

Effects of Weaning Time and Age on Fatty Acid Composition in Hu Sheep Lamb Muscle (Post-print)

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Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to investigate the effects of weaning time and age on fatty acid composition in the muscle of Hu sheep lambs. Fifty-four newborn Hu sheep male lambs with similar body weight [(3.51±0.57) kg] and good health status were selected for the experiment. Six lambs were slaughtered at 28 days of age. The remaining 48 lambs were divided according to the principle of homogeneity into a 28-day weaning group [(8.21±0.97) kg] and a 56-day weaning group [(8.06±0.53) kg], with 24 lambs in each group. Six lambs from each group were slaughtered at 42, 56, 70, and 84 days of age, respectively. After slaughter, the fatty acid composition of the longissimus dorsi muscle and the content of hydrogenating bacteria in the rumen were determined. The results showed: 1) The content of most fatty acids in muscle was higher in the 56-day weaning group than in the 28-day weaning group; with increasing age, the content of fatty acids in lamb muscle gradually increased, and the variety became more diverse. 2) At 70 days of age, the content of *Butyrivibrio fibrisolvens* in the rumen of the 28-day weaning group was significantly higher than that of the 56-day weaning group ($P<0.05$); the content of *Butyrivibrio fibrisolvens* in the 56-day weaning group at 42 days of age was significantly higher than at 70 and 84 days of age ($P<0.05$). It can be concluded that, compared with weaning at 28 days of age, weaning lambs at 56 days of age is more conducive to fatty acid deposition in muscle.

Full Text

Effects of Weaning Time and Age on Fatty Acid Composition in Muscle of Hu Lambs

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Abstract

This experiment investigated the effects of weaning time and age on fatty acid composition in muscle of Hu lambs. Fifty-four healthy newborn male Hu lambs with similar body weight [(3.51±0.57) kg] were selected and reared to 28 days of age, at which point six lambs were slaughtered. The remaining 48 lambs were divided according to homogeneity principles into a 28-day weaning group [(8.21±0.97) kg] and a 56-day weaning group [(8.06±0.53) kg], with 24 lambs per group. Both groups were slaughtered at 42, 56, 70, and 84 days of age (six lambs per time point). After slaughter, the fatty acid composition of the longissimus dorsi muscle and ruminal hydrogenating bacteria content were determined. The results showed: (1) Most fatty acid contents in muscle were higher in the 56-day weaning group than in the 28-day weaning group, and fatty acid contents gradually increased with age while species became more abundant. (2) At 70 days of age, *Butyrivibrio fibrisolvens* content in the rumen was significantly higher in the 28-day weaning group than in the 56-day weaning group (P<0.05); in the 56-day weaning group, the content at 42 days was significantly higher than at 70 and 84 days (P<0.05). These findings indicate that weaning at 56 days of age is more conducive to fatty acid deposition in lamb muscle compared to weaning at 28 days.

Keywords: weaning; age; fatty acid; hydrogenating bacteria

Introduction

As living standards improve, lamb meat has gained increasing popularity due to its rich nutritional value, desirable taste, and therapeutic benefits, making it a health-promoting meat product [1]. However, compared to developed sheep-producing countries, China still has substantial room for improvement in lamb quality. Fatty acid composition is a primary determinant of meat quality, making research on fatty acid deposition in lambs crucial for enhancing meat quality [2].

Manipulating weaning time has become a hotspot in ruminant nutrition for improving meat quality. Controlling weaning time alters feed intake in ruminants,

and dietary forage-to-concentrate ratio [3-4], energy level [5-6], and protein level [7] directly affect muscle fatty acid composition, thereby modifying fatty acid profiles and improving meat quality. Sañudo et al. [8] compared fatty acid composition between 56-day weaned and unweaned lambs, finding that unweaned lambs had higher contents of C12:0, C14:0, C15:0, C16:0, C16:1, and C18:3, but lower contents of C17:0, C18:0, C18:1, and C20:0 than the 56-day weaned group. After weaning, dietary fat sources change, and different fat sources affect muscle fatty acid composition. Supplementing various fat sources in diets can reduce saturated fatty acid (SFA) content while increasing monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents, particularly n-3 PUFA, elevating the PUFA/SFA ratio and decreasing the n-6/n-3 PUFA ratio, thus enhancing the nutritional value of ruminant meat products [9-10].

