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The Influence of Fattening by Eco–Feed Based on Okara on the Growth, Meat Quality and Histochemical Properties of the Longissimus Thoracis Muscle in Japanese Black Cattle

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The objective of this article is (1) to examine effects of eco–feed on histochemical properties in Longissimus thoracis muscle, (2) to identify the effectiveness of eco–feed utilization in composition of fattening concentrate and (3) to investigate quality of eco–feed beef taste. After 10 months reared, ten animals were divided into convention (steers, n=6) and eco–feed (steers, n=2; heifers, n=2) group, and fattened in similar feeding system based on numbers in each group. In eco–feed group, fattening concentrate was removed approximately 40 percent and replaced by eco–feed for slaughtered at age of 26 months and then all beef quality was evaluated. Samples from around 6 th thoracis in Longissimus muscle were performed in histochemical techniques. Quality of test was confirmed by the taste panel test from French and Italian restaurant in Fukuoka. The results showed that the body growth and quality of beef in both groups did not differ ($P<0.05$) in all categories. The composition of myofibre type I of steers (40%) in conventional group was larger than steers (25%) and heifers (29%) in eco–feed group ($P<0.01$). The size of myofibre type I had no significantly different for both steers and heifers between groups and the diameter type I, type ⅡA and type ⅡB in convention group was almost the same size, while in eco–feed the size of myofibre type ⅡA and ⅡB were larger than those in convention ($P<0.01$) group. The taste of eco–feed beef was extremely impressed by beef steak consumers at high confirmation level. As a result, eco–feed had no negative effect on the animal growth and its beef taste. The quality of beef of eco–feed were almost the same with common fattened; histochemical properties confirmed a slightly better in eco–feed animals in terms of beef quality grades, scores and levels.

INTRODUCTION

The longissimus thoracis muscle in the beef carcass indicates various factors for the classification of beef quality and grade. Zembayashi *et al*. (1994, 1995) have investigated many aspects of the Longissimus muscle in Japanese cattle including Japanese Black, Japanese Shorthorn, Holstein, and Japanese Cross–breed. Similarly, Gotoh (1994) and Iwamoto *et al*. (1991) evaluated groups of Japanese Black, Holstein and Japanese Brown, and Morita *et al*. (1999; 2000a,b) focused on the original Japanese cattle, Mishima. Quality grade determines the price of beef; based on the Japanese beef grading system, which was first launched in 1989 (JMGA, 1989), the quality grade is determined by the functioning of intramuscular fat in the Longissimus Thoracis muscle .

In addition, beef producers use many feeding techniques (e.g. feed composition and quantity) to produce well–marbled beef for the prime quality market in Japan. Zembayashi *et al*. (1987, 1994, 1995), Ozawa *et al*. (2000) and Gotoh (2003) confirmed that Japanese Black has advantages for both producers and consumers because of its capacity to produce high–fat content meat. Several studies have been carried out to identify factors that improve beef quality. Nishimura *et al*. (1998, 1999) examined the intramuscular connective tissue in aging beef, and that during the fattening period. The histochemical properties among beef breeds have been observed by Gotoh *et al*. (1994) and Iwamoto *et al*. (1991); myofiber types, diameter size and myofiber have been described for many types of muscle fiber and cattle breeds. Moreover, Zembayashi *et al*. (1987, 1994, 1995) have studied these properties in various cattle breeds, and have explored the nutritional composition within the Longissimus Thoracis muscle. Morita *et al*. (2000b) pointed out that the diameter of myofiber type I was shorter, and the density of myofiber type I higher, in Japanese Black cattle than for original Japanese cattle Mishima. Gotoh (2003) have reviewed research on the histochemical and myofiber distribution by gender, age and feeding system for different breeds of beef cattle.

Environmental issues have become a major concern worldwide. In Japan, many automobile and electronic companies have recently applied eco–care into their production systems, especially in the industrial sector. In addition, in the agricultural sector, eco–care has emerged over many years to tackle problems mainly related to food safety and energy sources. However, research covering aspects of the ecosystem is also being incorporated into many studies on agricultural products, not only by private companies, but also by public institutions. Research and development on livestock feeds has produced eco–feeds from food and brewery wastes for consumption by chickens, pigs and beef cattle. Japanese Black cattle have been used to verify the quality and effectiveness of eco–

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feed, and it is thought to have great benefits.

