



Effect of comfort conditions during steers fattening period on beef nutritional quality: fatty acid profile and healthy fat indices

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ABSTRACT. In intensive livestock farming is important that cattle have high space allowance and continuous availability of food to improve animal welfare. It is possible that such conditions may improve fat quality. The aim of this work was to evaluate the impact of concentrate diets supply alternatives and space allowances in beef finishing on meat nutritional quality through fatty acid profile analysis and healthy fat indices determination. Forty-eight Hereford steers aged 7.9 months and 175.0 ± 19.8 Kg body weight (BW) were allocated into four treatments in a 2×2 factorial arrangement: 100BF (food supply in feed bunks and 100 m² animal⁻¹), 100SF (food supply in self-feeders and 100 m² animal⁻¹), 10BF (food supply in feed bunks and 10 m² animal⁻¹), 10SF (food supply in self-feeders and 10 m² animal⁻¹). The fatty acid profile was measured and the healthy fat indices - atherogenic index (AI), thrombogenic index (TI), hypocholesterolemic/Hypercholesterolemic fatty acid ratio (h/H) and healthy fatty index (HFI)- were obtained. Treatments with self-feeders showed better AI, TI, h/H and HFI compared to groups with daily supply (0.5 vs. 0.6; 1.5 vs. 1.7; 1.6 vs. 1.4 and 1.6 vs. 1.7 respectively). In conclusion, animal production under comfortable conditions can contribute to the nutritional quality of meat.

Keywords: animal welfare; carcass; fat quality; feedlot; meat quality.

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Introduction

The globalization of the world and the trade policies of countries drive the evolution and dynamics of international meat markets. Moreover, consumer demands put pressure on production conditions and industrialization processes. Currently, there is an increasing preference for organic, eco-friendly, antibiotic-free meat products originating from production systems that minimize environmental impact and prioritize animal welfare (Organisation for Economic Co-operation and Development, & Food and Agriculture Organization, 2017).

Nalon et al. (2021) showed that the three most relevant themes in research since 1990 have to do with calf behavior and management, efficiency, and environmental sustainability, and the effects of transport and slaughter on meat quality. Research is also increasingly assessing aspects of beef cattle welfare, which are closely linked to meat quality, public health and environmental sustainability. While animal-source foods generally produce more greenhouse gas emissions per kilocalorie compared to plant-based foods, they also provide higher levels of several essential nutrients in bioavailable forms per kilocalorie, such as iron, calcium, and vitamin B12 (Place & Myrdal Miller, 2020). It is important to note that China, the world's second-largest beef consumer, will register a further 0.8 Kg year⁻¹ increase in its per capita consumption by 2032 (Food and Agriculture Organization, 2023).

Beef producing cattle are typically reared extensively during the early stages of life and are sometimes transferred to intensive systems during the fattening and finishing stages. Beef cattle finishing involves confining animals to pens and feeding them concentrates. In Argentina, beef farming has partially shifted from traditional extensive fattening systems towards intensive systems, following the global trend toward intensifying animal production. This transformation has led to a growing prevalence of feedlots. Argentine feedlots house larger groups (up to 200–250 animals/pen) with a higher space allocation per animal (10–20 m²/animal). Furthermore, these pens are based on a compact soil floor (Racciatti et al., 2022).

Feedlot systems increase efficiency, reduce pressure on pasture areas, minimize variability in final product characteristics and improve carcass quality among other things (Rodrigues Queiroz et al., 2022). However, few studies in South America have evaluated different comfort conditions during the fattening period and their potential impact on meat quality.

Feeding frequency and space allowance in fattening feedlots could affect animal welfare. Self-feeders can be used to increase feed intake frequency in larger lots with a more comfortable housing environment. In the present research, the authors propose evaluating a system where animals are fattened in a non-confined environment. The system is characterized by a space allocation of 100 m² per animal in sloping regions to reduce mud formation and ensure that animals have a dry resting area. The necessary infrastructure includes pickets built with electric fencing, mobile drinking troughs and self-feeders (Vittone et al., 2015). Thus, the aim of this work was to evaluate the impact of concentrate diets supply alternatives and space allowances in beef finishing on meat nutritional quality through fatty acid profile analysis and healthy fat indices determination.

