Study on Feed Processing and Application of Renewable Plant Humulus Scandens

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Abstract. In order to study the feed processing and development technology of the renewable plant Humulus scandens, Humulus scandens were collected, dried and comminuted at the stage of leaf unfolding, flower opening, flower blooming and yellow withering, and processed into direct raw material group, silage natural fermentation group and silage plus bacteria fermentation group. The contents of main nutrients and total flavonoids in Humulus scandens feed were determined and compared. The microbial quantity and fermentation quality of Humulus scandens in withered and yellow period in silage natural fermentation group and silage plus bacteria fermentation group were detected. The results showed that there was no significant change in calcium and phosphorus content in the three groups. The other nutrients in silage natural fermentation group and silage plus bacteria fermentation group were slightly lower than those in direct raw material group. The content of total flavonoids in silage plus bacteria fermentation group was significantly higher than that in other groups. In terms of fermentation quality and microbial quantity, the fermentation of silage natural fermentation group was not obvious, the pH value was on the high side, the fermentation of silage plus bacteria group was obvious, and the quality was also higher. In conclusion, if Humulus scandens is directly used as feedstuff, the best collection time is the blooming period of flowers. If silage and lactobacillus fermentation are used, the value of feedstuff is the highest, but the value of silage natural fermentation is the lowest, so it is not recommended to choose.

1. Introduction

The global livestock and fishery industry is in a period of vigorous development, the number of aquaculture is increasing, and form large-scale breeding, and the demand for forage feed is increasing. On the other hand, natural forage is seriously degraded, and the supply of forage is decreasing. This will have a serious impact on the development of the global livestock and fishery industry. There is an urgent need for new feed as a supplement. Humulus scandens is the dry overground part of Humulus scandens (Lour.) Merr. which belongs to the Humulus, a trailing herb of mulberry family. It is a perennial herb. It often grows in the ditch, wetland, ruins and other places. It has extremely strong vitality, strong renewability and high yield. Humulus scandens is distributed in many countries and regions in Asia and Africa. In addition to Xinjiang and Qinghai, all provinces and regions in China have different degrees of distribution. As a medicinal herb, valerian is included in many provincial and municipal floras such as “Fujian Folk Herbs”, “Jiangsu Wild Plants”, “Anhui Medicinal Materials”, etc., but it has less clinical use and occasional application by folks. Clear. However, valerian is rich in nutrients such as vegetable protein, fat, fiber and trace elements, and medicinal
compounds such as flavonoids, terpenoids, volatile oils and steroids, which have the functions of detoxification, antibacterial, anti-inflammatory and anti-viral. Waste is a treasure. If weaving grass to process and develop animal feed, it can be used as a daily feed to supplement nutrition, and it can also increase the disease resistance of livestock and fish, and the prospect is objective.\textsuperscript{[3-6]}

At present, most of the psyllium at home and abroad are focused on the study of its chemical properties, active ingredients and modern pharmacological effects. It has been found that flavonoids are a class of components with higher separation, and flavonoids are the main material basis for its pharmacological action such as heat-clearing and detoxification, water-saving, anti-bacterial, anti-inflammatory, anti-viral, etc., and its research as a feed processing method and application is less, has not been specifically reported, and is limited to raw materials directly as feed or feed additives.\textsuperscript{[7-10]}

In this study, weeds were used as test materials to prepare dry products of yarrow raw materials in different collection periods, and scientific processing technology was used to make silage natural fermentation group and lactic acid bacteria to prepare silage fermentation group, and the nutrients and totals were determined. The content of flavonoids, supplemented by the quantity of microorganisms and fermentation quality, comprehensively evaluate its quality, in order to provide a basis for accelerating the scientific feed of psyllium.

2. Experiment material
The test valerian samples were collected by the research team members at the north side of Hefei Railway Station at 5-10, which was the upper part of the grassland, and the weeds were removed. Dried, smashed into medium powder, spare.