Animal biochemical research on the histochemical properties of skeletal muscle in Japanese Black cattle has provided evidence that beef quality is affected by changes in feed intake and composition, e.g., animals fed only on silage grass, fattening by hay, and grazing on pasture. Nevertheless, there is a paucity of information on beef quality for cattle fed or fattened on eco–feed; this includes experimental work on the characteristics of myofiber types of eco–fed beef cattle.

Tofu is an important part of Japan's food culture. In recent times, tofu has become a popular soybean–derived health food. The extremely high production mass of tofu results in large amounts of a highly nutritional byproduct called "Okara" remaining after production. Many companies treat Okara as a waste product. The Asian Environmental Institute created a system for the production of eco–feed made from Okara using a special enzyme made by Natto bacteria (*Bacillus natto*). We used this eco–feed for fattening the cattle in our study.

The purpose of the study was to examine the effects on body growth and carcass quality, and the histochemical properties of the Longissimus Thoracis muscle in beef cattle fattened by eco–feed during the fattening period. We also investigated the quality of the taste of beef produced using eco–feed.

MATERIALS AND METHODS

Animals, eco–feed and muscle samples

Young calves (n=10), 10 months of age, were used for the study. They were fattened for another 16 months, which is a standard period for beef cattle fattening in Japan. The calves were divided into 2 groups, a conventional fattening system (steers, n=6) control group (group C), and an eco–feed fattening system (steers, n=2 and heifers, n=2) treatment group (group E).

The "Okara" byproduct eco–feed used for fattening the cattle (see Table1) is produced from a special enzyme made by Natto bacteria (Asian Environmental Laboratory Co., Ltd); the eco–feed is under patent application. The steers in group C were fed a concentrate diet of Toyonokuni formula, under the typical local fattening standard, as described in the Oita Prefectural Livestock Institute beef fattening guide (Figure 1). At each stage, the animals in group E were fattened on the same quantity of the diet.

Table 1. Contents of eco-feed made from Okara

Item (%)	Enzyme treatment	
	Before	After
moisture	78.2	8.4
crude protein	6.3	25.1
crude fat	3.5	12.6
crude fiber	3.2	12.8
crude ash	1.1	3.9
nitrogen-free extract	7.7	37.2
phosphate	0.078	0.320
calcium	0.160	0.460
metabolic energy (kcal/100 g)	114	478

Eco–feed was replaced by some middle fattening formula and finishing formula at the following proportions: maximum of 15% from 14 months, 45% from 15 months, and up to 50% from 16 months until slaughter at 26 months.

All animals were taken to the slaughter house after the 16 month trial. Frozen beef cut (sirloin) was purchased from the slaughter house as a sample for histochemical analysis. Data was taken from the beef quality grade report that had been attached to the beef carcass at the slaughter house. All purchased beef cuts were stored in a fridge at 0–4 °C prior to sampling.

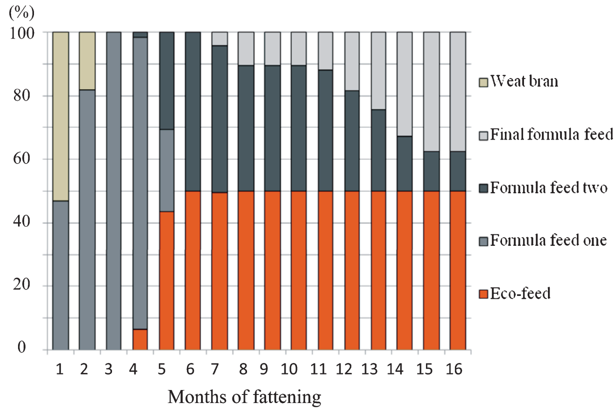

Fig. 1. Percentage of eco-feed composition in fattening intake during the 16 months fattening period (first fattening stage (11-15), middle fattening stage (16-22) and final fattening stage (23-26), approximately 40.2% in average of 16 months on trial).

Histochemistry

A muscle sample, around 1 cm^3 in volume size, was taken from the central part of the sirloin at the 6^{th} thoracis, in the Longissimus Thoracis muscle area. The sample was then frozen in liquid nitrogen and stored at –30 °C. The frozen sample was then cut transversely (i.e. standardized serial frozen section, thickness 8–10 μm) and stained by histochemical methods, which included the reduction of nicotine amide adenine dinucleotide dehydrogenase (NADH–DH) activities (Brooke and Kaiser, 1969), and histochemical reactions for myosin adenosine triphosphatase (ATPase) activities (Padykula and Herman, 1955) after acid (pH=4.3) preincubation (Brooke and Kaiser, 1969; Suzuki, 1976). Photographs (100×) were randomly taken at the same locations of three tissue samples stained with either NADH or ATPase. Myofiber type was divided into I, Ⅱ A and Ⅱ B, depending on the classification by Brooke and Kaiser (1969).

