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Full Length Article

Effect of Replacing Corn Silage with Various Forage Silages in the Diet on Carcass Parameters, Meat Quality, Fatty Acid Profile and Amino Acid Composition of Beef Cattle

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Abstract

Carcass parameters, meat quality, fatty acid profile, and amino acid composition of Simmental bulls fed a diet based on various forage silages (VS) compared with the one based on only corn silage (CS) were investigated. A total of thirty male Simmental (440.5 ± 11.5 kg) was selected and assigned randomly divided into two treatments. All animals were fed twice daily (0700 and 1700 h) and water was supplied *ad libitum*, feed considering 5 to 10% refusals. The period of 207 days fattening trial was divided into three stages as P1 (1 to 64 days), P2 (65 to 130 days), P3 (131 to 207 days). Six beef cattle were slaughtered from each group at the end of the experiment. Substituting CS with VS in the finishing diet did not have a significant effect on slaughter performance, nutrient content, fatty acids, and amino acids profile ($P > 0.05$). However, the intramuscular fat and connective tissue content of the VS diet was lower compared with the CS diet ($P < 0.05$). Also, beef cattle fed VS diet could improve eye muscle area, increase histidine content and diameter of muscle fiber. In conclusion, substituting corn silage with various forage silages in the diet of beef cattle could potentially reduce the negative effect under the studied conditions.

Keywords: Amino acids; Carcass characteristics; Fatty acids; Histological properties; Silage; Total mixed ration

Introduction

The herbivorous animal husbandry industry is undergoing the initial stage of transformation from "low quality forage + high levels of concentrate" to "high quality forage + low levels of concentrate" mode in China. In the development of modern herbivorous animal husbandry, the focus has been to reduce feed costs, increase economic benefits, expand the use of forage resources, and improve forage storage technology. A variety of forages such as alfalfa, oats, corn and sweet sorghum forage have been widely planted for silage production, bringing forage resource advantages to herbivorous livestock. The high-quality forages instead of concentrate were encouraged in herbivore diets to overcome the shortcomings such as occurrence of metabolic diseases, high feed costs, low nutrient conversion efficiency and poor economic benefits caused by high-level concentrate diets (Schwaiger *et al.* 2013; Touno *et al.* 2013; Alstrup *et al.* 2016; Marques *et al.* 2016).

The needs of consumers for high-quality and locally raised grass-fed beef have been paid attention to (Marques *et al*. 2016). Previous studies have recommended beef cattle fed with high-quality forage and a small number of concentrate (Vieira and Fernández 2014) or use local by-products on the farm (Casasús *et al.* 2012) to improve the economic benefits of beef production and obtain quality meat.

The carcass and meat quality of ruminants is affected by many factors (Dannenberger *et al.* 2006), and of these, diet is likely the foremost. The previous studies reported that bulls could adapt to different feeding strategies without significant effect on meat quality (Manni *et al*. 2018). Also, Alfaia *et al.* (2009) considered that a high-forage diet had nutritional advantages for ruminants and conducive for the activity of cellulose-decomposing bacteria, which synthesized intermediate isomers, biohydrogenated intermediate isomers *trans*11-18:1 (*t*11-18:1) and *cis*9, *trans*11-18:2 (*c*9, *t*11-18:2) and n-3 polyunsaturated fatty acid (PUFA) of meat.

In this context, considering the importance of the cattle industry and forage utilization needs of good beef as well as the continuous search for efficient and low-cost feed method, we evaluated the effect of replacing corn silage with various forage silage on slaughter performance

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and beef muscle quality, providing scientific and effective guidance for quality safety, and disease control of beef cattle. We hypothesized that beef cattle fed a TMR with various combinations of silage and concentrate had no negative effects on carcass parameter and beef quality compared with the corn silage on the diet.

Materials and Methods

This study was performed on basis of the animal care and use protocol approved by the Institute of Ruminants of Lanzhou University (China).

Experimental site

The experiment was conducted from May 2017 to December 2017 in Dingxi City, Gansu Province, in China. The experimental site (35°07'34" N, 104°59'23" E, altitude 1899 m) belongs to the typical place of the semi-arid and hilly region of the Loess Plateau, with an average annual temperature range of 5.7 to 7.7°C, frost-free period of 122 to 160 days, with an average annual rainfall range of 300 mm to 400 mm. The growing season for forage is short, only 120 to 180 days of the year, and droughts are common. More than 3 million acres of artificial forage were planted as alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), oats (*Avena fatua* L.), and sorghum (*Sorghum bicolor* L.).