Material and methods

Animals and experimental design

This research was carried out at the INTA Agricultural Experimental Station, located in Concepción del Uruguay, Entre Ríos, Argentina (32°48'S, 58°34'W). Forty-eight Hereford steers aged 7.4 to 8.7 months and 175.0 ± 19.8 Kg body weight (BW) were allocated into four treatments in a 2×2 factorial arrangement: 100FB (food supply in feed bunks and 100 m² per animal), 100SF (food supply in self-feeders and 100 m² per animal), 10FB (food supply in feed bunks and 10 m² per animal), 10SF (food supply in self-feeders and 10 m² per animal). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the INTA Institutional Animal Care and Use Committee (N° 11).

Diets and supply alternatives

In all treatments, 3.2% of the body weight (BW) was assigned to a diet formulated with 77% whole maize grain, 20% ground maize grain, 2% slow-release urea and 1% mineral premix, according to the mode of food supply (food supply in feed bunks or food supply in self-feeders). When the animals reached 280 Kg BW, slow-release urea was included at 1.5% to achieve a diet with 11% crude protein. In all cases, animals had access to clean drinking water suitable for consumption. Additionally, a mixture of NaCl and CaSO₄ was supplied with permanent availability.

Slaughter

The animals were slaughtered when they reached 370 – 390 Kg of BW. Final weight, backfat thickness and steak eye area were recorded prior to slaughter. Animals were slaughtered in a slaughterhouse officially authorized and controlled by SENASA, (National Control Service for Animal Sanitary Status).

Half-carasses were kept in cold chambers for 24h (internal temperature <5°C) and transported to a commercial packing house. Sections of 2.5 cm thick striploin (mainly *Longissimus dorsi* muscle), taken at 12th rib, were stored under frozen conditions until fatty acid profile analysis.

Fatty acid profile

Fatty acid methyl ester (FAME) was determined according to (O'Fallon et al., 2007). The FAME were separated by gas-chromatography using a PerkinElmer Clarus 680 model equipped with a flame ionization detector and a CombiPal automatic injection system using a HP-88 for FAME capillary column (100 m x 0.25 mm i.d., 0.25 m film thickness). The FAME preparation was injected in the split mode with a split ratio of 1:65. Nitrogen was used as the carrier gas with a 1.0 mL min.⁻¹ flow rate. Injector and detector were kept at constant temperatures of 250 and 270°C, respectively. The column oven temperature was programmed as follows: an initial increase at 4°C min.⁻¹ from 80 to 220°C, held for 5 min., then increased at 2°C min.⁻¹ to 240°C, and held for 10 min. Identification was achieved by comparing the retention time of unknown FAME with those of known FAME standard mix (37 FAME, Supelco Inc., Bellefonte, PA, United States). Conjugated linoleic acid (CLA) was identified using a standard mixture of *cis*- and *trans*-9,11- and -10,12-octadecadienoic acid methyl esters (O5632 Sigma). Quantitative analysis of FAME was performed using undecanoic acid as an internal standard.

Health indices

From the fatty acid profile, indices were determined to define the quality of the intramuscular fat of the *L. dorsi* according to:

PUFA/SFA = (Σ Polyunsaturated Fatty Acids/ Σ Saturated Fatty Acids)

ω - 6/ ω - 3 Ratio = (C18:2 ω -6 + C18:3 ω -6 + C20:2 ω -6 + C20:3 ω -6 + C20:4 ω -6 + C22:4 ω -6)/(C18:3 ω -3 + C20:5 ω -3) (Simopoulos, 2008).

Atherogenic index (AI) = (C12:0 + 4 × C 14:0 + C 16:0)/Σ UFA (Ulbricht & Southgate, 1991).

Thrombogenic index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5 × ΣMUFA) + (0.5 × Σω-6) + (3 × Σω-3) + (Σω-3/Σω-6)] (Samara et al., 2024).