Myofiber composition and diameter

The percentage distribution of fiber types for all animals in both groups was calculated from the number of each myofiber type out of 500–700 myofibers from each tissue. The calculation was repeatedly performed until 500–700 myofibers were included. The fiber sizes were determined by measuring the diameter at the maximum width, perpendicular to the length axis, of 50 randomly selected fibers of each type (i.e. type I, ⅡA and ⅡB) from each tissue sample (Brooke, 1970).

Beef taste panel test

Using beef from both groups, rump cuts were selected for a taste panel test. The cuts were stored in a freezer and then refrigerated until needed. They were kept at a constant temperature (15 °C) prior to cooking. The beef panel taste test was hosted by an Italian and a French restaurant in Fukuoka. Steak was the main menu item, and it was prepared and cooked by professional chefs using the standard techniques and styles at each restaurant.

As panelists, sixty–eight guests of the French restaurant and thirty five guests of the Italian restaurant were asked to taste the beef steak. After the dishes were set on a table, the panelists were given a questionnaire and the objective of the panel test was explained. The questionnaire consisted of the panelist's age and gender, and various taste categories for the steak (texture, flavor, juiciness, tenderness, tastiness and overall palatability). After tasting the beef steaks from each of the two groups, the panelists were requested to grade the beef based on three categories: distasteful, acceptable and extremely delicious.

Statistical analysis

Means and standard errors were calculated for myofiber type composition and diameter for each animal, and for myofiber type from each group. The significance of the comparisons was assessed by t–test analysis, and the results from the beef taste panel test confirmed, using the SPSS (2007) software program (SPSS Inc.,).

RESULTS

Body growth and beef carcass quality

All animals in group E ingested the feed containing 50% eco–feed. The body growth and quality of the beef carcasses from animals in groups C and E from the start until the finish of the fattening period are shown in Table 2. The mean bodyweight of steers in group C (n=6) was 277.17 kg, and for steers and heifers in group E (n=4) it was 302.50 kg and 273.00 kg, respectively. The mean height of animals in group C was 109.45 cm (steers, n=6), and for steers and heifers in group E (n=4) it was 108.90 cm and 105.95 cm, respectively. The mean chest round for steers in group C was 149.33 cm, and for steers and heifers in group E it was 152.00 cm and 146.50 cm, respectively.

At slaughter age (i.e. finishing point), the body growth was similar in the two groups. The final mean weight of steers in group C was higher (640.33 kg) than that in the steers and heifers in group E (628.00 and 566.00 kg, respectively). For mean height, the results showed that steers in group E (138.70 cm) were taller than those in group C (134.05 cm), but steers in group C were taller than heifers in group E. The mean chest round showed the same trend as height (149.33 cm in group C, and 152.00 cm (steers) and 146.50 cm (heifers) in group E).

Table 2. Average of body growth and quality of beef carcass of animals in group C and group E from the start until finishing fattening period

Group	C	E	
10 months of age: starting point of fattening			
Gender	Steers	Steers	Heifers
Weight (kg)	277.17	302.50	273.00
Height (cm)	109.45	108.90	105.95
Chest gut (cm)	149.33	152.00	146.50
Slaughter age (26 months of age): finishing point of fattening			
Weight (kg)	640.33	628.00	566.00
Height (cm)	134.05	138.70	130.25
Chest round (cm)	206.83	212.50	204.00
Beef carcass quality after slaughter			
Carcass weight	392.98	392.75	345.95
BMS	3 to 4	4 to 5	3 to 4
BGL	B3-A4	B4-A3	B3-A2
BSC	4	4	4
BFS	3	3	3

BGL: Beef grade level; BMS: Beef marbling score; BSC: Beef standard color; BFS: Beef fat score. Data of body growth, carcass weight and beef marbling score are shown Means ± standard errors. [a,b] the means with the same superscripts do not significantly differ in each animal within fiber type at (P<0.05).

