Effects of Dietary Supplementation with Artemisia annua Ethanol Extract on Milk Production Performance and Conjugated Linoleic Acid Content in Milk Fat of Dairy Cows: Postprint

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Abstract

This study aimed to investigate the effects of dietary supplementation with Artemisia annua ethanol extract on milk production performance and conjugated linoleic acid (CLA) content in milk fat of dairy cows. Fifteen lactating Holstein dairy cows with body weight $(600\$\pm29)kg, parity2–3, lactationperiod(158\pm3)d, andmilkyield(22.8\pm\$1.8)$ kg/d were selected and divided into 3 groups (n=5 per group) using a completely randomized block design for a 40-day feeding trial, with days 1–9 as the preliminary period and days 10–40 as the formal experimental period. The control group was fed a basal diet, while experimental groups 1 and 2 were supplemented with 96 and 160 g/(d · head) of Artemisia annua ethanol extract in the basal diet, respectively. The results showed that during the entire experimental period, there were no significant differences (P>0.05) between the two experimental groups and the control group in milk yield (on days 24, 31, and 40), milk fat percentage, milk fat yield, milk protein percentage, milk protein yield, non-fat solids content, or the contents of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, short-chain fatty acids, medium-chain fatty acids, and long-chain fatty acids in milk fat. The CLA content in milk fat of the two experimental groups increased by 30.7% and 38.6% compared with the control group, with experimental group 2 showing a significant difference from the control group (P<0.05). It can be concluded that dietary supplementation with an appropriate amount of Artemisia annua ethanol extract has no significant effect on milk production performance of dairy cows, but can increase the CLA content in milk fat, thereby optimizing the fatty acid profile in milk fat.

Full Text

Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Performance and Conjugated Linoleic Acid Content in Milk Fat of Lactating Cows

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Abstract

This study investigated the effects of dietary supplementation with Artemisia annua ethanol extracts on milk production performance and conjugated linoleic acid (CLA) content in milk fat of lactating dairy cows. Fifteen Holstein dairy cows with similar body weight [(600$±29)$kg$], $parity(2–3), $lactationstage[(158±3)$d$], $andmilkyield[(22.8±$1.8) kg/d] were randomly allocated into three groups of five cows each using a completely randomized block design. The feeding trial lasted 40 days, with days 1–9 serving as a preliminary period and days 10–40 as the experimental period. Cows in the control group received a basal diet only, while experimental groups 1 and 2 received the basal diet supplemented with 96 and 160 g/(d · head) of Artemisia annua ethanol extracts, respectively. The results demonstrated that throughout the entire experimental period, no significant differences were observed between the two experimental groups and the control group in milk yield (measured on days 24, 31, and 40), milk fat percentage, milk fat yield, milk protein percentage, milk protein yield, non-fat solids content, or the contents of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, short-chain fatty acids, medium-chain fatty acids, and long-chain fatty acids in milk fat ($P > 0.05$). However, the CLA content in milk fat increased by 30.7% and 38.6% in experimental groups 1 and 2, respectively, with experimental group 2 showing a significant difference compared to the control group ($P < 0.05$). These findings indicate that dietary supplementation with moderate amounts of Artemisia annua ethanol extracts does not significantly affect milk production performance but can enhance CLA content in milk fat, thereby optimizing the fatty acid profile.

Keywords: dairy cows; Artemisia annua ethanol extracts; milk performance; milk fat; conjugated linoleic acid

1. Materials and Methods

1.1 Experimental Material Artemisia annua was purchased from Nanjing, with the following composition: sesquiterpenes 35%, aromatics 29%, fatty acids 6%, steroids 6%, triterpenes 6%, aliphatics 4%, alkaloids 3%, phenols 5%, heterocyclics 2%, and others 4%. The ethanol extraction conditions were: 55% ethanol concentration, extraction temperature 95 °C, and extraction time 2 h.

1.2 Experimental Design and Animal Management Fifteen Holstein dairy cows with similar body weight $[(600\$\pm29)kg], parity(2–3), lactationstage[(158\pm3)d], andmilkyield[(22.8\pm$ kg/d] were randomly divided into three groups of five cows each based on similar milk production levels. The control group received a basal diet, while experimental groups 1 and 2 received the basal diet supplemented with 96 and 160 g/(d · head) of Artemisia annua ethanol extracts, respectively. Days 1–9 served as a preliminary period, and days 10–40 constituted the experimental period. All cows were managed uniformly with ad libitum access to feed and water, receiving two daily feedings and two daily milkings performed by dedicated staff.