Hypocholesterolemia/Hypercholesterolemic fatty acid ratio (h/H) = [(C 18:1ω-9 + C 18:1ω-7 + C 18:2ω-6 + C 18:3ω-6 + C 18:3ω-3 + C 20:3ω-6 + C 20:4ω-6 + C 20:5ω-3 + C 22:ω-6 + C 22:5ω-3 + C 22:6ω-3)/(C 14:0 + C 16:0)] (Samara et al., 2024)

Lipid Quality Index (LQI) = 100 × (C20:5 n-3)/(ΣSFA) (Dal Bosco et al., 2022a).

Healthy Fatty Index (HFI) = ((mg 100g⁻¹ of MUFA × 2) + (mg 100g⁻¹ of ω-6 × 4) + (mg 100g⁻¹ of ω-3 × 8) + (mg 100g⁻¹ ω-3 mg⁻¹ 100g⁻¹ of ω-6))/((mg 100g⁻¹ of SFA) + (mg 100g⁻¹ of MUFA × 0.5) + (mg 100g⁻¹ of ω-6 × 0.25) + (mg 100g⁻¹ of ω-3 × 0.125) + (mg 100g⁻¹ ω-6 mg⁻¹ 100g⁻¹ of ω-3)) (Dal Bosco et al., 2022a).

Statistical analysis

Statistical analysis was performed using the Infostat software (4). The model includes the effect of two housing areas, two feeding models and the interactions between them. The model also included the effect of time on data collection. The steers were considered as experimental units (n= 12). When interaction or main effects were significant (p<0.05), means were compared using the Tukey test. In addition, tendency at 0.05 < p < 0. were evaluated.

Results and discussion

The results show that there were no significant differences in the total saturated fatty acid (SFA) content of meat from among treatments studied (Table 1). However, lauric fatty acid (C12:0) was significantly higher in LD muscle from treatments with more space available.

Table 1. Effect of supply alternatives and space allowances on fatty acid profile (mg 100 g⁻¹ muscle) of the *Longissimus dorsi* muscle of beef cattle.

	Treatments				SEM	p-Value		
	100FB	100SF	10FB	10SF		FS	Space	FSxS
C12:0	3.2 a	2.9 a	2.2 b	1.7 b	0.3	0.6255	0.0387	0.8960
C14:0	24.6	22.9	22.7	21.8	10.9	0.8633	0.5949	0.5487
C15:0	25.8	37.57	29.05	25.8	3.2	0.6359	0.8484	0.3567
C16:0	881.7	1178.6	956.6	864.1	94.1	0.6038	0.5434	0.3269
C17:0	63.6	97.0	84.5	72.8	8.5	0.5573	0.8135	0.2965
C18:0	510.0	559.3	553.9	471.3	48.4	0.8732	0.8333	0.5306
C14:1	24.6	22.9	22.7	21.8	2.3	0.7976	0.7785	0.9331
C16:1	115.1	106.8	108.9	112.3	9.4	0.9063	0.9886	0.7790
C17:1	45.7	79.6	56.2	56.8	6.4	0.2417	0.9989	0.2548
C18:1ω-9t	76.6	116.3	153.5	109.4	15.0	0.8468	0.2389	0.3249
C18:1ω-9c	1203.6	1133.8	1351.0	1269.11	104.0	0.7403	0.5380	0.9789
C18:1ω-7c	48.8	50.1	52.0	51.2	3.7	0.9722	0.7936	0.8975
C18:2ω-6c	122.5	157.8	150.2	161.2	11.6	0.3427	0.5230	0.6167
C18:3ω-6	1.0	0.7	0.7	0.8	0.0	0.6086	0.4790	0.2287
C18:3ω-3	4.8 a	7.7 b	5.6 ab	4.4 a	0.6	0.9125	0.5940	0.0399
CLA c9, t11	6.6	9.3	7.7	5.4	1.1	0.6883	0.6352	0.5061
C20:0	1.8	1.9	1.7	1.5	0.2	0.7203	0.4904	0.6860
C20:1	1.7	2.9	2.8	1.9	0.3	0.8238	0.8773	0.0534
C20:2ω-6	0.6 a	1.0 b	1.1 b	0.5 a	0.1	0.7144	0.9420	0.0237
C20:3ω-6	8.9	10.5	6.8	9.0	0.6	0.1018	0.1207	0.7909
C20:4ω-6	42.0	42.5	31.8	46.2	2.5	0.1266	0.4950	0.1525
C20:5ω-3 EPA	3.1	3.8	2.2	2.4	0.3	0.4602	0.0947	0.7100
C22:4ω-6	4.2	5.1	3.3	4.2	0.3	0.1903	0.2299	0.9586
Σ SFA	1596.5	2139.2	1739.6	1530.6	172.6	0.6433	0.5194	0.3022
Σ MUFA	1515.7	1053.6	1747.1	1622.6	192.5	0.4540	0.9115	0.3415
Σ PUFA	193.5	235.6	209.2	234.1	15.3	0.2546	0.9137	0.7040
Σ FA	3305.7	4745.1	3695.89	3387.37	376.6	0.5656	0.8962	0.4061
Σω-6 PUFA	179.1	217.7	193.8	221.9	14.2	0.2685	0.7504	0.8608
Σω-3 PUFA	7.8	13.3	7.7	6.8	1.0	0.1907	0.0663	0.0734
ω-6/ω-3	24.9	17.3	40.2	39.4	5.1	0.6762	0.0752	0.7361
PUFA/SFA	0.14	0.13	0.12	0.15	0.0	0.2310	0.7803	0.1267

