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Comparative Analysis of Nutrients and Mineral Element Contents in Muscles of *Pelodiscus sinensis* Raised in a Paddy Field and on a Factory Farm

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XZ and YW contributed in study design. LY performed the study and wrote the original draft. KH and QZ provided help in software. JW, RL and MW analyzed the data. All authors have contributed to the manuscript and approved the final version.

Key words
***Pelodiscus sinensis*, Nutrient components, Amino acids, Fatty acids, Mineral elements**

ABSTRACT

The aim of this study was to investigate the nutritional value of muscles of the soft-shell turtle, *Pelodiscus sinensis*, raised in a paddy field and on a factory farm. The study revealed that crude protein and moisture contents in the muscle tissue from turtles raised in a paddy field were considerably lower and considerably higher, respectively, compared to those from turtles raised on a factory farm ($p < 0.01$). Furthermore, 16 amino acids, 14 fatty acids, and 10 mineral elements were detected in the muscle tissues from the turtles of the two farming methods. The muscle tissues of both groups of turtles contained available protein sources. PUFAs ($p < 0.05$) and total unsaturated fatty acid contents, iron, and copper ($p < 0.01$) were significantly higher, whereas phosphorus levels were significantly lower ($p < 0.05$) in turtles raised in a paddy field than in those raised on a factory farm. This study provides fundamental information for development and utilisation of agricultural resources and turtle resources.

INTRODUCTION

The soft-shell turtle (*Pelodiscus sinensis*), a common species of turtle, has been extensively consumed as a delicacy for a long time because of its high nutritional value and unique flavour (Tang *et al*., 2019). Turtles have become an important freshwater species cultured in China according to statistics from the China Fishery Statistical Yearbook (2022), the annual output of farmed turtles was 364,878 tonnes in 2021, representing an increase of 9.70% than that in 2020. The farming methods of turtles include pond farming, with turtles raised as the dominant farmed species, pond polyculture, greenhouse farming, factory farming, and paddy field farming (Wang *et al*., 2019). Among these, pond and greenhouse faming, which focus on producing large-sized and juvenile turtles, respectively, are the primary farming methods for turtles. In factory farming, heating equipment (e.g., boiler) is generally employed to maintain a constant temperature, compound feed is provided, and waste water for farming is disposed of or reused. Consequently, the water temperature remains stable and is maintained at a range suitable for turtle growth. Thus, the turtles can grow at a steady rate year-round with a high reproductive output, resulting in high economic profits. Paddy field turtle farming, which has long been practiced, has been transformed into an aquaculture activity conducted in a green, healthy, and eco-friendly manner over recent years to utilise agricultural resources. In paddy field farming, turtle growth and development conditions are considerably improved, and metabolites excreted by the turtles can facilitate the growth of rice plants, thereby achieving a high rice yield and productive turtle output and increasing the agricultural value of paddy fields (Shen and Gou, 2013).