3. Experiment method
3.1. Experiment design
The sample collection period was set as follows: the leaf stage (early May), the flower opening stage (early June), the flowering period (early July and early August), and the yellowing period (mid-occupation). The aboveground parts of the whole plant of alfalfa were collected in each period, dried and pulverized, and randomly divided into 9 parts in parallel, which were respectively made into small packets, and 3 small packets were taken as the direct raw material group; and 3 small packages were mixed uniformly according to the mass ratio of the liquid to 1:2.5. Allow it to naturally ferment for 30 days, and make it into the silage natural fermentation group; the remaining 3 packets are separately added with 5% of the lactic acid bacteria dilution of 4.6×10⁷ CFU/mL, and the concentration of the fermentation broth is 0.5 mg/mL, fermentation for 30 days, for the silage plus fermentation group. At the end of the fermentation, the samples were taken out and dried, and the contents of nutrients and total flavonoids in each packet were determined. The microbial quantity, pH value and organic acid content of the natural fermentation and silage plus fermentation group in the flowering stage were determined.\textsuperscript{[11-12]}

3.2. Evaluation indicators and methods
3.2.1. Determination of nutrients. The crude protein (CP), crude fat (EE), crude fiber (CF) and organic matter (OM) content of each packaged dry sample were determined according to the test method recommended in "Feed Analysis and Feed Quality Testing Technology"; The routine analysis method was used to determine the contents of trace elements calcium (Ca) and phosphorus (P).\textsuperscript{[12-13]}

3.2.2. Determination of total flavonoids. According to the results of preliminary experiments, the dry samples of valerian were ultrasonically added to 80% ethanol for 30 min according to the ratio of material to liquid, filtered, and the filtrate was continuously inspected. The rutin was used as the reference substance, the reagent was used as the blank control, the color was developed by Al (NO3)3
and NaOH, and the absorbance was measured by the colorimetric method at 510nm. The content of total flavonoids in the valerian was calculated by the standard curve method.  

3.2.3. Determination of microbial quantity, pH and organic acids. According to the literature method, the flowering stage silage natural fermentation group and the silage plus bacteria fermentation group were separately mixed with sterile water, divided into two parts, one diluted, using lactic acid bacteria, yeast, Escherichia coli, aerobic bacteria and heat resistance. The conventional culture method of oxygen bacteria was cultured for 48 hours and counted separately; the other was filtered, and the content of acetic acid (AA), propionic acid (PA), butyric acid (BA) and lactic acid (LA) was determined by high performance liquid chromatography. The pH was measured by a pH meter.

3.2.4 Data analysis. The test results data were statistically analyzed using Excel 2003 and SAS 9.0 software.

4. Test results and analysis

4.1. Comparison of the contents of nutrients and total flavonoids in direct raw materials at different collecting periods

The content of nutrients and total flavonoids in the direct raw material group in different collection periods is shown in Table 1. It can be seen from Table 1 that the CP and CF contents in the different collection periods are very different, and there are significant differences (P<0.05). The highest CP content is the flowering period, which is 20.57%, and the lowest is only 14.35%. The content of the content in the early flowering stage and the yellowing stage was higher, there was no significant difference (P>0.05), but there was significant difference between the leaf expansion stage and the flowering stage. The OM content was not significantly different between the leaf expansion stage and the flowering stage, but later The content decreased gradually and there was a significant difference between them. The difference of Ca and P content was small. The content of Ca was the highest in flowering stage, reaching 3.56%. There was no significant difference with other periods. P was the smallest in the leaf-expanding stage, only 0.21%. There are significant differences with other periods; the total flavonoid content is increased first and then decreased, and there is a significant difference between them. The highest content is flowering period, which is 2.01%. In general, the EE was slightly lower in the flowering period, and the other nutrients and total flavonoids were the highest.

| Ingredient | Leaf stage | Flower opening | Flowering period | Yellow period |
|------------|------------|----------------|------------------|--------------|
| CP         | 14.35±0.15<sup>d</sup> | 17.03±0.11<sup>c</sup> | 20.57±0.21<sup>a</sup> | 18.23±0.37<sup>b</sup> |
| CF         | 12.37±0.04<sup>c</sup> | 13.08±0.08<sup>b</sup> | 15.18±0.17<sup>c</sup> | 11.97±0.21<sup>d</sup> |
| EE         | 2.89±0.13<sup>b</sup> | 3.02±0.24<sup>a</sup> | 2.78±0.19<sup>c</sup> | 3.03±0.18<sup>d</sup> |
| OM         | 89.72±0.25<sup>a</sup> | 89.63±0.31<sup>a</sup> | 87.37±0.41<sup>b</sup> | 86.28±0.22<sup>c</sup> |
| Ca         | 3.34±0.07<sup>a</sup> | 3.48±0.21<sup>a</sup> | 3.56±0.52<sup>a</sup> | 3.38±0.09<sup>a</sup> |
| P          | 0.21±0.23<sup>b</sup> | 0.35±0.45<sup>a</sup> | 0.42±0.06<sup>a</sup> | 0.33±0.21<sup>c</sup> |
| Total flavon | 1.03±0.11<sup>d</sup> | 1.64±0.27<sup>c</sup> | 2.01±0.08<sup>a</sup> | 1.75±0.04<sup>b</sup> |

Note: The difference between the lower-case letters of the peer data indicates that the difference is significant (P<0.05), and the same letter of the shoulder mark indicates that the difference is not significant (P>0.05). Table 2, Table 3, and Table 4 are the same.