Different weaning times and age changes can alter rumen microbial flora, with most cellulolytic bacteria appearing after weaning and microbial diversity gradually increasing with age [11]. These shifts in rumen microbial populations significantly influence muscle fatty acid deposition [2]. Hu sheep, a Chinese indigenous breed, offers excellent meat quality and rapid growth [12]. However, few studies have examined how different weaning times affect fatty acid deposition in Hu lamb muscle. This experiment compared two weaning times (28 vs. 56 days of age) to investigate their effects on muscle fatty acid composition and rumen hydrogenating bacteria counts across growth stages, aiming to elucidate the process of fatty acid deposition and its underlying mechanisms, thereby providing a theoretical basis for regulating lamb muscle fatty acid composition through nutritional and management strategies.

1.1 Experimental Design and Diets

This study employed a single-factor design. Fifty-four newborn male Hu lambs with similar body weight [(3.51±0.57) kg] and good health were selected. After rearing to 28 days of age, six lambs were slaughtered. The remaining 48 lambs were divided according to homogeneity principles into a 28-day weaning group [(8.21±0.97) kg] and a 56-day weaning group [(8.06±0.53) kg], with 24 lambs per group. All experimental animals were supplemented with starter feed from 7 days of age and switched to fattening feed at 59 days of age with a 10-day transition period. Both groups were slaughtered at 42, 56, 70, and 84 days of age (six lambs per time point). All lambs were purchased from Jinchang Zhongtian Sheep Industry Co., Ltd. in Gansu Province.

The composition and nutrient levels of the starter feed are shown in Table 1, and those of the fattening feed in Table 2. The compression ratio for both diets was 1:6, with a pellet diameter of 2.5 mm.

1.2 Animal Management

Lambs were housed with their dams in the same pen, which contained creep feeders. From 08:00–10:00, 12:00–14:00, and 18:00–20:00 daily, lambs were confined in creep feeders to separate them from their dams, while during remaining hours they could suckle freely. When with their dams, lambs were prevented from consuming ewe feed but had access to creep feed and water ad libitum. Ewes were fed a conventional total mixed ration (TMR) consisting of corn silage (40%), oat hay (12%), alfalfa (10%), barley straw (8%), rapeseed straw (5%), bean dregs (13%), corn (9%), and soybean meal (3%), with nutrient levels of 7.38 MJ/kg digestible energy, 7.60% crude protein, 0.32% calcium, and 0.25% phosphorus. Pens were thoroughly disinfected every 15 days.

1.3 Sampling and Analysis

1.3.1 Sample Collection After slaughter, approximately 300 g of longissimus dorsi muscle was collected and stored at -20 °C for subsequent fatty acid analysis. The rumen was opened, and contents were homogenized, filtered through four layers of cheesecloth to obtain rumen fluid, which was then aliquoted into cryovials, placed in a liquid nitrogen tank, and stored at -80 °C until DNA extraction.

1.3.2 Muscle Fatty Acid Determination Sample Preparation: Total fat extraction and fatty acid methylation were performed following O' Fallon et al. [13]. One gram of semi-frozen sample was ground at room temperature for 10–15 s, then placed in a 16 mm × 125 mm screw-cap borosilicate tube containing 0.7 mL of 10 mol/L KOH solution and 6.3 mL of methanol. The tube was incubated in a 55 °C water bath for 1.5 h, vigorously shaken by hand for 5 s every 20 min to ensure proper permeation, dissolution, and hydrolysis. After cooling to room temperature in a tap water bath, 0.58 mL of 12 mol/L H₂SO₄ was added. The tube was mixed by inversion, producing K₂SO₄ precipitate, and incubated again at 55 °C for 1.5 h with vigorous shaking every 20 min. After fatty acid methyl esters (FAME) formed, the tube was cooled in tap water. Three milliliters of n-hexane was added, vortexed for 5 min, and centrifuged for 5 min. The n-hexane layer containing FAME was transferred to a GC vial, capped, and stored at -20 °C until analysis.

Gas Chromatography Analysis: FAME were analyzed using GC (Trace 1300, Thermo Fisher) with a 100 m × 0.25 mm × 0.2 μm capillary column (HP-88, Agilent). The temperature program was: initial 166 °C held for 39 min, increased to 230 °C at 10 °C/min and held for 10 min, then increased to 235 °C at 3 °C/min and held for 8 min. Injector temperature was 250 °C, flame ionization detector (FID) temperature was 250 °C, nitrogen carrier gas flow was 1.2 mL/min, and split ratio was 25:1. FAME standards were purchased from Sigma.

1.3.3 Rumen Hydrogenating Bacteria Quantification Real-time quantitative PCR (qRT-PCR) was used to determine the relative abundance of *Butyrivibrio fibrisolvens* and *Clostridium proteoclasticum* relative to total rumen bacteria.