The nutritional components and heavy metal

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contents of these turtles can vary with different farming environments, modes, and cycles. Therefore, exploring the nutritional value of turtles remains a concern for consumers (Wang *et al*., 2020). Numerous studies on the nutrient components of the muscle tissues of turtles have been reported. For example, an analysis revealed that the muscle tissues of soft-shell turtles from Qingxi and Japanese are rich in nutritional components and are good protein source (Chen *et al*., 2015). Similarly, a muscle quality evaluation of soft-shell turtles from the Huaihe, Yellow River, and Japanese varieties established that the amino acid contents were high and relatively balanced in turtles of all three varieties (Yu *et al*., 2019). Further analysis detecting the nutritional value of turtles raised in different farming environments detected 32 fatty acids in the muscle tissues of wild soft-shell turtles; however, only 25 fatty acids were detected in turtles raised in a biotic environment; moreover, the polyunsaturated fatty acid (PUFA) content in wild soft-shell turtles was evidently higher than that in turtles raised in a biotic environment (Song *et al*., 2019). The results of nutrient component identification in the muscle tissues of turtles raised in different modes suggested that the total amino acid, monounsaturated fatty acid (MUFA), and PUFA contents in turtles raised in an ecological environment were higher than those in turtles raised in a pond or greenhouse (Wang *et al*., 2013). Another analysis of nutrient component content in male and female turtles raised in biotic and greenhouse environments revealed that the fat content in turtles raised in a biotic environment was higher than that in turtles raised in a greenhouse (He *et al*., 2013). In addition, a comparison of the nutrient component contents of female turtles showed that those raised in a greenhouse had higher amino acid and fatty acid contents and lower dry matter contents (particularly protein content) in the muscle tissues than those raised in polyculture in a crab pond (Long *et al*., 2018). It was recently detected the nutritional qualities of muscle tissues of turtles raised in a paddy field, a greenhouse, and a wild environment to compare nutrient components. The results showed that the nutritional qualities and volatile flavours of the muscles of turtles raised in a paddy field and wild environment were comparable or superior to those of turtles raised in a greenhouse (Wang *et al*., 2019). Regarding the detection of heavy metal and mineral element contents in two turtle varieties (Qingxi soft-shell turtles and soft-shell turtles of the Japanese variety), turtles raised in three farming environments (paddy field, greenhouse, and wild) and those raised using three farming modes (paddy field, pond with a micro-flowing water system, and common pond farming) were comparatively analysed; results suggested that common nutritional elements necessary for humans were abundant in the turtles (Wang *et al*., 2019; Chen *et al*., 2015). With increasing urbanisation and industrialisation, rice–turtle polyculture technologies and factory farming technologies have become increasingly sophisticated, and paddy field and factory farming have become the major farming modes of turtles. Nonetheless, comparative analyses of the nutritional qualities and flavours of muscle tissues and the mineral element contents of turtles raised in paddy fields or on factory farms have rarely been reported. Therefore, in this study, we compared the differences in nutritional quality and mineral element contents of the muscle tissues between turtles raised in a paddy field and those raised on a factory farm, thereby providing a reference basis for improving the muscle tissue quality of turtles in the future.

MATERIALS AND METHODS

Experimental materials

All soft-shell turtles (mean body weight: 610.90±49.15 g; farming time: September through December) were obtained from Huizhou Caixing Industry Co., Ltd. (Huizhou city, Guangdong Provinces, China). The paddy field covered an area of approximately 1,334 m^2, and 500 soft-shell turtles (Ages:18 months), raised in a greenhouse in the early stage, were stocked. The factory farm covered an area of 2,000 m^2, and the workshop had a height of 3.8 m, with an arched trellis structure; a pond covered an area of 180–200 m^2, with a water depth of 1.2 m. The water temperature was maintained at approximately 30 °C, and the stocking density was 70 turtles/m^2. In September through October, the dedicated compound feed (protein: 60%, starch: 20%, soybean meal: 10%; other components included multi-vitamins, amino acids, mineral elements, and yeast powder) was administered based on 0.3% of the average body weight for turtle cultured in paddy field, and the amount of compound feed provided daily was approximately 1.0% of the average body weight for turtle cultured in factory farm (protein: 60%, starch: 20%, soybean meal:10%; other components included multi-vitamins, amino acids, mineral elements, and yeast powder). During October through December, the dedicated compound feed was not administered. Muscle mass from the abdominal segments of five individuals which belongs to the same farm model was put together for one sample, with three repetitions for measurement in each aquaculture model. Approximately 50 g of muscle tissue was taken from each model and stored at −20 °C for later use.

Conventional analysis of nutrient components

The crude fat content of muscle tissue samples was determined through Soxhlet extraction (GB/T5009.6-2003) (2003) with a Soxtec 2055 Auto Fat Extraction System (Foss Tecator AB, Sweden). Crude protein content was determined using a Kjeltec 2400/2460 Auto Sampler System (Foss Tecator AB, Sweden; GB/T5009.5-2003) (2003). Moisture content was determined using the oven-drying method at atmospheric pressure (GB/T5009.3-2010) (2010) and ZRD-A780 automated blast air oven, and the ash content was determined using the high-temperature ashing method and a B180 muffle furnace (Nabertherm, Germany; GB/T5009.4-2010) (2010).