Table 3. Percentage of myofiber composition in the longissimus thoracis muscle between animals in convention and eco-feed group

Cattle no: (gender)	Percentage of composition		
	Type I (%)	Type IIA (%)	Type IIB (%)
Group C			
278 (♂)	24.2	19.4	56.4
279 (♂)	41.8	21.1	37.1
281 (♂)	27.7	14.4	57.9
283 (♂)	36.6	22.3	41.1
284 (♂)	33.6	17.5	48.9
285 (♂)	39.9	24.8	35.6
Combined	33.9 ± 6.9	19.9 ± 3.7[b]	46.2 ± 9.7
Group E			
295 (♂)	22.8	28.3	48.9
296 (♂)	27.2	31.4	41.5
Average	24.9	29.8	45.2
1117 (♀)	30.8	33.5	35.7
1119 (♀)	26.8	25.4	47.8 l
Average	28.8	29.4	41.8
Combined	26.9 ± 3.2	29.6 ± 3.1[a]	43.5 ± 6.1

All data present in Means ± standard errors. [a,b] data with the same superscripts significantly differ between groups of myofiber type at (P<0.05). However, the combined data in group E consisted of data from Steers and Heifers while data in group C is only steers.

[2] In Japanese beef grade (BGL) was defined as A, B and C, with A the highest out five levels from 1–5 representing the worst to best grades. BMS was rated from 1–12, with 1 the best and 7 the worst. BSC had a range from 1–7, with 1 the best, and 7 the poorest. BFC varied from 1–7, symbolizing bright white to yellow for the best and worst grades, respectively.

Carcass quality was based on the current beef grading system[2]. The data for all categories showed no difference between groups, except for carcass weight including that for females. Carcass weight was significantly higher in group C than in group E including that for heifers; the difference in the mean weight was 23 kg. However, the Beef Marbling Score (BMS), Beef Grade level (BGL), Beef Standard Color (BSC) and Beef Fat Score (BFS) between group C and group E were not significantly different (Table 3).

Histochemical fiber types

Myofibers were classified as myofiber type I or type II based on a negative test for alkaline ATPase activity and a positive test for acid–ATPase activity, respectively. Some myofibers show moderate or strong reactions after ATPase activity; these myofibers were classified as type I on the same basis as the NADH–DH activity (Figure 2). We carried out an analysis of acid–ATPase activity and NADH–DH activity because, in cattle, alkaline ATPase and acid ATPase activity show the relationship exactly. For NADH–DH activity, the strongest reacting myofibers were also categorized into myofiber type I, and the moderate and weakest reactions were categorized as myofiber type IIA and myofiber type IIB, respectively.

Myofiber type I was observed as a black spot, caused by formazan granules from NADH–DA activity, across the entire cross–sectional transverse area. Myofiber type IIA was identified by an unclear dot within the central area, which stretched around the corner of the granules. Myofiber type IIB was recognized by no or very few granules as a result of very weak NADH–DH activity. Type I myofibers were often distributed across the transversal section, with a connection to a minimum of 3 myofibers if they were gathered in a group. Likewise, single and groups of 2 type I myofibers occurred at low frequencies, and they were always surrounded by other fiber types.

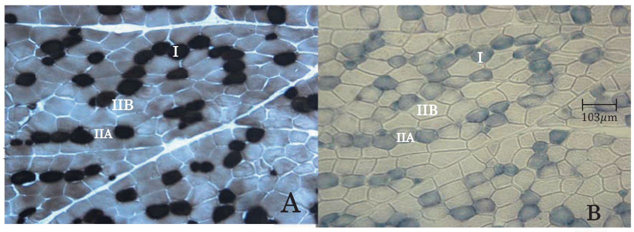

Fig. 2. Enzyme activities on the serial transverse sections (thickness: 10 μm per section) in longissimus thoracic muscle. ×100. Serials cross-sections were stained by reaction for alkaline-ATPase by preincubation activity at pH4.3 (A). NADH-dehydrogenase activity (B), as shown in location of type I, IIA and IIB. These indicate longissimus thoracis muscle of heifers in group E.

1.1 Myofiber type composition and diameter

All myofiber types were observed in the Longissimus Thoracis muscle from each animal in both group C and group E. The percentage of myofiber types in each animal in group E, including heifers, is shown in Table 3. The composition of myofiber type I in group C was 33.69±6.9%, and showed a higher density than that in steers and heifers in group E (steers, 24.9%; heifers, 28.8%; steers and heifers combined, 26.9±3.2%).