DNA Extraction: Total genomic DNA from rumen fluid samples was extracted using a Stool DNA Kit (Omega) following the manufacturer's instructions.

PCR Primer Design: Primers for total rumen bacteria, *B. fibrisolvens*, and *C. proteoclasticum* were designed using Primer 5.0 and synthesized by Sangon Biotech (Shanghai). The sequences were: - Total bacteria: 5'-CGGCAACGAGCGCAACCC-3' (forward), 5'-CCATTGTAGCACGTGTGTAGCC-3' (reverse) - *B. fibrisolvens*: 5'-GCCTCAGCGTCAGTAATCG-3' (forward), 5'-GGAGCGTAGGCGGTTTTAC-3' (reverse) - *C. proteoclasticum*: 5'-TCCGGTGGTATGAGATGGGC-3' (forward), 5'-GTCGCTGCATCAGAGTTCC-3' (reverse)

qRT-PCR Reaction System and Conditions: Reactions were performed on a Bio-Rad CFX 96 system using SYBR Premix Ex Taq™ (Beijing TransGen Biotech) in a 20 µL volume containing 10 µL SYBR Green I Mix, 0.4 µL each of forward and reverse primers, 2 µL DNA template, and 7.2 µL double-distilled deionized water. Cycling conditions were: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, annealing at 60 °C (total bacteria)/57 °C (*B. fibrisolvens*)/55 °C (*C. proteoclasticum*) for 30 s, and 72 °C for 30 s with fluorescence acquisition; followed by melting curve analysis.

Calculation Formula: The abundance of the two rumen hydrogenating bacteria was expressed as a percentage relative to total rumen bacterial 16S rDNA using the formula:

$$\text{Target bacteria content (\%)} = 2^{-(\text{Ct}_{\text{target}} - \text{Ct}_{\text{total}})}$$

where Ct_target is the threshold cycle for target bacteria primers and Ct_total is the threshold cycle for total rumen bacteria primers.

1.4 Statistical Analysis

All data were initially organized in Excel 2010. SPSS 18.0 was used for one-way ANOVA to analyze age effects, with Duncan's multiple comparison test. Independent t-tests were used for inter-group comparisons between weaning treatments. Differences were considered significant at $P < 0.05$ and non-significant at $P > 0.05$.

Results

2.1 Effects of Weaning Time and Age on Longissimus Fatty Acid Composition

The effects are shown in Table 4. No significant differences in PUFA and SFA contents were observed between weaning groups at 28, 42, and 56 days of age ($P>0.05$). However, at 70 and 84 days, the 56-day weaning group had significantly higher PUFA and SFA contents than the 28-day weaning group ($P<0.05$). For odd-numbered fatty acids (OFA) and MUFA, the 56-day weaning group showed significantly lower contents at 42 days ($P<0.05$). OFA contents did not differ significantly at other ages ($P>0.05$), while MUFA contents were significantly higher in the 56-day weaning group at 70 and 84 days ($P<0.05$). The PUFA/SFA ratio showed no significant differences between groups at any age ($P>0.05$). The n-6/n-3 PUFA ratio was significantly lower in the 56-day weaning group at 70 days ($P<0.05$) but not at 84 days ($P>0.05$).

For individual fatty acids at 42 days, C18:0, C18:3n-6, and C18:3n-3 were significantly higher in the 56-day weaning group ($P<0.05$), while C16:0, C16:0anteiso, C18:1t11, C18:2n-6, C20:1, C20:3, C22:1, and C20:5n-3 were significantly lower ($P<0.05$). At 56 days, only C18:1t11 and C18:3n-3 were significantly lower in the 56-day weaning group ($P<0.05$). At 70 days, the 56-day weaning group had significantly higher contents of C10:0, C12:0, C14:0, C15:0, C16:0anteiso, C16:0, C17:0iso, C18:2n-6, C18:1c9, C20:3, C22:1, and C20:5n-3 ($P<0.05$), but lower contents of C17:0, C17:1, and C18:1t11 ($P<0.05$). At 84 days, C16:0, C17:0iso, C17:0, C18:0, C18:2n-6, C18:3n-3, and C22:1 were significantly higher in the 56-day weaning group ($P<0.05$), while C15:0 and C20:0 were significantly lower ($P<0.05$).