Analysis of amino acid and fatty acid compositions

Approximately 10 g of the frozen muscle sample from each turtle was lyophilised to a constant weight using a vacuum lyophiliser (OlaiBo, Model OLB-FD10P, China). After being pulverised, the samples were screened using an 80-mesh sieve. An appropriate amount of the lyophilised sample was weighed and hydrolysed with hydrochloric acids. Subsequently, the contents of 14 amino acids, except methionine (Met), were determined using an automated amino acid analyser (Hitachi, Model 835-50) (Chen *et al*., 2007). Met content was determined based on performic acid oxidation and hydrolysis as previously described (Splindler *et al*., 1984). Fatty acids were extracted via hydrolysis, and the fatty acid content was determined using a 7890A gas chromatograph (Agilent, United States) according to GB/T22223-2008 (2008). The amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) were calculated using the following formulas (Wang *et al*., 2014):

$$AAS = \frac{0.625 \times aa}{AA(FAO/WHO)}$$

$$CS = \frac{0.625 \times aa}{AA(egg)}$$

$$EAAI = \sqrt[n]{\frac{100A}{AE} x \frac{100B}{BE} x \frac{100C}{CE} \cdots x \frac{100G}{GE}}$$

where aa is the amount of amino acids per test protein (mg g^{-1} protein); AA (FAO/WHO) is the amount of amino acid per nitrogen based on the FAO/WHO reference pattern for adults (mg g^{-1} N); AA(egg) is the amount of amino acids per nitrogen in whole egg protein (mg g^{-1} N); n is the amount of amino acids; *A, B, C, G* are the amount of essential amino acids per test nitrogen (mg g^{-1} N); and *AE, BE, CE, ,GE* are the amount of essential amino acids per test nitrogen in whole egg protein (mg g^{-1} N).

Determination of mineral element contents

A total of 0.5 g of the muscle tissue sample from each turtle was weighed and placed in a 200 mL beaker with a watch glass. Subsequently, 12 mL of HNO_3 and 6 mL of $HClO_4$ were added, and the beaker was covered with the watch glass and placed on a temperature-controlled electric hot plate for heating and dissolution until approximately 2–3 mL of the solution was left. After observation, heating was discontinued if the solution was clear; otherwise, the beaker was taken off and allowed to cool, and then, 3 mL of $HClO_4$ solution was added for heating until the solution was completely clear. After cooling, the solution was transferred to a 25-mL volumetric flask to dilute it to a constant volume and mixed well for later use. The mineral and trace elements were detected using an AA-6800 Atomic Absorption Spectrophotometer (Shimadzu, Japan) according to the method provided in GB/T 5009 (13/14/87/90/91/92)-2003 (Xiao *et al*., 2002).

Data processing

Experimental data were recorded and saved using Microsoft Office Excel 2019 and statistically analysed with SPSS 22.0 Statistics software (spss, Chicago, IL, USA). All data are expressed as the mean and standard error. If the data failed to meet the homogeneity of variance criteria, percent data were transformed to square root values, and a significance analysis was performed using an independent sample *t* test. Differences were considered to be significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS

Essential nutrient components in muscle tissue

Table I shows the essential nutrient components of muscle tissue from turtles raised in the paddy field and on the factory farm. The crude fat and ash contents of muscle tissue from turtles raised in the paddy field were lower than those of turtles raised on the factory farm (0.70% and 0.96% vs. 1.40% and 1.03%, respectively); however, these differences were not significant ($p > 0.05$). The crude protein content of muscle tissues from turtles raised in the paddy field was significantly lower than that of turtles raised on the factory farm (17.47% vs. 18.57%; $p < 0.01$). The moisture contents of muscle tissues from turtles raised in the paddy field and on the factory farm were also highly significantly different (80.63% vs. 77.87%; $p < 0.01$).

Table I. Basic nutrient composition in a paddy field and on a factory farm.