a, b: mean values represented by different letters in the rows indicate statistical differences detected by the Tukey test (p<0.05). 100FB: food supply in feed bunks and 100 m² per animal, 100SF: food supply in self-feeders and 100 m² per animal, 10FB: food supply in feed bunks and 10 m² per animal, 10SF: food supply in self-feeders and 10 m² per animal. SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

The total unsaturated fatty acid (MUFA and PUFA) content was not affected by the space and feeding system studied. However, C18:3 ω -3 content was significantly higher in the 100SF treatment compared to 100FB and 10SF. A trend was observed for C20:1 fatty acid content, which tended to be higher in the 100SF and 10FB associated with the interaction of the main factors. A similar trend was observed in C20:2 ω -6, which was significantly higher for the same treatments (100SF and 10FB). The C20:5 ω -3 EPA showed a trend of higher content in the *LD* muscle of animals housed in 100 m² compared to those housed in 10 m².

Although total polyunsaturated fatty acid (PUFA) content was not significantly different between treatments, it was observed that, the $\Sigma\omega$ -3 PUFA tended to be higher in the *LD* muscle from 100SF treatment. Consequently, this treatment also exhibited a tendency toward to lower ω -6/ ω -3 index compared to the confined groups.

The qualitative and nutritional indices obtained from the fatty acid composition were significantly affected by the feeding system, with the best indices observed in the 100SF treatment (Table 2). Treatments with self-feeders had better AI, TI, h/H and HFI regarding the groups with daily supply (0.5 vs. 0.6; 1.5 vs. 1.7; 1.6 vs. 1.4 and 1.6 vs. 1.7 respectively), while more space allowance tended in better LQI (2.6 vs. 1.6-2.0).

Table 2. Effect of supply alternatives and space allowances on health lipid indices of the *Longissimus dorsi* muscle of beef cattle.

	Treatments				SEM	p-Value		
	100FB	100SF	10FB	10SF		FS	Space	FXS
AI	0.57 a	0.51 b	0.55 ab	0.52 ab	0.0	0.0070	0.7658	0.3182
TI	1.70 a	1.49 b	1.64 ab	1.53 ab	0.0	0.0029	0.8170	0.3415
h/H	1.43 a	1.58 b	1.45 a	1.56 b	0.0	0.0104	0.6564	0.8738
LQI	0.54	0.54	0.33	0.45	0.0	0.5488	0.0823	0.3216
HFI	1.60 a	1.71 b	1.59 a	1.72 b	0.0	0.0067	0.9258	0.8505

a, b: mean values represented by different letters in the rows indicate statistical differences detected by the Tukey test ($p < 0.05$). 100FB: food supply in feed bunks and 100 m² per animal, 100SF: food supply in self-feeders and 100 m² per animal, 10FB: food supply in feed bunks and 10 m² per animal, 10SF: food supply in self-feeders and 10 m² per animal. AI: atherogenic index, TI: thrombogenic index, h/H: hypocholesterolemic/Hypercholesterolemic fatty acid ratio, LQI: lipid quality index, HFI: healthy Fatty Index.

