than that of group 3 ($p<0.05$) in the early lactation and the milk density of group 1 and 2 was higher than that of group 3 ($p<0.05$) in the middle lactation. TP and SNF in milk of group 2 was highest and milk fat percentage of group 3 was highest whether in the early lactation or the middle lactation ($p>0.05$).

Table 1: Ration composition and DM intake in each trial group (DM (kg))

		The early lactation			The middle lactation		
Items	DM (%)	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Fresh alfalfa	21.4	0.00	2.50	5.00	0.00	2.50	5.00
Straw and silage	78.7	4.71	3.62	0.68	4.91	4.00	1.00
Brewers grains	23.4	2.45	2.45	2.45	2.45	2.45	2.45
Concentrate	88.0	6.00	6.00	6.00	6.00	6.00	6.00
Average intake	-	13.16	14.57	14.13	13.36	14.95	14.45

Table 2: The milk yield and composition of dairy cows in each trial group

	The early lactation			The middle lactation		
Items	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Yield (kg)	15.61±1.92	17.86±4.06	17.60±3.91	11.39±4.31	16.01±2.78	13.06±4.52
Fat (%)	3.27±0.41	3.01±0.60	3.47±0.49	3.21±0.32	3.18±0.48	3.52±0.29
Protein (%)	2.28±0.07	2.35±0.05	2.26±0.13	2.32±0.08	2.35±0.02	2.26±0.09
Density	25.49±0.45^{ab}	26.53±0.62^{b}	24.95±1.50^{a}	25.91±1.08^{c}	26.22±0.52^{bc}	24.96±0.98^{a}
SNF (%)	8.13±0.23	8.29±0.15	8.02±0.34	8.18±0.22	8.28±0.03	8.02±0.27

Table 3: The concentration of metabolite level of serum in each trial group

	The early lactation			The middle lactation		
Items	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
TP (g L^{-1})	76.38±1.79^{a}	82.75±4.590^{b}	80.73±5.110^{ab}	82.65±6.68	82.67±4.980	82.67±7.830
ALB (g L^{-1})	35.54±0.25	33.87±1.920	33.73±2.360	33.69±2.13	32.22±2.250	34.19±1.350
ALP (IU L^{-1})	43.54±6.63	46.73±12.58	46.60±12.77	41.20±4.03	46.73±13.39	47.14±5.020
CO_2CP (mmol L^{-1})	22.68±0.46^{b}	21.74±0.550^{a}	22.56±0.280^{b}	21.80±0.95	21.28±0.510	22.11±0.450
BUN (mmol L^{-1})	6.73±0.73^{a}	6.91±0.880^{ab}	7.92±0.790^{b}	5.88±1.07^{Aa}	6.56±0.790^{ABa}	7.88±0.730^{Bb}
T_3 (ng mL^{-1})	1.66±0.18	1.56±0.230	1.68±0.430	1.59±0.31	1.43±0.140	1.82±0.660
T_4 (ng mL^{-1})	52.76±5.93	53.18±4.020	55.68±8.070	54.31±5.33^{ab}	51.05±8.000^{a}	62.11±10.38^{b}
INS (μ IU mL^{-1})	35.63±5.75	38.05±8.130	35.61±5.510	41.81±6.96^{b}	35.67±10.93^{ab}	26.22±7.700^{a}

Table 4: The composition and relative content of fatty acid in the milk of each trial group (%)

	The early lactation			The middle lactation		
Items	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Laurica (12:0)	2.84±1.39	2.97±1.14	2.83±1.38	2.13±0.29	2.75±0.58	2.56±0.42
Tridecanoic acid (13:0)	0.07±0.02	0.08±0.00	0.08±0.01	0.05±0.02	0.05±0.01	0.07±0.02
Myristic acid (14:0)	10.78±2.27	9.88±2.31	10.44±3.04	9.19±1.01	9.54±1.73	9.77±0.57
Palmitic acid (16:0)	29.98±2.74	29.50±4.26	29.80±3.07	29.48±3.59	28.44±4.13	29.50±1.37
Heptadecanoate (17:0)	0.86±0.17	0.39±0.13	0.55±0.09	0.73±0.22	0.65±0.12	0.76±0.07
Stearic acid (18:0)	11.90±2.51	11.25±3.55	12.15±1.39	16.05±1.39	12.87±2.43	14.20±1.50
Arachidic acid (20:0)	0.35±0.25	0	0	0.37±0.08	0.10±0.01	0.23±0.02
Palmitoleic acid (16:1)	1.66±0.14	1.31±0.22	2.05±0.31	1.80±0.75	1.99±0.74	1.93±0.21
Oleic acid (18:1)	29.50 ±2.68	29.60±5.58	29.06±2.94	29.05±3.22	29.26±3.43	29.30±1.15
Linoleic acid (18:2)	2.85±0.45	3.34±2.16	2.83±0.89	3.16±1.03	3.19±0.35	3.36±0.87
Linolenic acid (18:3)	0.16±0.06	0.34±0.15	0.30±0.17	0.23±0.11	0.46±0.38	0.47±0.33
Others	9.05±1.55	11.34±12.34	9.91±4.34	10.56±2.84	10.71±9.61	7.85±0.83
SFA	56.78	54.07	55.85	58.00	54.39	57.09
MUFA	31.16	30.91	31.11	30.85	31.25	31.23
PUFA	3.01	3.68	3.13	3.59	3.65	3.83
UFA	34.17	34.59	34.24	33.44	34.90	35.06
U/S	0.60	0.64	0.61	0.58	0.64	0.61