Experimental animals, diets, and design

The thirty male Simmental (440.5 ± 11.5 kg) were selected and randomly divided into two treatments, each group consisting of fifteen animals. A total mixed ration (TMR) with single corn silage + alfalfa hay + concentrate (CS), and TMR with various silage (corn silage + oat silage + alfalfa silage) + wheat straw + concentrate (VS) was used to feed the animals. The fifteen animals in each group were fed in a 100-m^2 housing unit, each animal was fed in an individual pen, and was allowed access only to its individual ration. All animals were fed twice daily (0700 and 1700 h) and water was supplied *ad libitum*, feed considering 5 to 10% refusals. The period of 207 days fattening trial was split into three stages as P1 (1 to 64 days), P2 (65 to 130 days), P3 (131 to 207 days), and the formulations were adjusted according to the nutritional requirements of beef cattle at different growth stages using the NRC guidelines (2000). During each fattening phase, the level of concentrates in the diets increased from 52 to 61.75%, and finally to 69.35% in the CS diet, and from 38 to 42%, and finally to 50% in the VS diet. Diet composition and nutritional levels are shown in Table 1 and 2.

Slaughter, carcass measurement, and sample collection

Six bulls from each group were weighed as live BW after 12 h fast and slaughtered by the Islamic Halal way within 12 h at the end of the experiment. The 12^{th} and 13^{th} ribs of the left carcass were selected to measure the eye muscle area (EMA) and backfat thickness (BFT). *Longissimus lumborum* (*LL*) was sampled from the left side of the carcass. Four steaks were collected from each *LL* sample, one for estimations of meat color and pH, the second piece for measurement of cooking losses and followed by Warner-Bratzler shear force (WBSF) determinations, the third piece for measurement of water loss rate in muscle, and the last piece for analysis of the routine composition, fatty acids, and amino acids. All TMR diets were sampled 4 times a month (once a week) and stored at -20°C until mixed in units of one fattening period. The content of fatty acids and amino acids were determined.

pH value and meat color measurements

The pH was determined at 45 min, 24 h, and 48 h postmortem, by inserting a portable pH meter (Testo 205, TestoAG, Schwarzwald, Germany) probe into the muscle. The meat color (L^* = lightness, a^* = redness, b^* = yellowness) was measured using a CM-2600d spectrophotometer (Minolta CR-400, Japan) at 45 min postmortem. Each meat sample was determined three times and the average value was taken as the final value of pH and meat color.

Warner-Bratzler shear force, water holding capacity measurements

The WBSF was determined using a meat tenderness tester (RH-N50, Nanjing Xiyi Instrument equipment Co. Ltd, China) following the method (Zhao *et al*. 2015) at 45 min postmortem. Briefly, the meat samples were heated in a constant temperature water bath at 85°C. The samples were taken out and cooled to room temperature after the central temperature reached 70°C. Then, the steak was cut into six cylindrical cores through a round sampler (diameter = 1.27 cm) for WBSF measurement, the value of WBSF was the average value after six measurements.

For the cooking loss analysis at 45 min postmortem, 300 g of muscle was cooked in 85°C water bath to a 70°C central temperature then the sample was taken out, blotted dry with filter paper, and weighed again. The value of the cooking loss was the percentage of weight change before and after cooking.

At 45 min postmortem, 3 × 3 × 1 cm meat slices were taken from the meat samples and weighed (W1). Then at room temperature, 18 layers of neutral filter paper were put on the top and bottom of the meat sample, and a 35 kg pressure was applied to the meat sample with a meat quality hydraulic tester (RH-1000, Chongqing Corod Technology Co., Ltd. China). After waiting for 5 min, the meat sample was taken out to weigh again (W2), and calculated according to the following formula:

$$\text{Water loss rate (\%)} = (W1-W2)/W1\times100\%.$$

Table 1: Composition and nutrient levels of experimental diets (DM basis)