The myofiber types and diameters in the Longissimus Thoracis muscle from animals in group C and group E are shown in Table 4. The mean diameter of type I myofiber was slightly higher in group E (steers, 57.8 μm and heifers, 59.8 μm) than group C (54.9 μm). For myofiber types IIA and IIB, the size of the myofibers was larger in group E than group C. For the combined data, the mean diameter of type IIA myofibers was smaller (53.8±1.6 μm) in group C than group E (68.4±4.3 μm). For myofiber type IIB, the combined data had a mean diameter of 56.6±1.1 μm in group C and 75.9±3.5 μm in group E.

Table 4. Myofiber types and myofiber diameters in the longissimus thoracis muscle of animals in convention and eco-feed group

Cattle no: (gender)	Fiber diameter		
	Type I (μm)	Type IIA (μm)	Type IIB (μm)
Group C			
278 (♂)	58.9	54.9	57.7
279 (♂)	56.4	54.4	65.1
281 (♂)	55.8	53.2	58.5
283 (♂)	47.0	51.7	56.4
284 (♂)	56.8	52.4	57.1
285 (♂)	54.9	55.9	44.5
Combined	54.9 ± 1.3	53.8 ± 1.6[b]	56.6 ± 1.1[b]
Group E			
295 (♂)	58.4	63.7	70.8
296 (♂)	57.3	74.2	77.7
Average	57.8	68.9	74.3
1117 (♀)	56.5	67.9	76.3
1119 (♀)	63.2	67.7	78.7
Average	59.8	67.8	77.5
Combined	58.8 ± 3.0	68.4 ± 4.3[a]	75.9 ± 3.5[a]

All data present in Means ± standard errors. [a,b] data with the same superscripts significantly differ between groups of myofiber type at ($P<0.05$). However, the combined data in group E consisted of data from Steers and Heifers while data in group C is only steers.

Beef taste panel test

The results of the beef taste panel test are shown in Figure 3. Although about 10% of the panelists from the French restaurant were unimpressed with the texture of the beef steak from the eco–fed beef, the percentage of panelists who graded the beef as distasteful for flavor, tenderness, juiciness and tastiness was less than 5%. The panel graded the beef in the extremely delicious category for flavor, juiciness and tastiness at less than 30–45%, while the majority graded it as acceptable for flavor and juiciness. For texture and tenderness, most panelists enjoyed the taste and graded the beef as extremely delicious. For overall palatability, 60% of panelists graded the taste of eco–feed beef steak as extremely delicious, and 40% graded it as acceptable.

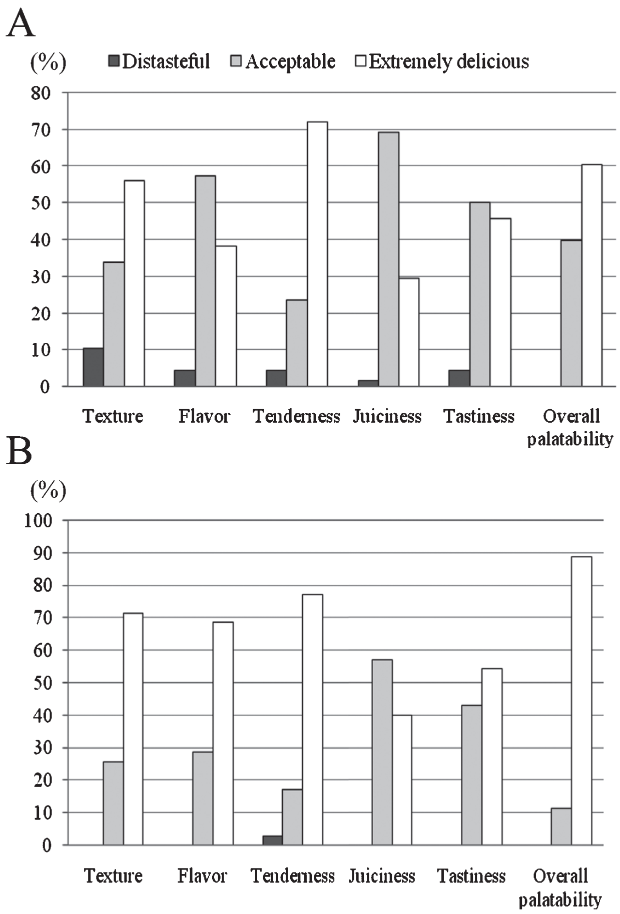

Fig. 3. The percentage of beef taste panel test in eco-feed beef. A: French restaurant. B: Italian restaurant in Fukuoka.