Age significantly affected fatty acid composition. In the 28-day weaning group, OFA content was significantly higher at 56 and 70 days than at 28 and 42 days ($P<0.05$). MUFA content was significantly higher at 42, 56, and 84 days than at 28 and 70 days ($P<0.05$). PUFA content was significantly higher at 56 days than at 28, 42, and 84 days ($P<0.05$). SFA content was significantly higher at 56 days than at other ages ($P<0.05$) and lowest at 28 days ($P<0.05$). The PUFA/SFA ratio was significantly higher at 28 days than at other ages ($P<0.05$). The n-6/n-3 PUFA ratio was significantly higher at 70 days and lower at 28 days compared to other ages ($P<0.05$).

In the 56-day weaning group, OFA content was significantly higher at 84 days and lower at 28 and 42 days compared to other ages ($P<0.05$). MUFA content was significantly higher at 70 days and lower at 28 and 42 days ($P<0.05$). PUFA and SFA contents were significantly higher at 70 and 84 days and lower at 28 and 42 days ($P<0.05$). The PUFA/SFA ratio was significantly higher at 28 days than at other ages ($P<0.05$). The n-6/n-3 PUFA ratio was significantly lower at 28 days than at other ages ($P<0.05$).

Definitions: OFA (odd-numbered fatty acids) = sum of C15:0, C17:0iso,

C17:0anteiso, C17:0, and C17:1. MUFA = sum of C17:1, C18:1t11, C18:1c9, C20:1, and C22:1. PUFA = sum of C18:2n-6, C18:3n-6, C20:3, and C20:5n-3. SFA = sum of C8:0, C10:0, C12:0, C14:0, C15:0, C16:0anteiso, C16:0, C17:0iso, C17:0anteiso, C17:0, C18:0, and C20:0. n-6 PUFA = sum of C18:2n-6 and C18:3n-6. n-3 PUFA = sum of C18:3n-3 and C20:5n-3.

Values in the same row with different superscripts differ significantly ($P < 0.05$); values in the same column marked with * differ significantly ($P < 0.05$).

2.2 Effects of Weaning Time and Age on Rumen Hydrogenating Bacteria

Effects on *B. fibrisolvens* and *C. proteoclasticum* are shown in Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper], respectively. At 70 days, *B. fibrisolvens* content was significantly higher in the 28-day weaning group ($P < 0.05$), with no significant differences at other ages ($P > 0.05$). *C. proteoclasticum* content did not differ significantly between groups at any age ($P > 0.05$).

Age affected rumen bacterial populations. In the 28-day weaning group, neither hydrogenating bacterium changed significantly with age ($P > 0.05$), though *B. fibrisolvens* peaked at 42 days and *C. proteoclasticum* peaked at 56 days. In the 56-day weaning group, *C. proteoclasticum* content showed no significant change with age ($P > 0.05$), peaking at 56 days, while *B. fibrisolvens* content was significantly higher at 42 days than at 70 and 84 days ($P < 0.05$).

Values within the same group with different letters differ significantly ($P < 0.05$); values at the same age marked with * differ significantly ($P < 0.05$).

Discussion

3.1 Nutrient Intake

In another study by our research group analyzing nutrient intake under identical conditions, statistical analysis was not performed due to group feeding with dams. From 1-28 days, dry matter intake was low and similar between groups (36.88 g/d vs. 40.01 g/d). From 29-70 days, intake was higher in the 28-day weaning group (205.05-643.62 g/d) than in the 56-day weaning group (152.59-569.03 g/d). From 71-84 days, intake was lower in the 28-day weaning group than in the 56-day weaning group (1,008.33 g/d vs. 1,156.41 g/d) [18].

3.2 Effects of Weaning Time and Age on Longissimus Fatty Acid Composition

Improving lamb meat quality aims to increase unsaturated fatty acids (UFA) while decreasing SFA proportion. In this study, the UFA proportion was higher in the 28-day weaning group. Chai et al. [14] compared 30- and 60-day weaning, finding higher UFA content at 90 days in the 30-day weaning group, consistent with our results. This may occur because early weaning at 28 days promotes solid feed intake, accelerating rumen microbial colonization [5]. Earlier establishment of rumen microbiota in young ruminants correlates with higher muscle UFA proportions [14]. No significant differences in PUFA/SFA ratio between groups at any age suggest weaning time does not affect this ratio. The 56-day weaning group had significantly lower n-6/n-3 PUFA ratio at 70 days (less than 5). Enser et al. found that dietary n-6/n-3 PUFA ratios below 5 may prevent certain cancers [15]. At 84 days, the 56-day weaning group showed 138.83%, 125.00%, 127.71%, 126.25%, 144.59%, 42.15%, and 28.39% higher contents of C16:0, C17:0iso, C17:0, C18:0, C18:2n-6, C18:3n-3, and C22:1, respectively, compared to the 28-day weaning group.