Neville et al. (2020) noted that while comfort conditions may not impact performance during the fattening period, they can influence product quality. Ha et al. (2017) reported that increased space per animal in feedlots can improve animal welfare, since they can express their natural behavior, tend to increase their social behavior and decrease antagonistic events.

There is strong evidence that even-numbered saturated fatty acids (lauric, myristic, and palmitic) raise total and LDL cholesterol concentrations, and there is some evidence that these same fatty acids increase coagulation, inflammation, and insulin resistance. In accordance with these effects, a high exposure to saturated fatty acids, in particular, to lauric, myristic, and palmitic acids is associated with higher risk of coronary heart disease (CHD), cardiovascular disease (CVD), and type 2 diabetes (Calder, 2015). In this study, saturated fatty acid levels, except for lauric acid (C12:0), were not affected by housing space or feeding system.

Monounsaturated fatty acids (MUFAs) are recognized for their role in mitigating noncommunicable diseases, including cardiovascular disorders (Billingsley et al., 2018). Oleic acid (18:1 ω -9) is the most abundant *cis* MUFA in the human diet and, for many individuals, the most prevalent dietary fatty acid. Palmitoleic acid (C16:1 ω -7) is present in small amounts in animal fats. Significant proportions of oleic acid and smaller amounts of palmitoleic acid are found in many cell membrane phospholipids (Calder, 2015). A systematic review of 32 cohort studies demonstrated that higher oleic acid intake is associated with a reduced risk of CHD, cardiovascular events, and cardiovascular mortality (Schwingshackl & Hoffmann, 2014). Additionally, studies suggest a positive correlation between improved insulin sensitivity and higher levels of circulating 'free' palmitoleic acid in humans (Stefan et al., 2010). In this study, all treatments exhibited similar levels of oleic acid and palmitoleic acid.

Linoleic acid (C18:2 ω -6) is the most prevalent ω -6 PUFA in the human diet. It plays an important role in skin barrier function, while its derivative, arachidonic acid (C20:4 ω -6), is essential for brain development and function. One of the primary health benefits of linoleic acid is its ability to lower LDL cholesterol levels when it replaces major dietary saturated fatty acids, which is strongly associated with a reduced cardiovascular risk. Linoleic acid may also positively influence other cardiovascular risk factors (Calder, 2015). In this study, linoleic acid content tended to be higher in treatments with food supply in self-feeders, while arachidonic acid did not show a clear trend.

Omega-3 fatty acids have positive health effects in reducing the risk of several diseases, such as coronary heart disease and strokes, chronic inflammation, and several cancers. They are also important for the development and functionality of the brain and retina, as well as for reproductive functions (Akonjuen et al.,

2023). These fatty acids are not synthesized in the body and must be consumed directly from the diet. Major ω - 3 fatty acids include α -linolenic acid (ALA- C18:3 ω -3), eicosatetraenoic acid (EPA- C20:5 ω -3), and docosahexaenoic acid (DHA- C22:6 ω -3) (Harauma et al., 2023). In this study, the meat of animals housed in larger areas and fed through a self-feeders system showed the highest levels of ALA. And EPA content tended to be higher in treatments providing 100 m² per animal compared to those housing animals in 10 m².

In agreement with what was published by Dal Bosco et al. (2022a, 2024), in the present work, nutritional indices were determined to understand the effect of concentrate diet supply alternatives and space allowances in beef cattle finishing on meat nutritional quality.

The PUFA/SFA ratio is the most used index for evaluating the impact of a particular food on cardiovascular health, assuming that all PUFAs can reduce low-density lipoprotein cholesterol and serum cholesterol, whereas all SFAs can contribute to increasing serum cholesterol. Thus, this is a direct index: higher values indicate a better (positive) effect given by a certain meat or meat product intake (Dal Bosco et al., 2022). No changes in the PUFA/SFA ratio were evident due to the diet supply model and the animal housing area.

