Comparative Study on Nutritional Compositions and Pathogenic Microorganism Isolated from Green Turtle Egg and Chicken Egg

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Abstract
Exploitation of marine turtles is getting more severe as their population is starting to decline by years. The consumption of their eggs by the public is one of the factors that lead to this matter. However, there is not much research done so far on its nutritional composition and how it affects public health. A comparative study between turtle egg and chicken egg was done to evaluate the egg’s nutrient content and pathogenic microorganism. A total of 30 turtle eggs were purchased from the wet market located in Terengganu to determine the percentage of fatty acid and crude protein within the eggs. There was no significant difference between the crude protein percentage in chicken and turtle eggs; 11.99% and 11.58%. In this study, the composition of fatty acids, Omega-3 (N3PUFA) and Omega-6 polyunsaturated (N6PUFA), monounsaturated (MUFA), and saturated fatty acids (SFA) were determined. From the findings, there was a significant difference in saturated and monounsaturated fatty acids where the composition was higher in turtle eggs (35.27% and 48.79%, respectively) as compared to chicken eggs (31.99% and 45.3%, respectively). On the other hand, turtle eggs were higher in Omega-3 but lower in Omega-6 FAs (13.35%, 2.58%), which contrary to the chicken eggs (6.44%, 16.27%). In bacterial isolation, none of the isolates were positive for Salmonella sp. and the majority of the isolates were positive with Proteus mirabilis in both chicken and turtle eggs. The presence of this bacteria in turtle eggs might pose certain health risks as they are commonly consumed raw by the public. In conclusion, these findings related to the composition of fatty acid, protein and the existence of bacteria in the turtle egg would allow us to increase the conservation effort to keep the population from extinction.

Keywords: fatty acids, protein, bacterial isolation, turtle eggs, chicken eggs

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hatchlings, disturbed and degraded nesting beaches, fishery-related mortality, pollution, development on the coastal areas, and most importantly, poaching of eggs by humans for consumption [3]. Human consumption of marine turtle eggs is becoming a trend because in certain Malaysian state laws, the eggs can be marketed legally. Therefore, the public has no doubt about consuming the eggs.

In Peninsular Malaysia, egg collector should get a license from the States Fisheries Department as a permit for them to collect the eggs legally. Eggs collected must then be sold back to the Fisheries Department and incubated in the hatchery. However, the presence of many illegal egg collectors and sometimes lack of funding from the government to buy the eggs for incubation had resulted in a high number of eggs being sold in the local market. Pasar Payang, one of the famous markets in Kuala Terengganu, has become the hub for egg’s trading. Unfortunately, there is no law in the state comprehensively bans the selling and consumption of turtle eggs [3]. Eggs from different species of marine turtle are still broadly promoted, particularly in Terengganu, Malaysia except for leatherback turtle's eggs are banned from open trade [4]. Due to lack of law and enforcement, harvesting of turtle eggs had contributed as one of the significant figures to the decline of sea turtle nesting in Peninsular Malaysia.

According to one study [5], green turtles have been recognized as a potential bio-indicator of marine pollution because of the exposure due to their long life-span and long-distance migratory route. It might be related to their diet or characteristics of pollution at their foraging habitats from different places [6]. Different diet practices by different species of animals obviously influenced the nutritional composition in their body. Marine turtles are mostly omnivorous (feed on algae and plants from a lower level of the food chain) and some of them are carnivorous according to the species (feed on molluscs, crustaceans). If pollution happens at their pit stop, such pollutants either from water or food can pass through their digestive tracts and deposited in the body.

With respect to the utilization of an assortment of species reptile caught from the wild, turtles (meat and eggs) are likely the most intensely misused globally [7]. Consumption of raw turtle eggs may be linked to the beliefs on the nutritive values that give humans health benefits, including improving fertility and acting as a natural aphrodisiac. However, there is still a limited study to support these ideas. Their preferences on this type of food were highly influenced by their cultural and environmental perceptions, socioeconomic characteristics, access and abundance of marine turtle eggs. Unfortunately, this practice may be exposing humans to the risk of pathogenic microorganisms such as bacteria. Thus, the present study aimed to determine the protein and fatty acids content in turtle eggs. The quantitative results of the nutrient content of turtle eggs particularly their fatty acids and protein, are compared with similar nutrient parameters in chicken eggs commonly consumed by humans. This study is coupled with bacterial isolation and identification of Salmonella sp. of the turtle egg content through bacterial culture and biochemical tests. Positive results from the bacterial culture will prove that consumption of turtle egg exposes humans to the risk of Salmonellosis; thus, discouraging humans’ consumption of turtle eggs. This study is parallel to environmental efforts aimed at minimizing the harvesting of turtle eggs for human consumption.