Fan [16] reported that high-energy milk replacer increased UFA proportion in lamb muscle compared to low-energy diets. Different weaning times directly affect nutrient composition intake, particularly energy level, which critically influences muscle fatty acid development. In this study, the 28-day weaning group had higher energy intake after weaning (3.13 MJ/d) than the 56-day group (2.32 MJ/d) [18], consistent with Fan's findings [16]. Dietary protein level, source, and forage-to-concentrate ratio also modify muscle fatty acid composition by affecting rumen microbiota [14]. Hadjipanayiotou et al. [17] found higher MUFA and PUFA contents in high-protein diets. In our study, the 28-day weaning group had higher MUFA and PUFA contents at 42 days, corresponding to higher crude protein intake (42.06 g/d) compared to the 56-day group (31.26 g/d) [18], supporting Hadjipanayiotou's results [17]. Demirel et al. [10] showed that low-concentrate diets increased PUFA proportion and decreased SFA proportion compared to high-concentrate diets. Post-weaning, diets shifted from milk plus starter to starter only, changing protein sources from milk to plant-based and altering protein levels, inevitably modifying rumen microbiota. Rumen microbes, especially hydrogenating bacteria, directly alter UFA hydrogenation pathways, changing muscle fatty acid composition [19].

Age also affected fatty acid composition. Li et al. [20] reported that increasing age elevates fatty acid content and diversity. In our study, C15:0, C17:0iso, and C17:0 were absent at 28 days. For the 28-day weaning group, these fatty acids appeared at 42 days, while for the 56-day weaning group, C17:0iso and C17:0 were not detected until 56 days. Both groups showed increasing fatty acid diversity with age, consistent with Li et al. [20]. Ruminant muscle contains odd-numbered and branched-chain fatty acids (OBCFA), absent in monogastric animals, due to rumen microbes. Fiber-degrading bacteria are rich in branched-chain fatty acids (e.g., C15:0iso, C17:0iso), while starch-degrading bacteria contain odd-

numbered branched-chain fatty acids (e.g., C15:0, C17:0) [21]. Earlier weaning at 28 days may have promoted earlier establishment of OBCFA-associated microbes, explaining earlier OBCFA detection. Additionally, MUFA, PUFA, and SFA contents at 84 days increased by 55.14%, 49.45%, and 287.48% compared to 28 days in the 28-day weaning group, and by 123.25%, 157.91%, and 770.95% in the 56-day weaning group, consistent with Li et al. [20]. This likely reflects progressive fatty acid deposition with age. The greater deposition in the 56-day weaning group suggests that rational dietary regulation becomes increasingly important as lambs age.

3.3 Effects of Weaning Time and Age on Rumen Hydrogenating Bacteria

Weaning time and age did not significantly affect *C. proteoclasticum* abundance, but at 70 days, *B. fibrisolvans* was significantly higher in the 28-day weaning group. Age also affected *B. fibrisolvans* in the 56-day weaning group. Zhao [19] observed minimal variation in *C. proteoclasticum* across treatments, consistent with our findings. Jami et al. [11] reported decreased *B. fibrisolvans* after weaning in calves. In our study, increased feed intake after weaning in the 56-day group (from 333.83 g/d to 569.03 g/d) [18] may have reduced rumen pH, inhibiting *B. fibrisolvans* growth and contributing to the difference observed at 70 days.

Weaning significantly impacts rumen microbiota [22], and dietary changes with age and intake variations also alter microbial populations [23-24]. Conjugated linoleic acid (CLA) and its precursor C18:1t11, beneficial for human health, are produced through incomplete hydrogenation of dietary PUFA in the rumen [24]. Two bacterial groups participate: Group A (primarily *Butyrivibrio* spp.) hydrogenates C18:3n-3 and C18:2n-6 to C18:1t11, while Group B (primarily *C. proteoclasticum*) hydrogenates C18:1t11 and C18:1c9 to C18:0 [26]. In our study, at 70 days, the 28-day weaning group had lower C18:3n-3 and C18:2n-6 contents but higher *B. fibrisolvans* abundance and higher C18:1t11 content than the 56-day weaning group, demonstrating that *B. fibrisolvans* influences muscle fatty acid composition through its hydrogenation pathway.

Conclusions

1. Most fatty acid contents in the longissimus muscle remained significantly higher in the 56-day weaning group than in the 28-day weaning group at 84 days, indicating that 28-day weaning is less conducive to fatty acid deposition.
2. With increasing age, most fatty acid species contents in the longissimus muscle gradually increased.

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