Strains	Crude protein	Crude fat	Moisture	Ash
Turtle of paddy field	17.47± 0.09	0.70± 0.00	80.63± 0.38**	0.96± 0.00
Turtle of factory farm	18.57± 0.19**	1.40± 0.31	77.87± 0.38	1.03± 0.03

Amino acid composition and content in muscle tissue

Table II presents the amino acid contents in the muscle tissues of the turtles raised in the paddy field and on the factory farm. Sixteen total amino acids were detected, including seven essential and non-essential amino acids and two semi-essential amino acids. A comparison of these 16 amino acids suggested that significant differences were present in the glutamic acid (Glu), glycine, histidine, and leucine contents between muscle samples of turtles raised in the paddy field and those raised on the factory farm ($p < 0.05$). Furthermore, marked significant differences were present in the arginine, lysine, tyrosine, Met, alanine, valine, serine, aspartic acid, phenylalanine, isoleucine, and threonine contents between groups ($p < 0.01$); no marked differences were found in the proline content ($p > 0.05$). Among the contents of 16 amino acids in all turtles, Glu content was the highest, and Met content was lowest. The contents of total essential, semi-essential, non-essential, flavour-associated, and pharmacodynamic amino acids in the muscle tissues from turtles raised in the paddy field were lower than those from turtles raised on the factory farm; however, these differences were not significant ($p > 0.05$). Specifically, the comparisons of total amino acids, total flavour-associated amino acids, and total pharmacodynamic amino acids in muscle tissues from turtles raised in the paddy field and on the industrial aquaculture were 15.57 vs 17.30%, 6.58 vs 7.13%, and 10.53 vs 11.69%, respectively.

Table II. Hydrolytic amino acid compositions in a paddy field and on a factory farm (dry weight %).

Amino acid	Turtle of paddy field	Turtle of factory farm
Arg ×$	0.98±0.00	1.12±0.01**
Lys *$	1.57±0.01	1.73±0.02**
Tyr # $	0.54±0.00	0.62±0.01**
Met *$	0.38±0.00	0.50±0.01**
Ala #¤	0.94±0.01	1.02±0.01**
Glu #¤$	2.68±0.03	2.88±0.04*
Val *	0.77±0.01	0.91±0.01**
Ser #	0.69±0.00	0.77±0.01**
Gly #¤$	0.78±0.02	0.87±0.01*
Asp #¤$	1.56±0.01	1.74±0.02**
Pro #¤	0.62±0.01	0.62±0.01
His ×	0.51±0.02	0.61±0.02*
Phe *$	0.68±0.01	0.76±0.01**
Leu *$	1.38±0.02	1.48±0.02*
Ile *	0.71±0.00	0.83±0.01**
Thr *	0.77±0.00	0.85±0.01**
TEAA	6.27±0.16	7.06±0.08
Semi-TEAA	1.48±0.24	1.72±0.03
TNEAA	7.80±0.29	8.52±0.07
TDAA	6.58±0.38	7.13±0.05
TPAA	10.53±0.24	11.69±0.13
TAA	15.57±0.09	17.30±0.17**

*, essential amino acids (EAA); ×, semi-essential amino acids (Semi-EAA); #, non-essential amino acids (NEAA); ¤, diliacate amino acids (DAA); $, Pharmacodynamics amino acids (PAA).

Table III. Contents of fatty acid in the muscle of paddy field and factory farm (dry weight %).

Amino acid	Turtle of paddy field	Turtle of factory farm
$C_{14:0}$	0.97±0.00	1.26±0.08*
$C_{16:0}$	15.73±0.19	20.13±0.19**
$C_{18:0}$	11.97±0.09**	7.62±0.25
$C_{20:0}$	0.22±0.00	0.15±0.00
$C_{16:1}$	5.72±0.69	7.02±0.56
$C_{20:1}$	0.30±0.00	0.84±0.16*
$C_{24:1}$	1.68±0.02*	0.96±0.20
$C_{18:1n9c}$	20.00±0.25	30.50±1.70**
$C_{22:1n9}$	1.69±0.01	1.03±0.25
$C_{18:2n6c}$	25.57±1.27**	14.37±0.74
$C_{18:3n3\,\alpha}$	0.70±0.00	1.15±0.02**
$C_{20:4n6}$	6.61±0.07**	1.32±0.17
$C_{20:5n3}$ (EPA)	6.27±0.03	6.88±0.51
$C_{22:6n3}$ (DHA)	6.35±0.05	6.78±0.51
SPA	28.89±0.12	29.09±0.18
MUFA	55.66±0.63	55.87±1.19
PUFA	19.23±0.09*	14.98±1.43
UFA	74.89±0.66**	70.85±0.25