4.2. Comparison of nutrient content and total flavonoids content in silage natural fermentation group in different collection periods
The contents of nutrients and total flavonoids in the natural fermentation group of silage in different collection periods are shown in Table 2. It can be seen from Table 2 that the content of CP and CF in different collection periods is quite different, and the flowering period is significantly different from other periods (P<0.05). Both of them have increased first and then decreased, and the highest content is in flowering period. The CP was 17.31% and the CF was 16.25%, which was lower than the raw material period. The EE and OM contents were reduced to different extents relative to the raw material group, and the EE was the lowest in the leaf-expansion period, only 2.75%, and the other three phases were significantly different, but the difference was not significant in the other three periods (P>0.05). There was no significant difference in OM content between the leaf-expanding stage and the flowering stage, but there was a significant difference between the flowering period and the yellowing stage. The former content was higher. Ca, The content of P and total flavonoids was very close to that of alfalfa raw materials, and there was no change at all, indicating that the natural fermentation of silage had no obvious effect on the components. In general, the nutrient composition and raw material group of silage natural fermentation group decreased slightly, but the contents of Ca, P and total flavonoids did not change much. The overall period was the flowering period.

Table 2. Comparison of nutrient content and total flavonoids content in silage natural fermentation group at different collection periods (%).

| Ingredient | Leaf stage | Flower opening | Flowering period | Yellow period |
|------------|------------|----------------|------------------|--------------|
| CP         | 12.61±0.18c| 16.13±0.21b    | 17.31±0.19a      | 16.48±0.23b  |
| CF         | 13.58±0.12c| 14.08±0.03b    | 16.25±0.12a      | 13.47±0.58c  |
| EE         | 2.75±0.16b | 2.94±0.07a     | 2.88±0.62a       | 2.93±0.37a   |
| OM         | 88.87±0.14a| 88.71±0.27a    | 85.96±0.53b      | 85.82±0.07b  |
| Ca         | 3.31±0.11a | 3.45±0.18a     | 3.41±0.09a       | 3.40±0.13a   |
| P          | 0.22±0.31b | 0.33±0.37a     | 0.41±0.12a       | 0.33±0.47a   |
| Total flavon | 1.09±0.25d | 1.58±0.06c     | 2.09±0.38a       | 1.81±0.67b   |

4.3. Comparison of nutrient content and total flavonoids in silage and fermentation groups in different collection periods

Lactic acid bacteria were fermented into silage fermentation group in different collection period, and the contents of nutrients and total flavonoids were determined and compared. The results are shown in Table 3. It can be seen from Table 3 that the CP content in silage and bacteria fermentation in different collection periods is slightly lower than that in direct raw materials, which is significantly higher than that in silage. The highest value is in the flowering period, reaching 22.37%, and the lowest is the leafing stage, which is 15.57. %, flower blooming period was significantly different from other time periods (P<0.05); CF, EE, OM content decreased slightly compared with silage natural fermentation group, CF was the highest in flowering period, and significant difference with other periods. EE in flower The content of the initial opening period was the highest, but there was no significant difference between the initial flowering period and the flowering period. There were significant differences with other periods. The OM content was high-low-high-low, and the lowest was 85.31%. Significant difference, but there was no significant difference in leaf-expanding stage, initial flowering period and flowering period; there was no change in Ca and P content after fermentation; the total flavonoid content increased in different degrees at different times, and the flowering period was the highest. It reached 2.26%, which was significantly different from other periods.
Table 3. Comparison of nutrient content and total flavonoids in silage-fermented fermentation group at different collection periods (%).