The same letters represent no significant difference ($p>0.05$), the different letters in the same line represent difference significantly ($p<0.05$) and the different capital letters in same line represent difference significantly ($p<0.01$)

Blood indexes: The effect of the fresh alfalfa on blood indexes was shown in Table 3. The level of serum BUN of group 3 ($p<0.05$) and group 2 ($p>0.05$) was higher than that of group 1 in the early lactation. The level of serum TP of group 2 ($p<0.05$) and group 3 ($p>0.05$) was higher than that of group 1 in the early lactation. The CO_2CP of group 2 and was significantly lower than that of group 1 and 3 in the early lactation ($p<0.05$). The level of serum T_4 of group 3 was higher than that of group 2 ($p<0.05$) and group 1 ($p>0.05$) in the middle lactation. The level of serum INS of group 1 was higher than that of group 3 ($p<0.05$) and group 2 ($p>0.05$) in the middle lactation.

The fatty acid profiles of milk: The effect of the fresh alfalfa on the fatty acid profiles of milk was shown in Table 4. The fatty acid profiles of in milk changed little in every group. The level of PUFA (C18:2 and C18:3) of group 2 was higher than that of group 1 in the early lactation ($p>0.05$). The level of PUFA (C18:2 and C18:3) of group 2 was higher than that of group 1 in the early lactation ($p>0.05$). Compared with group 1, the level of SFA of group 2 and 3 was lower ($p>0.05$), the level of UFA of them was higher ($p>0.05$) and U/S had a tendency of increase. From the experiment, it could be showed that there were 11 kinds of fatty acid in the milk including 7 kinds of Saturated Fatty Acid (SFA) (about 55%) acid and 4 kinds of Unsaturated Fatty Acid (USFA) (about 35%). In SFA, palmitic acid (16:0) was highest then stearic acid (18:0) and myristic acid (14:0) and three of them occupied >90% of SFA. USFA gave priority to oleic acid (18:1), the content of linoleic acid (18:2), palmitoleic acid (16:1) and linolenic acid (18:3) decreased in turn.

DISCUSSION

Effect of fresh alfalfa on milk yield and milk composition: Compared with common quality of forage (wheat straw and whole-plant corn silage), fresh alfalfa as high quality of forage had a tendency of increasing the milk yield. This results was same of obtained data by Lin *et al*. (2004). In this experiment, fresh alfalfa had a tendency of increasing the milk fat percentage because fresh alfalfa increased forage to concentrate ratio and Acetate to Propionate ratio (A/P) and produced large numbers of Volatile Fatty Acid (VFA) increased milk fat synthesis (Brito *et al*., 2008).

Effect of fresh alfalfa on blood indexes: Fresh alfalfa increased the level of serum BUN. Elizalde *et al*. (1999) reported that fresh alfalfa had the high degradability in the rumen and the efficiency of AA and ammonia nitrogen produced to rumen microbe. Superfluous NPN could not be utilized effectively, came into the blood by the capillary vessel of the rumen wall thus, the content of serum BUN was increased. In the study, fresh alfalfa increased the level of serum T_4. It was T_4 enhanced the metabolism and accelerated growth, development of economy and histodifferentiation. And it be showed that fresh alfalfa, fed to dairy cows could promoted their metabolism.

Effect of fresh alfalfa on the fatty acid profiles of milk: Fatty acid profiles and relative content of milk affected directly the quality of milk (Chilliard *et al*., 2000). The exorbitant SFA would increase the cholesterin content in the blood and cardiovascular diseases of human and on the contrary increasing USFA could make for the health (Whiting *et al*., 2004). It was a good approach to increase the the content of USFA, especially PUFA in the milk by the nutrition manipulation. In this study, fresh alfalfa had a tendency of increasing PUFA (C18:2 and C18:3) and decreasing SFA.

And it was same of obtained data by Whiting *et al*. (2004) and Zhu (2007). The average content of linoleic acid was 3.12% and it was close to that (3.8%) in Beijing, Inner Mongolia and Gansu (Wang, 2002, 2003) but was under to that (8.5%) reported by Zhu *et al*. (2000).

CONCLUSION

This study showed feeding fresh alfalfa increased the DMI of dairy cows. Fresh alfalfa had a tendency of increasing the milk yield and the milk fat percentage, the content of USFA of milk and it increased the level of BUN in the serum.

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