MUFA is the content mo-mounsaturated fatty acid; PUFA is the content of polyunsaturated fatty acid; SFA is the content of saturated fatty acid. UFA is the conten unsaturated fatty acid.

Fatty acid composition of the muscle tissue

The fatty acid compositions of the muscle tissue samples are presented in Table III. Fourteen fatty acids, including four saturated fatty acids, seven MUFAs, and three PUFAs, were detected in the muscle tissues from turtles raised in the paddy field and on the factory farm. The contents of arachidic acids, palmitoleic acids ($C_{16:1}$), erucic acids, eicosapentaenoic acids (EPAs), and docosahexaenoic acids (DHAs) in muscle tissue samples were identical between farming environments; however, the contents of the other nine fatty acids were highly significantly different ($p < 0.01$) or significantly different ($p < 0.05$). The contents of MUFAs and unsaturated fatty acids were 55.00% and more than 70.00%, respectively, in the muscle tissue samples of paddy field raised turtles than in those raised on a factory farm. Among fatty acids, linoleic acid ($C_{18:2n6c}$) ranked at the top (25.57%) and arachidic acid ($C_{20:0}$) ranked at the bottom (0.22%) in terms of percentage of the total fatty acid content in muscle tissue samples from turtles raised in the paddy field; meanwhile, oleic acid ($C_{18:1n9c}$) ranked at the top (30.50%) and arachidic acid ($C_{20:0}$) ranked at the bottom in terms of percentage of total fatty acid content in the muscle tissue of turtles raised on the factory farm.

MUFA is the content mo-mounsaturated fatty acid; PUFA is the content of polyunsaturated fatty acid; SFA is the content of saturated fatty acid. UFA is the conten unsaturated fatty acid.

Analysis of mineral element content

Five common elements (sodium, potassium, calcium, magnesium, and phosphorus) and five trace elements (iron, copper, zinc, manganese, and selenium) were detected in the muscle tissues from turtles raised in the paddy field and on the factory farm (Table IV). Among them, phosphorus content was the highest (1,560 mg kg^{-1} and 1,730 mg kg^{-1}) and selenium content was the lowest (0.21 mg kg^{-1} and 0.19 mg kg^{-1}) in the samples from turtles raised in the paddy field and on the factory farm, respectively. Potassium, iron, copper, zinc, and selenium contents in muscle tissues from turtles raised in the paddy field were higher than those in turtles raised on the factory farm, without being significantly different ($p > 0.05$); and iron and copper contents were markedly significantly different ($p < 0.01$). In samples from turtles raised on the factory farm, sodium, calcium, magnesium, phosphorus, and manganese contents were higher, without being significantly different ($p > 0.05$); while magnesium and phosphorus contents were markedly significantly ($p < 0.01$) and significantly higher ($p < 0.05$), respectively, than those in samples from turtles raised in the paddy field.

Table IV. Composition and contents of mineral in turtle muscle of paddy field and factory farm.

Type of element	Turtle of paddy field	Turtle of factory farm
Na (mg/kg)	77.53±0.55	87.60±4.73
K (mg/kg)	308.70±0.88	303.30±4.18
Ca (mg/kg)	83.37±1.11	101.30±13.08
#Fe (mg/kg)	52.23±0.94**	13.67±0.60b
Mg (mg/kg)	199.00±2.65	225.70±1.45**
#Cu (mg/kg)	0.58±0.00**	0.43±0.11
#Zn (mg/kg)	23.00±0.44	21.97±2.14
P (mg/kg)	1.56±0.04×10³	1.73±0.02×10³*
#Mn (mg/kg)	0.30±0.00	0.32±0.01
#Se (mg/kg)	0.21±0.01	0.19±0.01

#, represents the microelement.