1.3 Basal Diet The concentrate and forage used in the experiment were sourced from Saihan District Ranch in Huhhot. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.4 Sample Collection and Analysis Milk yield was recorded 3–4 days per week starting from the preliminary period. Milk samples were collected on days 1, 10, 17, 24, 31, and 40 for milk composition analysis. Daily milk yield was recorded using a graduated milking cup. On each sampling day, morning and evening milk samples were collected, mixed in a 1:1 ratio, and analyzed for milk fat percentage, milk protein percentage, total solids content, non-fat solids content, and lactose percentage using a milk composition analyzer (Mickro-FT120, FOSS). Simultaneously, mixed milk samples were frozen at -20 °C for subsequent analysis of milk fatty acid composition.

Fatty acids were extracted from samples as follows: frozen milk was thawed in cold water, and 2 mL of milk sample was mixed with 4 mL of n-hexane/isopropanol solution, followed by addition of 2 mL of sodium sulfate (Na_2SO_4) solution and centrifugation at 5,300 r/min for 20 min at room temperature. The supernatant was then transferred to a 20 mL hydrolysis tube, mixed, and dried under nitrogen.

For methylation, 2 mL of sodium methoxide ($NaOCH_3$)/methanol solution was added to the dried hydrolysis tube and incubated in a 50 °C water bath for 15 min. After cooling, 2 mL of hydrochloric acid/methanol solution was added and incubated in an 80 °C water bath for 1.5 h. The mixture was then cooled to room temperature, and 3 mL of water and 6 mL of n-hexane were added, shaken, and allowed to separate by standing or centrifugation. The upper layer was aspirated (as completely as possible), brought to a final volume of 10 mL,

dried with anhydrous Na_2SO_4, and analyzed using a gas chromatograph (Agilent 6890N).

1.5 Statistical Analysis Data were initially processed using Excel 2007 and subsequently analyzed using one-way ANOVA with SAS 9.2 software. Differences were considered significant at $P < 0.05$.

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2. Results

2.1 Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Yield As shown in Figure 1 [Figure 1: see original paper], milk yield in experimental group 2 and the control group increased over time, while milk yield in experimental group 1 exhibited an initial increase followed by a decrease. Table 2 reveals that at the end of the experiment (day 40), milk yield in experimental group 1 was lower than that in the control group, but the difference was not significant ($P > 0.05$). Milk yield in experimental group 2 was higher than that in the control group, though this difference was also not significant ($P > 0.05$). Over the experimental period, milk yield in the control group and experimental group 2 (except on day 24) was higher than at the start of the experiment (day 1). In contrast, milk yield in experimental group 1 on days 24, 31, and 40 was significantly lower than at the start of the experiment ($P < 0.05$).

2.2 Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Composition Table 3 shows that compared with the control group, experimental groups 1 and 2 exhibited increases in milk fat percentage of 14.7% and 22.4% ($P > 0.05$), respectively, and increases in milk fat yield of 4.2% and 4.8% ($P > 0.05$), respectively. Milk protein percentage increased by 10.6% and 6.1% ($P > 0.05$), and milk protein yield increased by 3.5% and 1.3% ($P > 0.05$), respectively. Non-fat solids content increased by 12.2% and 6.7% ($P > 0.05$), respectively. Total solids content in experimental groups 1 and 2 showed a decreasing trend ($P > 0.05$). Lactose percentage in experimental group 1 showed an increasing trend ($P > 0.05$), while that in experimental group 2 showed a decreasing trend ($P > 0.05$).

2.3 Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Fatty Acid Composition Table 4 demonstrates that compared with the control group, both experimental groups showed an increasing trend in milk fat CLA content, with experimental group 2 showing a significant difference ($P < 0.05$). The CLA content in experimental groups 1 and 2 increased by 30.7% and 38.6%, respectively. Compared with the control group, experimental groups 1 and 2 showed decreasing trends in saturated fatty acids, unsaturated fatty acids, and monounsaturated fatty acids, though these differences were not significant ($P > 0.05$). Polyunsaturated fatty acid content was higher in both experimental groups than in the control group, increasing by 28.1% and 12%,

respectively, but these differences were not significant ($P > 0.05$). Long-chain fatty acid content in experimental groups 1 and 2 showed an increasing trend, though not significant ($P > 0.05$), with increases of 34.5% and 24.5% compared to the control group. Short-chain and medium-chain fatty acid contents in experimental group 1 showed increasing trends, while those in experimental group 2 showed decreasing trends, but these differences were not significant ($P > 0.05$).

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3. Discussion

3.1 Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Yield Jiang et al. [7] investigated the addition of different proportions (0, 1.0, 1.7, and 2.5 kg) of extruded full-fat soybeans to total mixed rations (TMR) and reported that milk yield significantly increased in the extruded full-fat soybean groups compared with the control group. Wang et al. [8] added whole flaxseed at 972 g/(d · head) and whole cottonseed at 2,169 g/(d · head) to dairy cow diets and found that the mixture of flaxseed and cottonseed increased milk yield. These studies increased milk fat CLA content by adding vegetable oils or oilseeds to dairy cow diets. The present study achieved similar results using plant extracts to increase milk fat CLA content, suggesting that regulating milk fat CLA content through plant extracts may also increase milk yield. However, milk yield in experimental group 1 was significantly lower than at the start of the experiment, and the reasons for this require further investigation.