Ingredients (DM/%)	CS			VS		
	P1	P2	P3	P1	P2	P3
Corn silage	40.00	33.50	26.80	27.00	30.00	30.00
Oats silage				15.00	14.00	10.00
Alfalfa silage				10.00	9.00	8.00
Alfalfa hay	8.00	4.75	3.85			
Wheat straw				10.00	5.00	2.00
Corn	31.00	40.10	46.95	20.00	23.00	30.00
Bran	9.00	6.00	6.00	9.00	6.00	6.00
Soybean meal	3.00	4.30	4.30	1.00	2.00	2.00
linseed meal	5.00	6.34	7.20	5.00	6.34	7.20
$NaHCO_3$	0.80	1.09	1.00	0.80	1.09	1.00
$CaHPO_4$	0.40	0.50	0.50	0.40	0.50	0.50
NaCl	0.20	0.30	0.30	0.20	0.30	0.30
Premix[1)]	2.50	3.10	3.10	2.50	3.10	3.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient level						
ME (MJ/kg)	13.34	14.07	14.46	13.32	14.07	14.45
CP (%)	12.89	13.18	13.80	12.86	13.16	13.78
Ca (%)	0.40	0.36	0.36	0.39	0.37	0.39
P (%)	0.41	0.35	0.38	0.36	0.38	0.39
Neutral detergent fiber (%)	32.77	27.62	24.08	38.90	29.30	25.45
Acid detergent fiber (%)	17.39	13.96	11.63	19.61	15.07	13.60

CS group: trench-style corn silage, VS group: wrapped corn silage

Premix provided the following per kg of the diet: VA 160 KIU, VD_3 50 KIU, VE 900 IU, VB_1 120 mg, nicotinic acid 500 mg, Fe 1200 mg, Cu 150 mg, Zn 1000 mg, Mn 500 mg

Table 2: Fatty acid and amino acid content of experimental finishing diets fed to beef cattle during different fattening phases

Parameter	P1		P2		P3	
	CS	VS	CS	VS	CS	VS
Fatty acid profile (g/kg) FAME[1)]						
C16:0	158.10	175.23	147.00	162.00	182.23	177.45
C18:0	110.80	77.30	121.20	80.34	118.00	95.56
C18:1	218.60	233.90	205.00	224.12	214.11	231.00
C18:2	528.00	522.00	524.11	516.34	509.00	518.70
C18:3	75.22	57.73	66.12	54.23	76.32	59.80
Amino acids (mg/g)						
Lysine	6.12	8.40	8.76	8.78	9.02	8.98
Arginine	5.72	9.67	10.45	10.12	10.44	10.89
Methionine	2.12	3.02	2.27	2.34	2.67	2.56
Threonine	5.02	5.24	5.78	5.68	6.01	6.12
Isoleucine	0.55	0.58	0.57	0.51	0.66	0.61
Leucine	1.01	0.81	0.82	0.79	1.00	0.96
Phenylalanine	0.35	0.52	0.49	0.51	0.65	0.76
Aspartic acid	10.70	9.67	10.23	10.26	11.23	11.21
Proline	9.89	10.01	9.45	9.59	8.87	8.79
Histidine	2.03	2.31	2.56	2.68	2.90	2.96
Cystine	0.72	0.58	0.45	0.54	0.67	0.78
Valine	4.27	4.34	3.98	4.06	3.86	3.69
Serine	3.12	3.04	3.67	3.36	3.78	3.56
Glutamate	11.20	11.56	11.03	11.23	10.76	10.91
Glycine	2.13	2.02	1.89	1.87	2.04	2.45
Alanine	1.23	1.34	1.45	1.61	1.56	1.76
Tyrosine	1.81	1.73	1.92	1.88	2.02	2.00

CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)

[1)] FAME = fatty acid methyl esters

Nutritional composition of beef muscle

The muscle fresh samples were brought back to the laboratory for freeze-drying and ground into a powdered form for the test. Protein, intramuscular fat (IMF), and ash contents of meat were analyzed following AOAC (1995) method. The analyses were performed in triplicate.

The fatty acids (FA) were extracted through the fatty acid methyl ester (FAME) synthesis modified version method of O'Fallon *et al.* (2007). A 0.5 g desiccated sample was put into a 10 mL glass tube with a stopper, 6.3 mL methanol solution (0.1 mol/L) and 0.7 mL KOH (10 mol/L) was added, and subjected to incubation in a water bath for 90 min at 55°C, the glass tube was shaken for 5 s every 20 min. After the water bath, the glass tube was taken out, cooled to room temperature, and then 0.58 mL H_2SO_4 (12 mol/L) was added. The glass tube was subjected to 55℃ for 90 min and shaken for 5 s every 20 min. After the second water bath, the glass tube was taken out, cooled to room temperature, then added 3 mL n-hexane, shaken, and transferred the solution to a centrifuge tube. Finally, centrifuged at 3000 r/min for 5 min, then filtered the supernatant into a sample bottle by the organic phase filter membrane, and placed at -20°C for GC detection. The internal standard C19:0 methyl ester (standard no. N-21M, Nu-Chek, USA) was added to n-hexane (1 g/L) in advance. The 37 FAME Standards (Supelco, USA) and mixture standard of conjugated linoleic acid (CLA) (Sigma-Aldrich Chemie, Germany) were used. The FA analysis was performed by Agilent technologies 6890N gas chromatograph (Thermo Scientific, TRACE, 1300, Milan, Italy). The chromatographic column model is HP-88 (100 m × 0.25 mm × 0.20 μm, Agilent Technologies, USA), compensation gas flow rate: 40.0 mL/min; flow rate of hydrogen: 35.0 mL/min; air flow rate: 35.0 mL/min; detector temperature: 250°C; inlet temperature: 250°C; split ratio: 15:1. FA analysis was an automatic sampler and reference fatty acid standard for retention time identification of individual FA.