DISCUSSION

Essential nutrient components are major indicators used to measure nutritional value and quality, and different farming methods, feed components, and fish sizes affect the nutrient components in the muscle tissues of aquatic organisms (Liu *et al*., 2011; Feng *et al*., 2017). A comparative analysis of multiple farming methods (greenhouse, ecological, and paddy field farming) and essential nutrient components in wild soft-shell turtles established that nutrient components in the muscle tissues of turtles can be significantly different (Wang *et al*., 2019; He *et al*., 2013). In this study, we comparatively analysed the essential nutrient components in the muscle tissues of turtles raised in a paddy field and on a factory farm and found that the crude fat and ash contents in the muscle tissues were not significantly different between turtles raised in the paddy field and on the factory farm. However, the crude protein and moisture contents of muscle tissue samples from turtles raised in the paddy field were significantly lower and significantly higher, respectively, than those in samples from turtles raised on the factory farm. During paddy farming, the turtles were confined to a large space and required an enormous amount of energy, resulting in the consumption of nutrient components (e.g., fat and proteins). After the onset of winter, the water temperature decreased in the paddy field, and the turtles discontinued feed intake and hibernated. Because of this decreased nutrient intake, low nutrient and high moisture contents were observed in muscle tissues. In factory farming, the turtles were confined to a small space maintained at an appropriate temperature, provided with sufficient food, and consumed less energy. Consequently, numerous nutrients accumulated in the turtle tissues. Additionally, the crude protein and ash contents in muscle tissues of factory farmed turtles were lower than those found in muscle tissue from turtles raised in a paddy field, and the crude fat content in the former was considerably lower than that in the latter (0.70 vs. 2.04%, respectively) (Wang *et al*., 2019). Regarding the turtles raised in the paddy field in this study, the initial mean body weight was approximately 610 g, and the farming period was 4 months. The initial mean body weights and feeding period for previously reported studies on turtles raised in a paddy field were not described (Wang *et al*., 2019). To establish the reliability of our study results, we accessed information pertaining to essential nutrient components for turtles raised in greenhouse, ecological, and wild environments (He *et al*., 2013) and found that the crude protein, crude fat, and moisture contents should be within the ranges of 16.61–24.00%, 0.31–0.89%, and 78.52–81.38%, respectively. The study results reported herein were within the aforementioned ranges of nutrient contents in turtles.

Proteins play an integral role in living organisms, and amino acids are the basic building blocks of proteins. Therefore, amino acid contents are major indicators used to measure the nutritional value of aquatic products. In this study, the amino acid composition and contents were determined based on the method provided in "Determination of Amino Acids in Food" (GB 5009.12-2016); however, the method failed to determine the cystine and tryptophan contents. A comparison of the 16 detected amino acids (Supplementary Table I) showed that the essential amino acid contents in the muscle tissues from turtles raised in the paddy field and factory farm comprised 41.00% of the total amino acid content, and the ratios of essential and non-essential amino acids were 0.82 and 0.83, respectively. According to the reference protein pattern proposed by the FAO/WHO, essential amino acids from a high-quality food protein source should account for approximately 40.00% of the total amino acid content and the ratio of essential to non-essential amino acids should be greater than 0.60 (Seligson and Mackey, 1984). We found that the essential amino acid contents in muscle samples from turtles raised in the paddy field and greenhouse complied with these criteria. Moreover, an evaluation and analysis of the nutritional value related to amino acids in the muscle tissues (Supplementary Table II) suggested that the contents of Met and other essential amino acids (i.e., isoleucine, leucine, threonine, phenylalanine, tyrosine, lysine, and valine) in the muscle tissues from turtles raised in the paddy field and greenhouse were lower and higher, respectively, than the values specified in the criteria proposed by the WHO/FAO. Furthermore, we evaluated the protein qualities in the muscle tissues from turtles raised in the paddy field and greenhouse primarily based on the scoring criteria of the WHO/FAO, whole-egg protein pattern, and AAS, CS, and EAAI values. The calculated AAS results showed that the scores for essential amino acids, except Met, in the muscle tissues from turtles raised in the paddy field and greenhouse were more than 0.90, and the calculated CS values of amino acids in these animals were greater than 0.67 and had a range of 0.75–1.44. This indicated that the amino acid compositions in the muscle tissues of turtles raised in the paddy field and greenhouse complied with the criteria of the WHO/FAO and whole-egg protein pattern. The EAAI is an indicator of the similarity between the essential amino acid content and standard protein content. An EAAI of more than 0.95 denotes a high-quality protein source, 0.86–0.95 denotes a good protein source, and 0.75–0.86 denotes an available protein source. The EAAIs in the muscle tissue from turtles raised in the paddy field and greenhouse were 0.86 and 0.83, respectively, implying that the meat from these turtles was considered a source of available protein (Supplementary Table II).