The ω -6/ ω -3 ratio has become a widely used method for evaluating the nutritional quality of foods. A lower ω -6/ ω -3 ratio is more desirable for reducing the risk of many chronic diseases prevalent in Western societies and developing countries (Timmis et al., 2022). The larger animal housing area and the self-feeders system showed the lowest ω -6/ ω -3 ratio.

The AI (Atherogenic Index) indicates the relationship between the sum of SFAs, excluding stearic acid (C18:0), which is not considered pro-atherogenic due to the human capacity to desaturate it to oleic acid (C18:1 ω -9). In contrast, lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids favor the adhesion of lipids to circulatory and immunological cells, promote the accumulation of atherogenic plaques, and reduce the levels of phospholipids and esterified fatty acids (Omri et al., 2019). The Thrombogenic Index (TI) characterizes the thrombogenic potential of fatty acids, separating them based on the effects triggered by some derivatives (eicosanoids) into pro-thrombogenic (C12:0, C14:0, and C16:0) and anti-thrombogenic FAs, such as MUFAs and ω -3 and ω -6 PUFAs. Foods or products with a lower TI or AI are beneficial for human health (indirect indices; Dal Bosco et al., 2022). The larger animal housing area and the self-feeders system showed the lowest TI and AI values.

The h/H ratio focuses on the relationships between dietary fatty acids and plasma low-density lipoproteins, relating to hypocholesterolemic fatty acids (C18:1 ω -9 and PUFA) and hypercholesterolemic fatty acids (C12:0, C14:0, C16:0). Foods or products with a higher h/H ratio are beneficial for human health. The larger animal housing area and the self-feeders system showed the highest h/H ratio.

The Healthy Fatty Index (HFI) differentiates the various classes of FA (by unsaturation and by the position of the double bonds) and considering the different classes of FA and their role in cardiovascular diseases (CVD). The rationale of this index is to underline recent knowledge on the nutritional and health value of some fatty acid classes regarding CVD onset, not only based on their biological and metabolic properties, but also considering their quantities weighted with multiplication coefficients. In the numerator are reported the FA classes multiplied by the relative positivity coefficients, while in the denominator, the FA classes are multiplied by fractional coefficients of negativity, except for the SFA, which are multiplied by 1, to indicate the maximum level of attention for the aforementioned diseases. The HFI, in contrast to the AI and TI, is a direct index because the higher values correspond to healthier foods (Dal Bosco et al., 2024). In the present study, the HFI is higher for meat from animals housed in larger areas with the self-feeders system.

The animals in this study had similar performance but different behavior patterns during the fattening period. Animals with more space allowance showed increased movement and resting behaviors. Even more, self-feeders contributed to a more uniform food intake throughout the day. Differences in behavior affected the *Longissimus dorsi* area, back fat thickness and carcass yield (Munilla et al., 2023). Dong-Hyun et al. (2018) found that walking activity can influence physical fitness and improve energy balance in Holstein cows. Furthermore, the sedentary lifestyle of ruminants consuming concentrated diets is indicative of poor health, and the fatty acid profile may be impacted by exercise levels (Zhang et al., 2022).

Conclusion

While there are numerous studies on the effects of diet types, slaughter weight, and crossbreeding on fat quality, there is little information regarding how comfort conditions influence the fatty acid profile and,

consequently, health indices. In this research, treatments with self-feeders showed better AI, TI, h/H and HFI and a higher space allowance tended to improve LQI. According to the results observed in the fatty acid profile and the indices, it could be inferred that the larger animal housing area with the self-feeder system promotes the production of meat with better healthy characteristics. In the future, it will be important to evaluate the additional comfort factors, such as thermal conditions and diets formulation even from the cattle breeding stage to improve the meat quality even more. Even more, it will be important to assess what happens with other breeds and other scales where the allocation of surface area in commercial lots and the size of the herd can also affect the results.

Data availability

Data will be made available on request.

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