The amino acids were determined by the hydrochloric acid hydrolysis method. The desiccated sample was weighed at 10 mg, and 10 mL HCl (6 mol/L) was added, then added 0.2 mL phenol solution, vacuumed and filled with nitrogen, hydrolyzed at 110°C for 24 h, and then filtered into 50 mL volumetric bottle. After vacuum drying, it was washed with a small amount of deionized water. After steaming, 1 mL HCl was added (0.02 mol/L) and put through a 0.22-μm filter membrane. Hitachi 835-50 amino acid automatic analyzer was used (Jiao *et al.* 2020). The ion-exchange chromatographic column (650–0042, MembraPure, Bodenheim, Germany) was used, where the amino acids were eluted by the natrium buffer system. After reacting with ninhydrin, the derivatives were tested at 570 nm.

Muscle histological properties

The three pieces muscle (1 × 1 × 1 cm) of each sample was fixed in 4% formalin and stained using the hematoxylin-eosin (HE) staining method to make sections (Wang *et al.* 2009). After the slices were prepared, it was observed with a

Table 3: Slaughter performance of beef cattle fed TMR with various forage silage (n = 6 per group)

Parameter	Groups	
	CS	VS
Slaughter BW[1)] (kg)	723.94 ± 14.73	730.50 ± 11.10
Carcass weight (kg)	415.18 ± 8.89	422.50 ± 6.99
Slaughter rate (%)	57.35 ± 0.69	57.84 ± 0.32
Backfat thickness (mm)	10.53 ± 0.96	10.55 ± 1.08
Eye muscle area (cm^2)	137.87 ± 16.55[b]	149.59 ± 4.10[a]

CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)
[1)] BW: body weight
[a, b] Means with different superscript letters in the same row differ from each other ($P < 0.05$)

Table 4: Effects of TMR with various forage silage on meat quality of *Longissimus lumborum* from fattening beef cattle (n = 6 per group)

Parameter		Groups	
		CS	VS
Meat color	L* (lightness)	30.95 ± 1.62	29.15 ± 0.73
	a* (redness)	7.54 ± 1.09	6.50 ± 0.46
	b* (yellowness)	9.20 ± 1.80	7.80 ± 0.51
pH	0 h	6.01 ± 0.11	5.93 ± 0.13
	24 h	5.57 ± 0.19	5.54 ± 0.22
	48 h	5.41 ± 0.13	5.48 ± 0.17
Water loss rate (%)		5.09 ± 0.56	5.89 ± 0.51
Cooked meat rate (%)		61.94 ± 0.26	62.83 ± 0.19
Shearing force (N)		58.86 ± 0.09	58.24 ± 0.15

CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)

Table 5: Effect of TMR with various forage silage on the chemical composition of muscle (*Longissimus lumborum*) from fattening beef cattle (n = 6 per group) (DM basis, %)

Parameter	Groups	
	CS	VS
Protein	76.84 ± 0.99	77.13 ± 0.89
Intramuscular fat	7.15 ± 0.49	6.14 ± 0.54
Ash	4.41 ± 0.04	4.34 ± 0.04

CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)

microscope (ScopeIm-age 9.0) at 4 × 10 magnification and stored for photos. For the measurement method of muscle fiber diameter, area, and density referenced to Wang *et al.* (2018). Briefly, three areas were selected randomly from each sample, and not less than 300 roots muscle fibers were observed, 30 roots muscle fibers were randomly measured for muscle fiber diameter and muscle fiber area. ScopeIm-age 9.0 was used to measure the long and short diameters of muscle fiber cross-sections, both the geometric mean was regarded as the muscle fibers diameter, and the muscle fibers area of the cross-section was directly measured. The number of muscle fibers in each visual field was measured, and a final, converted to the number of roots per square millimeter to serve as the muscle fiber density of the sample.

Statistical analysis

Statistical analysis was performed by independent sample *t*-test with S.P.S.S. (v. 19.00). The normality test is carried out before the test, and further analysis can be carried out after the normality is satisfied. Each animal was taken as the experimental unit, *P*-value of 0.05 as the significance criterion.