Fatty acids comprise saturated and unsaturated fatty acids, and unsaturated fatty acids consist of MUFAs and PUFAs. Among these, an abundance of PUFAs is necessary to significantly improve the fat flavour and can reflect the juiciness of meat (Bin *et al*., 2005). In this study, we found that the total PUFA and unsaturated fatty acid contents (19.23% and 74.89%, respectively) in the muscle tissues from turtles raised in the paddy field were significantly and highly significantly increased compared to those in the muscle tissue from turtles raised on the factory farm. It was reported that PUFAs can exert significant blood lipid-lowering, anti-tumour, and immunoregulatory effects and can markedly reduce the incidence of cardiovascular diseases (Huang *et al*., 2001). Additionally, the fat tissues of turtles contain abundant PUFAs that produce a considerable amount of succinic acid after degradation, which can add flavour and nutritional value to the meat (Qian and Zhu, 2002). We hypothesised that the nutrient components in the muscle tissues of turtles raised in the paddy field would be superior to those of turtles raised on the factory farm in terms of reducing blood lipids, lowering the incidence of cardiovascular diseases, and preventing tumours. Additionally, EPA and DHA are PUFAs with extensive physiological activities in humans. Between them, DHA, one of the major constituents of the human brain accounting for approximately 10% of components, can help promote brain cell growth, maintain the physiological activities of brain cells, and prevent ageing of brain tissue. In this study, no notable differences were observed in the muscle tissue contents of EPA and DHA between the turtles raised in the paddy field and on the factory farm (6.27% and 6.35% vs. 6.88% and 6.78%, respectively). However, EPA and DHA contents were higher in the tissues from turtles raised in the paddy field and on the factory farm compared to reported values from turtles raised in an ecological environment (2.17% and 3.69%, respectively), a pond (2.05% and 3.74%, respectively) and a greenhouse (2.02% and 2.83%, respectively) (He *et al*., 2013). This suggests that turtles raised both in paddy fields and on factory farms can provide dietary and health benefits.