3.2 Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Composition Milk fat and protein are important components of milk and dairy products. Research has shown that adding vegetable oils to dairy cow diets to regulate milk fat CLA content can increase milk fat percentage, but milk protein decreases significantly compared with the control group, whether used alone or with sunflower oil [9]. These findings differ from the present study, possibly due to the use of different sources of substances to regulate milk fat CLA content. Wang [6] found that adding 7.26 and 12.10 g/d of Artemisia annua extract to dairy goat diets significantly increased milk fat yield in both experimental groups, with experimental group 2 showing a significant difference from the control group. Both experimental groups also showed higher milk protein yield than the control group, with experimental group 2 being significantly higher. Milk fat percentage was higher in both experimental groups than in the control group, with experimental group 2 showing a significant difference ($P < 0.05$). Milk protein percentage was higher in both experimental groups than in the control group, but the difference was not significant. Lactose percentage was also higher in both experimental groups than in the control group, but the difference was not significant. Non-fat solids content was higher in both experimental groups than in the control group, but the differences were not significant. The present study found that compared with the control group,

experimental groups 1 and 2 showed increasing trends in milk fat percentage, milk fat yield, milk protein percentage, milk protein yield, and non-fat solids content, but these differences were not significant, consistent with the findings of Wang [6]. Lactose percentage in experimental group 1 showed an increasing trend, while that in experimental group 2 showed a decreasing trend, which differs from the results of Wang [6] and requires further investigation.

3.3 Effects of Dietary Artemisia Annua Ethanol Extracts on Milk Fatty Acid Composition Dietary fat supplementation significantly influences milk fatty acid composition. Numerous studies have demonstrated that supplementing dairy cow diets with vegetable oils or oilseeds can regulate milk fat CLA content. For example, Yin et al. [10] reported that adding sunflower seeds to the diet increased milk fat CLA content by 98.28%. In the present study, dietary supplementation with Artemisia annua ethanol extracts increased milk fat CLA content, with experimental group 2 showing a significant increase. Experimental groups 1 and 2 increased CLA content by 30.7% and 38.6%, respectively, compared with the control group. Wang [6] found that adding 7.26 and 12.10 g/d of Artemisia annua extract to dairy goat diets resulted in significantly higher milk fat CLA content in both experimental groups compared with the control group, consistent with the present findings. The mechanism of CLA formation in milk fat involves two pathways: first, the desaturation of trans-11-C18:1 fatty acid to CLA by *Butyrivibrio fibrisolvens* in the rumen, and second, the desaturation of trans-11-C18:1 fatty acid to CLA by stearoyl-CoA desaturase (SCD) in the mammary gland. Wallace [12] reported that the mechanism by which Asteraceae plant extracts increase milk fat CLA content involves increasing the population of *Butyrivibrio fibrisolvens* bacteria in the rumen that are associated with CLA synthesis. The possible mechanism by which Artemisia annua ethanol extracts regulate milk fat CLA content in the present study may be similar, as Artemisia annua belongs to the Asteraceae family.

Compared with the control group, experimental groups 1 and 2 showed decreasing trends in saturated fatty acids, unsaturated fatty acids, and monounsaturated fatty acids. The present study also found that dietary supplementation with Artemisia annua ethanol extracts increased polyunsaturated fatty acid content in milk fat. Yin et al. [11] added different oilseeds (sunflower seeds, flaxseeds, and rapeseeds) as exogenous fat sources to dairy cow diets and increased polyunsaturated fatty acid content in milk fat, consistent with the present results. Wang [6] found that adding 7.26 and 12.10 g/d of Artemisia annua extract to dairy goat diets resulted in a non-significant increasing trend in medium- and short-chain fatty acid content in experimental group 1 and a non-significant decreasing trend in experimental group 2, consistent with the present study. Previous research has confirmed that nearly all fatty acids below C14:0 and approximately half of C16:0 fatty acids are synthesized de novo in mammary tissue from acetate and β-hydroxybutyrate produced by rumen fermentation, while long-chain fatty acids in milk fat are primarily derived from blood lipids, which mainly originate from the diet. The present study showed that dietary

supplementation with Artemisia annua ethanol extracts significantly increased long-chain fatty acid content in milk fat in experimental groups 1 and 2.

Conclusion

Dietary supplementation with Artemisia annua ethanol extracts at 96 and 160 g/(d · head) did not significantly affect milk production performance in dairy cows. However, supplementation at 160 g/(d · head) significantly increased CLA content in milk fat. These results suggest that Artemisia annua ethanol extracts can be used as a functional feed additive to enhance the nutritional value of dairy products by increasing their CLA content without compromising overall milk production.

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Note: Figure translations are in progress. See original paper for figures.