| Ingredient | Leaf stage | Flower opening | Flowering period | Yellow period |
|------------|------------|----------------|------------------|--------------|
| CP         | 13.57±0.23c | 16.98±0.15b   | 18.47±0.24a      | 17.22±0.14b  |
| CF         | 13.05±0.12c | 14.09±0.11b   | 15.53±0.05a      | 12.98±0.32c  |
| EE         | 2.52±0.23b  | 2.81±0.62a    | 2.72±0.08a       | 2.51±0.37b   |
| OM         | 88.19±0.18a | 87.59±0.27a   | 87.71±0.26a      | 85.31±0.43b  |
| Ca         | 3.24±0.11a  | 3.38±0.54a    | 3.34±0.17a       | 3.29±0.13a   |
| P          | 0.20±0.19b  | 0.34±0.09a    | 0.35±0.28a       | 0.34±0.57a   |
| Total flavon | 1.37±0.16c | 1.87±0.64b    | 2.26±0.32a       | 1.98±0.14b   |

4.4. Benchmarking analysis of quality and microbial quantity of alfalfa silage after fermentation

Based on the previous test data, the mashing feed, fermentation or not, the best selection period is the flowering period. In this experiment, the fermentation quality of the natural fermentation of silage and the fermentation of silage and fermentation materials and the number of main microorganisms were compared. The results are shown in Table 4. From Table 4, the fermentation quality was improved. The PA and LA in the silage plus fermentation group increased significantly. The AA, BA and pH values decreased significantly. BA can decompose protein, its content decreased, and the protein loss will be reduced. The pH value will drop to 4.05. It meets the requirements of conventional silage pH value of 4.0-4.2. From the perspective of microbial quantity, the number of lactic acid bacteria and yeast in silage and bacteria fermentation group increased significantly, and Escherichia coli, aerobic bacteria and heat-resistant aerobic bacteria decreased significantly. On the whole, the degree of fermentation was significantly higher than that of natural fermentation, and the fermentation quality was better than natural fermentation.

Table 4. Comparison of nutrient content and total flavonoids content in silage fermented feed group at different collection periods (%).

| Strain | Silage natural fermentation group | Silage plus fermentation group | Ingredient | Silage natural fermentation group | Silage plus fermentation group |
|--------|---------------------------------|--------------------------------|------------|---------------------------------|--------------------------------|
|        | Number of microorganisms (lgCFU/gFM) | Lactic acid bacteria | 3.05±0.14b | 4.21±0.08a | PA | 1.34±0.03b | 3.01±0.12a |
| Yeast E.coli Aerobic bacteria | 2.25±0.06b | 3.48±0.11a | Fermentation quality (mg/g) | LA | 12.29±0.13b | 15.74±0.25b |
| Aerobic bacteria | 0.53±0.21a | 0.61±0.18a | AA | 0.22±0.07a | 0.17±0.16b |
| Heat-resistant aerobic bacteria | 3.82±0.15a | 2.63±0.13b | BA | 1.06±0.12a | 0.14±0.05b |
|       | 0.41±0.08a | - | pH value | 4.67±0.03a | 4.05±0.09b |

5. Results and discussion

As a renewable resource, valerian can be used as a good feed for livestock and fish. This study found that it is rich in nutrients such as crude protein, crude fiber and organic matter. It also contains a variety of amino acids in combination with the literature, and its nutritional value is high. The antibacterial, antiviral, anti-oxidant and anti-aging effects of total flavonoids can greatly increase the disease resistance of livestock and fish.

As a feed development, valerian can directly dry, pulverize and store raw materials. This method is simple and feasible, and can retain its original color, aroma and taste, but the nutritional value and disease resistance are significantly lower than the fermentation group. If this method is selected, the
comprehensive nutrient content and total flavonoid content, the optimal collection period is in the flowering period; the natural fermentation of silage is compared with the unfermented, the difference of total flavonoids and trace elements Ca and P is small, not significant. The crude fiber increased slightly, but the protein, fat and organic matter decreased to varying degrees. Among them, the CP decreased most obviously, and the color was dim, the aroma disappeared, and the acidity increased. Therefore, the comprehensive evaluation of valerian is not suitable for silage natural fermentation and feed; Compared with the direct raw material group and the silage natural fermentation group, the content of total flavonoids was significantly increased, and the contents of trace elements Ca and P were basically unchanged. Other nutrients were reduced to varying degrees, but the overall content did not change much, and the protein content was higher than that of the silage natural fermentation group. The fermentation degree, fermentation quality and microbial quantity were also significantly better than the silage natural fermentation group. The herbicide feedstock not only considers the nutrient and digestibility of the material, but also increases the disease resistance of the animal and fish. Therefore, the best method is to select the flower in the flowering period and add the lactic acid bacteria to make the feed.

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