Results

Slaughter performance of beef cattle

As expected, the slaughter BW, carcass weight, slaughter rate, and BFT of the Simmental were similar between the two groups (Table 3). The EMA was significantly greater in the VS group (149.59 cm^2) compared with the CS group (137.87 cm^2) ($P < 0.05$) (Table 3).

Meat quality of beef cattle

Table 4 showed that neither treatment nor acid drainage time showed significant differences in muscle pH value. We found that the shear force values were not significantly different between the two groups. The type of diets did not affect the WHC of beef muscle. No difference was observed in the cooking loss of beef between the two groups in the present study. And more, the L* (lightness), a* (redness), and b* (yellow) chromaticity of meat were not influenced by diet composition.

Nutrient content, fatty acid profile, amino acid composition of the longissimus lumborum

Nutrient content: There were no significant differences in the protein, IMF, and ash content between the two groups ($P > 0.05$), however, the content of IMF was 6.14 and 7.15% in the VS group and CS group, respectively (Table 5).
Fatty acid profile: The diet treatment did not significantly affect the fatty acid composition of the muscle (Table 6). The main fatty acid included palmitic (C16:0), stearic (C18:0), and octadecenoic (C18:1n9c) acids, which together accounted for 75.27% of the total fatty acids. The main intermediate products of rumen biohydrogenation were *trans*-vaccenic acid (C18:1n9t), vaccenic acid (C18:1n9c), linolelaidic acid (C18:2n6), conjugated linoleic acids (CLA), and linolenic acid (C18:3n3), which accounted for 44.89 and 47.04% of the total fatty acids in the CS and VS groups respectively. The CLA which have great benefits for human health was 0.25 and 0.32% in the CS and VS groups respectively. Overall, the saturated fatty acid (SFA) was 47.41% in the CS vs. 45.44% in the VS group of the total fatty acids, the monounsaturated fatty acids (MUFA) were 39.40% in the CS *vs*. 40.02% in the VS group, and polyunsaturated fatty acids (PUFA) were 12.94% in the CS *vs*. 14.23% in the VS group. Within PUFA, the n-6: n-3 PUFA were 3.32 and 3.02 in the CS and VS group, respectively. Likewise, the ratio of P/S was 0.27 and 0.31 in the CS and VS group.
Amino acid composition: There were no significant

Table 6: Effect of TMR with various forage silage on the fatty acid profile (mg/g, DM basis) of muscle (*Longissimus lumborum*) from fattening beef cattle (n = 6 per group)

Parameter	Groups	
	CS	VS
∑Fatty acids (mg/g)	100.21 ± 2.56	92.79 ± 2.16
C10:0	0.64 ± 0.06	0.60 ± 0.07
C12:0	0.59 ± 0.05	0.48 ± 0.04
C14:0	2.22 ± 0.10	1.90 ± 0.09
C14:1	0.27 ± 0.01	0.29 ± 0.01
C15:0	0.34 ± 0.02	0.30 ± 0.02
C16:0	25.32 ± 0.15	22.77 ± 0.17
C16:1	3.14 ± 0.16	2.66 ± 0.14
C17:0	1.33 ± 0.10	1.13 ± 0.11
C17:1	0.65 ± 0.05	0.52 ± 0.04
C18:0	16.88 ± 0.16	14.90 ± 0.18
C18:1n9t	1.81 ± 0.12	1.67 ± 0.09
C18:1n9c	33.42 ± 0.26	31.99 ± 0.23
C18:2n6t	2.37 ± 0.11	2.11 ± 0.10
C18:2n6c	5.85 ± 0.07	5.64 ± 0.09
CLA	0.45 ± 0.06	0.30 ± 0.01
C18:3n3	1.91 ± 0.06	1.94 ± 0.08
C20:0	0.09 ± 0.01	0.08 ± 0.01
C20:1	0.12 ± 0.02	0.12 ± 0.02
C20:3n6	0.68 ± 0.03	0.61 ± 0.01
C20:4n6	1.74 ± 0.09	1.56 ± 0.12
C20:5n3	0.67 ± 0.03	0.65 ± 0.01
C22:5n3	0.71 ± 0.04	0.69 ± 0.02
∑SFA	47.41 ± 1.26	42.16 ± 1.16
∑MUFA	39.41 ± 0.76	37.13 ± 0.87
∑PUFA	12.94 ± 0.56	13.20 ± 0.56
n-6/n-3PUFA	3.32 ± 0.02	3.02 ± 0.06
P/S	0.27 ± 0.04	0.31 ± 0.03