For the Italian restaurant, less than 30% of panelists graded the taste of eco–feed beef steak as acceptable for texture, flavor, tenderness and overall palatability. In contrast, 57% and 43% of panelists were not satisfied with juiciness and tastiness, respectively. However, over 68% of panelists graded the beef as extremely delicious for tenderness and juiciness. Finally, the results for overall palatability from the Italian restaurant showed that 89% of panelists graded the eco–feed beef steak as extremely delicious.

DISCUSSION

Positive aspects of byproduct feedstuffs

To date, there has been no specific research aimed at incorporating byproducts into the beef production system. Cattle at the Illinois State University farm were initially used for trialing feed, reproduced as an ensilage from recycled food waste and paper (Hoelting and Walker, 1994). Joshi and Sandhu (1996) reported that crude protein, produced as a byproduct from fermentation of apple pomace, was enriched 3–fold. In our research, crude protein from an eco–feed byproduct of tofu (Okara) was enriched 3–fold. This means that the eco–feed byproduct has considerable potential for the beef production system. The results for body growth, carcass weight, and quality grade proved that the eco–feed can be used as a feedstuff mixture to produce feeds of a quality similar to that used in conventional feeding systems.

In economic terms, eco–feed (Okara) provides options for profitable investment by beef production farmers. In 2008, the ASIA Environmental Laboratory, an eco–feed company, informed that the eco–feed (Okara) produced using a waste energy recycling system, would cost about ¥25–35 per kilogram on the local market. In contrast, imported feedstuffs are expensive at ¥80 per kilogram (local retailer's price in the third quarter of 2008). Hence, eco–feed has positive attributes as a feedstuff produced from food waste in the form of a byproduct. It is not only a high quality feed, but it is also a worthy feed product that can reduce the reliance of the beef production system on imported feedstuffs.

Panel test

We assessed consumer response to the quality of the taste of eco–feed beef using test panels in a French and an Italian restaurant; the restaurants offered different cuisine and cooking styles. The panelists reported a very positive response to the eco–feed beef steak at both restaurants. This indicates that eco–feed beef has a high quality taste. However, there was a small percentage of negative responses from the French restaurant; this may be because some of the panelists were at this restaurant for the first time, and they might not have been familiar with French food.

Function of myofiber type

Males and females from group E had less type I than type IIA myofiber. However, more investigations are needed by increasing the number of test animals. The percentage of myofiber type IIB may not be different between the two groups. For the steers, myofiber size in group C was relatively large for all myofiber types: $54.9\pm1.3\,\mu$m, $53.8\pm1.6\,\mu$m and $56.6\pm1.1\,\mu$m for myofiber type I, type IIA and type IIB, respectively. The sizes were larger for fibers from the steers ($57.8\,\mu$m, $68.9\,\mu$m and $76.3\,\mu$m for myofiber type I, type IIA and type IIB, respectively) and heifers ($59.8\,\mu$m, $67.8\,\mu$m and $77.5\,\mu$m for myofiber type I, type IIA and type IIB, respectively) in group E. To date, there is no research that indicates that byproduct affects the composition, size or the histochemical properties of the myofibers.

This study will provides new ideas and fundamental information for further investigating the positive or negative effects of eco–feed on the histochemical properties of beef. However, to draw strong conclusions, such a study will need to reconsider the experiment design, number of animals, the affects of gender and animal breed, and the percentage composition of eco–feed.

CONCLUSION

The benefits from the eco–feed byproduct are vital for the improvement of the beef product system. The maximum use of eco–feed was as high as 50%; at this level, the eco–feed did not delay developmental growth, or meat quality, as shown in the carcass reports, or destroy the tastiness of the beef. Therefore, further investment should be made into research to confirm the quality of eco–feed. The nutritional performance of eco–feed remains uncertain; it could lead to a change in the histochemical properties of beef cattle. Our study indicates that eco–feed may influence the composition and enlargement of myofiber types. Eco–feed is still under development; however, it has potential to be a profitable feedstuff option at a competitive retail price. It is environmental– friendly, and can reduce reliance on imported feed.

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