Mineral elements, which are vital for maintaining tissue function and normal physiological functions, are one of the seven essential nutrients for humans and cannot be synthesised by humans; they must be supplied by outside sources. Therefore, mineral element contents remain fundamental in the daily diets of humans (Lu *et al*., 2011). Ten different mineral elements were present in the muscle tissues of turtles (Table IV). Among the common mineral elements, phosphorus content was the highest, followed by potassium, magnesium, calcium, and sodium contents. Phosphorus is a fundamental component in the structure of the human body and plays a critical role in maintaining the osmotic pressure and acid–base balance in humans (Dunn and Rustad, 2007). The phosphorus content in the muscle tissues of turtles raised in the paddy field was evidently lower than that of turtles raised on the factory farm, it is thus speculated that the nutrient components in the muscle tissues of the turtles raised on the factory farm may be superior to those of turtles raised in the paddy field in terms of regulating osmotic pressure and maintaining the acid–base balance in humans. Among trace elements, iron content was the highest, followed by zinc, copper, manganese, and selenium contents. Iron, an essential component of haemoglobin, can carry oxygen and facilitate the exchange between oxygen and carbon dioxide and is vital for producing energy. If iron deficiency occurs in humans, symptoms will occur (e.g., anaemia, fatigue, nausea, and cold sensitivity) (Goldhaber, 2003). The iron content in the muscle tissues from turtles raised in the paddy field was significantly higher than that in the turtles raised on the factory farm (52.23 mg kg^{-1} vs. 13.67 mg kg^{-1}, respectively) and was significantly higher than the reported iron contents in muscle tissues from turtles raised in a wild environment or a greenhouse (52.23 mg kg^{-1} vs. 5.13 mg kg^{-1} and 3.19 mg kg^{-1}, respectively) (Wang *et al*., 2019). Similar to iron, copper, a component of some metalloenzymes, participates in the haematopoietic process in the human body and catalyses the synthesis of haemoglobin. If copper content decreases in the human body, oxidative metabolism will no longer be regulated and symptoms will occur (e.g., incoordination and mental aberration) (Chen *et al*., 2015). The copper content in the muscle tissues of turtles raised in the paddy field was significantly higher than those in muscle tissues from turtles raised on the factory farm, in a wild environment, and in a greenhouse (0.58 mg kg^{-1} vs. 0.43 mg kg^{-1}, 0.14 mg kg^{-1}, and 0.08 mg kg^{-1}, respectively), suggesting that the turtles raised in paddy fields and on factory farms can provide a wealth of trace elements for the human body, and consuming turtles raised in paddy fields could be more beneficial to human health because copper and iron contents are higher.

CONCLUSION

In this study, we comparatively analysed the effects of paddy field and factory farming on nutrient components in the muscle tissues of turtles. We established that the muscle tissues of turtles raised in the paddy field and on the factory farm are available protein sources. Muscles of turtles raised in the paddy field can provide more moisture, mineral element (iron and copper), PUFA and total unsaturated fatty acid, but less crude protein contents than that on the factory farm. This study helps to provide a reference basis to scientifically evaluate the muscle qualities of turtles raised in these environments.

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Supplementary material

There is supplementary material associated with this article. Access the material online at https://dx.doi.org/10.17582/journal.pjz/20230801010244

Statement of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

Comparative Analysis of Nutrients and Mineral Element Contents in Muscles of *Pelodiscus sinensis* Raised in a Paddy Field and on a Factory Farm

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Supplementary Table I. Components of different amino acid of the muscle in the paddy field and factory farm.

Amino acid	Turtle of paddy field	Turtle of factory farm
TEAA/TAA	0.41	0.41
Semi-TEAA/TAA	0.10	0.10
TNEAA/TAA	0.50	0.49
TDAA/TAA	0.41	0.41
TPAA/TAA	0.62	0.62
TEAA/TNETAA	0.82	0.83
TDAA/TPAA	0.66	0.66

*Essential amino acids (EAA); ˣ, semi-essential amino acids (Semi-EAA); #non-essential amino acids (NEAA); ¤ diliacate amino acids (DAA); $ Pharmacodynamics amino acids (PAA)

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Supplementary Table II. Components and evaluation of essential amino acid of turtle in the paddy field and factory farm.

Essential amino acid	FAO/WHO standard (mg/g pro)	Whole egg protein standard (mg/g pro)	Turtle of paddy field			Turtle of factory farm		
			Content (mg/g pro)	AAS	CS	Content (mg/g pro)	AAS	CS
Ile	40	54	45.83	1.15	0.85	48.17	1.20**	0.89**
Leu	70	86	88.65	1.27	1.03	85.55	1.22	0.99
Thr	40	47	49.68	1.24	1.06	49.13	1.23	1.05
Phe+Tyr	60	93	77.94	1.30	0.84	79.38	1.32	0.85
Lys	55	70	100.6	1.83	1.44	99.8	1.81	1.43
Val	50	66	49.68	0.99	0.75	52.61	1.05*	0.80*
Met	35	57	24.63	0.70	0.43	29.09	0.83**	0.51**
EAAI				0.86			0.83	