CS group: TMR with single corn silage; VS group: TMR with various silage (corn silage, alfalfa silage, and oat silage).
CLA: *cis*-9. *trans*-11 conjugated linoleic acid
SFA: Saturated fatty acid
MUFA: Monounsaturated fatty acids
PUFA: Polyunsaturated fatty acids
P/S: Polyunsaturated fatty acids/ Saturated fatty acid

differences in the contents of amino acids, which are essential amino acid (EAA), nonessential amino acid (NEAA), delicious amino acid (DAA), and functional amino acid (FAA) between the two groups, except histidine ($P > 0.05$). The content of histidine in the VS group was significantly higher than that in the CS group ($P < 0.05$). The content of EAA was 41.54 and 42.62% in the CS group and VS group respectively. The ratio of EAA/NEAA in the two groups was about 0.91 (Table 7). Also, the content of TAA and the percentage of each amino acid in the VS group were higher than that in the CS group.

Muscle histological properties

The diameter of muscle fiber was greater for the VS group compared to the CS group ($P = 0.04$), and connective tissue content was lower ($P = 0.05$) (Table 8; Fig. 1).

Discussion

Carcass weight is an individual trait influenced by factors such as heredity, breeding method, live weight before slaughter, and market time (Petit 2005). The slaughter rate should be more than 55%, and was larger in beef cattle when the BW is more than 600 kg before slaughter, consistent with the results of this study. Back fat is an important parameter to reflect fat deposition, as a higher back fat was negatively related to the lean meat percentage. Lerch *et al.* (2015) found that the BFT of beef cattle was greatly affected by breed. In the present study, the experimental animals were Simmental; the BFT had no significant difference between the two groups. Besides, there is a positive correlation between EMA, as a growth index, and meat production performance, and lean meat percentage. In the present study, the EMA in the VS group was higher compared with the CS group; illustrating that TMR with various forage silage might be beneficial for increasing meat yield. It should be related to the VS group increased dry matter intake with an increase in roughage proportion compared with the CS group. Further studies should analyze the relationship between the forage ratio and meat yield.

Neither treatment nor acid drainage time showed significant differences in muscle pH, which was consistent with previous studies (Blanco *et al*. 2018), only a steady decrease in meat pH was observed in both groups. The final pH value (5.41 and 5.48 for CS group and VS group respectively) is range from 5.4 to 5.6 after slaughter 72 h within the normal range (Judge *et al.* 1988). In the present study, the shear force value (approximately 58.6 N) indicated that the meat not be called tender meat with a shear force value equal to or less than 40 N (Perry *et al.* 2001). Meat tenderness is affected by many factors, including diet, age, growth rate, and length of fattening period (Campo *et al*. 2008). Previous reports indicated that beef tenderness decrease with the length of the fattening time (French *et al*. 2000). Probably, the animals are fed for a relatively long period to obtain greater slaughter weight, may help explain greater shear force values in the present study.

The water holding capacity (WHC) of meat may be influenced by the rate and extent of pH descends (Oliveira *et al*. 2018). In the present study, the type of diets did not affect the WHC of beef muscle, possibly due to a lack of significant differences in the final pH of the meat. Additionally, there was a negative correlation between WHC and the water loss rate of meat. According to Luciano *et al.* (2009) and Schafer *et al.* (2002), the loss of water on the meat could decrease the heme content in the muscle, affecting the superficial color and weight of the meat, and reducing the economic benefit.

The lower the cooking loss, the less loss of protein and fat, and the higher the nutritional value of the muscle (McKenna *et al*. 2005). No difference was observed in the cooking loss of beef between the two groups in the present study. Similarly, Wales *et al.* (1998) and Dewhurst *et al.* (2009) also reported that, little evidence was known as diet composition influenced cooked meat percentage.

The meat color may be affected by diet, especially grass-fed (Priolo *et al*. 2001). Meat color is not only related

Table 7: Effect of TMR with various forage silage on the total amino acid profile of muscle (*Longissimus lumborum*) from fattening beef cattle (n = 6 per group) (DM basis)

Parameter	Groups	
	CS	VS
Essential		
Threonine (%)	4.41 ± 0.09	4.54 ± 0.04
Valine (%)	4.66 ± 0.08	4.76 ± 0.05
Methionine (%)	0.84 ± 0.05	0.78 ± 0.08
Phenylalanine (%)	3.75 ± 0.08	3.84 ± 0.03
Isoleucine (%)	4.49 ± 0.09	4.59 ± 0.04
Leucine (%)	7.82 ± 0.16	8.05 ± 0.07
Lysine (%)	5.60 ± 0.12	5.76 ± 0.05
Histidine (%)	3.88[b] ± 0.05	4.09[a] ± 0.04
Arginine (%)	6.10 ± 0.12	6.23 ± 0.07
Nonessential		
Aspartic acid (%)	8.93 ± 0.17	9.22 ± 0.09
Serine (%)	15.99 ± 0.40	16.40 ± 0.19
Glutamate (%)	3.65 ± 0.07	3.76 ± 0.04
Proline (%)	3.65 ± 0.07	3.68 ± 0.06
Glycine (%)	4.23 ± 0.06	4.31 ± 0.10
Alanine (%)	5.51 ± 0.09	5.63 ± 0.05
Tyrosine (%)	3.00 ± 0.05	3.09 ± 0.03
Cystine (%)	1.82 ± 0.03	1.85 ± 0.01
Total AA (mg/g)	868.10 ± 16.56	887.11 ± 9.12
Limited Amino Acids (%)	6.44 ± 0.13	6.53 ± 0.12
Essential amino acids (%)	41.54 ± 0.79	42.62 ± 0.43
Taste amino acids (%)	41.60 ± 0.83	42.56 ± 0.50
Functional amino acids (%)	29.91 ± 0.68	30.67 ± 0.33
EAA/NEAA	0.91 ± 0.04	0.91 ± 0.05

CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)
AA: amino acid;
EAA/NEAA: essential amino acid/nonessential amino acid
[a,b] Means with different superscript letters in the same row differ from each other ($P < 0.05$)

Table 8: Effects of histological characteristics of lion-eye muscle beef cattle fed TMR with forage silage (n = 6 per group) (4 × 10 μm)

Parameter	Groups	
	CS	VS
Diameter of muscle fiber (μm)	25.24[b] ± 0.56	30.00[a] ± 1.01
Area of muscle fiber (μm^2)	557.14 ± 2.06	812.79 ± 1.56
Density of muscle fiber (mm^{-2})	1109.10 ± 10.06	1070.43 ± 9.56
Connective tissue content (%)	41.21[a] ± 0.96	29.23[b] ± 1.16

CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)
[a,b] Means with different superscript letters in the same row differ from each other ($P < 0.05$)

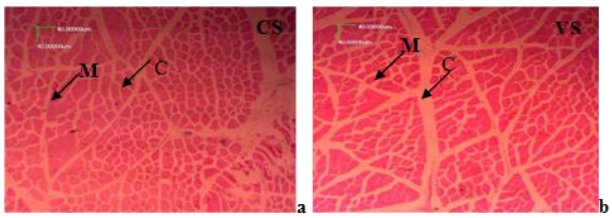

Fig. 1a, b: Histological characteristics of beef muscle
CS group: TMR with single corn silage; VS group: TMR with various silages (corn silage, alfalfa silage, and oat silage)
M: Muscle fibers
C: Connective tissue

to intramuscular fat content, muscle pH value, animal age, and carcass weight (Priolo *et al*. 2001), but also changes when beef is oxidized by air (Liu *et al*. 1995). In this study, the L* (lightness), a* (redness), and b* (yellow) chromaticity of meat were not influenced by diet composition. The study results are consistent with previous findings that forage itself had little effect on muscle color (Duckett *et al*. 2007, 2013). Our findings may also be due to the fact that most factors affecting meat color are not affected by dietary treatment.

Kobayashi *et al.* (2012) reported that IMF in the muscle was positively related to the level of dietary concentrate level. Similarly, the IMF content was higher in the muscle of cattle fed with concentrate than cattle fed with roughage (Dannenberger *et al*. 2004). Thus, differences in the ratio of concentrate could lead to differences in the IMF content.

The composition of fatty acid affects the health of meat, and is closely related to the flavor, tenderness, and juiciness of the meat (Fisher *et al*. 2000). The World Health Organization (WHO) (2003) indicates that the nutritional value of beef is positively correlated with the PUFA ratio, especially the n-3 PUFA ratio. Researchers generally believed that fatty acid composition was significantly affected by diet factors (Enser *et al*. 1998; Scollan *et al*. 2001; Dannenberger *et al*. 2004; Nuernberg *et al.* 2005). French *et al.* (2000) found that fatty acid composition in beef could be improved by adding forage in the diet. According to Yu *et al.* (1995); Wood *et al.* (2004), compared with concentrate-based feeding method, forage feeding could affect some meat quality indicators, including meat color, flavor, and fatty acid composition. Previous studies' results showed that the n-6: n-3 PUFAs were higher in some high-concentrate diets, while that of livestock mainly fed forage was only about 1.2 (Joy *et al*. 2014; French 2000). The n-6: n-3 PUFAs ratio of 4:1 is recommended by the WHO in a healthy diet (2003). The present study showed that the n-6: n-3 PUFAs of the two groups was 3.32 (CS) and 3.02 (VS) respectively, which were close to the recommended value. The WHO (2003) recommended that PUFA/SFA (P/S) should be higher than 0.40. In this experiment, the ratio of P/S (average 0.30) was lower than 0.40, which was still far from the reasonable nutritional recommendation value of 0.40. As has been also reported the generality of livestock products for the P/S lower than 0.40, which was necessary to devote ourselves to producing high-quality livestock products in the future.

The amino acid is the basic unit of protein, and important indicators for evaluating the nutritional value of protein, which directly affects the nutritional value of beef (Ludden and Kerley 1998; Gan *et al*. 2010). The EAA (lysine, threonine, leucine, isoleucine, valine, methionine, tryptophan, phenylalanine, *etc*.) are the basic indicators for assessing the bioavailability of proteins. They are important nutritional and physiological effects that can only be obtained from outside the body. Histidine and arginine are semi-essential amino acids in the human body, which cannot meet the needs of the human body, and need to be

taken from food, acting on the regulation of metabolism, dilated blood vessels, cut down blood pressure (O'Connor *et al*. 1993; Semba *et al*. 2016). The DAA includes glycine, glutamate, alanine, and aspartic acid, their content influences the degree of freshness to some extent (Li *et al*. 2001).

The content of histidine in the VS group was significantly higher than that in the CS group, indicating that increase with the content and type of silage forage in the diet could potentially reduce the negative effect. The content of EAA was 41.54 and 42.62% in the CS group and VS group, respectively, which are close to the ideal amino acid value (40%). The ratio of EAA/NEAA in the two groups was about 0.91, which was greater than the recommended value (approximately 0.6) of FAO (1973), indicated that the two groups diet from this study produces relatively nutritious meat. Thus, the content of amino acid in the VS group was more likely to approach or exceed the corresponding content of amino acid in the ideal protein, which may be beneficial to improve the utilization of protein in the human body, resulting from the overall interaction effect between nutrients in different feeds combinations.

Previous studies had shown that the histological characteristics of muscle fibers determined meat quality and were closely related to food quality traits (Zeng *et al*. 1999). The muscle fibers could be affected by the nutritional status, breed, age, and athletic ability of the animal. Guo and Li (2008) showed that muscle fibers were related to the shear force and tenderness of muscle. Also, the muscle fiber diameter of meat products was negatively correlated with muscle fiber density. There was a positive correlation between the diameter of muscle fibers and the slaughter rate and eye muscle area for the same species (Li *et al*. 2017). Zhao and Yang (2003) showed that muscle fiber area increased with an increase of the diet nutrient levels in pigs of the same weight. Also, Swatland (1997) found that the structural distribution and content of connective tissue are related to water loss and muscle succulence. Hence, the results of this study indicate that diets with various silage matchings, a small number of concentrates in beef cattle can improve muscle fiber, and thus meat quality.

Conclusion

Beef cattle can be fed TMR including corn silage, or a mixture of corn silage, alfalfa silage, and oat silage during the fattening stages with little difference in beef quality. A TMR based on various forage silages can improve eye muscle area of beef cattle, increase histidine content in muscle, and diameter of muscle fiber. Furthermore, connective tissue content of the VS group was lower compared with the CS group. In conclusion, substituting corn silage with various forage silages in the diet for beef cattle could be a feasible strategy to raise fattening beef cattle in the intensive feedlot system.

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Author Contributions

Hu-Cheng Wang conceptualized the work and provided laboratory facilities for analyzing and financial support; Xia Zhang collected and analyzed the data, and visualized the results; Xia Zhang and Mahaboubil-haq Muhaiden wrote and edited the paper.

Conflict of Interest

There is no conflict of interest among the authors and institutions where the research has been conducted

Data Availability Declaration

Primary and supplementary data reported in this article are available with the corresponding authors

Ethics Approval

This study involving animals was reviewed and approved by the Institutional Animal Care and Use Committee of the author(s). Animals were humanely sacrificed as necessary to ameliorate suffering. Procedures were performed in accordance with the ARRIVE guidelines available at: PLoS Bio 8(6), e1000412,2010 (authors are strongly encouraged to carefully read these guidelines)

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