2. Material and Methods

2.1. Sample Preparation

A total of 15 turtle eggs and 15 chicken eggs were used for Salmonella sp. isolation from the shell and egg content. Both samples were also used for crude protein and fatty acids analysis. The turtle eggs were purchased from the commercial market in Terengganu, where the eggs were collected from the beach area in the state. The chicken eggs were also purchased from the regular local wet market. For all procedures, the egg contents were put in sterile plastic bags and were homogenized using a food stomacher.

2.2 Sample Analysis

2.2.1. Determination of Crude Protein Content

Kjeldahl method was used to determine crude protein in the samples by measuring the total nitrogen and multiplying by 6.25. This method includes the following step; (i) digestion, (ii) distillation, (iii) titration. Each sample was analyzed by doing three replicates. The percentage of nitrogen and protein content of the sample was calculated using the formula below:

\[
\text{Nitrogen content} (\%) = \frac{(V1-V2) \times 0.1 \times 14}{1000 \times \text{Weight of sample taken (g)}} \times 100
\]

\[V1 = \text{Titration volume for sample (mL)}\]
\[V2 = \text{Titration volume of blank (mL)}\]

\[6.25 = \text{Nitrogen conversion factor.}\]

2.2.2. Determination of Fatty Acid Content

The total fatty acids were extracted from the previously homogenized egg content using chloroform: methanol 2:1 (v/v) based on the method of Folch et al. (1957) modified by Raji (1985). The preparation of fatty acid methyl ester (FAME) was carried out according to methods in AOAC (2007). Next, the methyl esters were quantified by GC (Agilent 7890N) using a 30m x 0.25mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA).

2.2.3. Isolation of Pathogenic Microorganism

Ten turtle eggs and ten chicken eggs were analyzed for isolation of bacteria which carried out for both shell surface of the turtle egg and also the egg content. The eggshell was scrubbed all over its surface by using the shell swab and cultured onto pre-enrichment culture (non-
selective liquid media), buffered peptone water and then incubated at 37°C for 24 hours. The pre-enrichment culture is then sub-cultured into selective enrichment media, Rappaport Vassiliadis soy broth (RVS) and incubated at 37°C for 24 hours. One ml of the RVS was inoculated onto two selective agar media; Xylose-Lysine Desoxycholate (XLD) agar and Brilliant Green agar and incubated at 37°C for 24 hours. Salmonella colonies were identified on each agar media and findings were recorded. Plates with no presumptive Salmonella sp. colonies were cultured onto non-selective media; blood agar prior to confirmatory biochemical testing. The same turtle eggs and chicken eggs shell were sterilized using 70% alcohol and the content was collected in a sterile plastic bag and labeled accordingly. A stomacher machine was used to homogenize the egg yolk and the white (albumin) part. One ml of the eggs was transferred by using a pipette, into pre-enrichment culture (non-selective liquid media), buffered peptone water and then incubated at 37°C for 24 hours. This is followed by the same steps done for Salmonella sp. isolation of the eggshell.

3. Statistical Analysis

Independent T-Test was carried out for normally distributed data, whereas Mann-Whitney U test for non-normally distributed data. All parameters were analyzed by using SPSS 20.0 (SPSS software for Windows, IBM Corp) and p values of <0.05 were considered statistically significant.

4. Results

From the crude protein analysis, Figure 1 shows the crude protein percentage is higher in chicken eggs (11.99%) as compared to turtle eggs (11.58%). However, no significant difference at (P<0.05) was recorded between the two samples. The fatty acid profile (Figure 2) for the turtle egg was dominated by monounsaturated fatty acid (48.79%), which significantly higher than a chicken egg (45.30%). As for the Omega-6 PUFA, the value was detected at very low concentrations (2.58%) in contrast with the value recorded for essential fatty acid of Omega-3 PUFA (13.35%) in turtle egg samples. In comparison to chicken egg samples, the amounts of saturated fatty acid in turtle eggs (35.27%) were much higher than Omega-6 PUFA, Omega-3 PUFA and SFA itself (16.27%, 6.44% and 31.99%, respectively).

Figure 3 summarizes the percentage of bacteria isolated from the shell. Both turtle and chicken eggs were free from Salmonella either in the egg content or outside the eggshell. The highest percentage of isolated bacteria in turtle and chicken eggs (shell=62%; 31% and egg content=46%; 48% respectively) was found to be Proteus mirabilis. For bacterial isolated from the egg content, Figure 4 shows that both turtle and chicken eggs were negative for Salmonella sp. and the majority of the isolates were positive for P. mirabilis. Other bacteria species include Klebsiella pneumonia, Escherichia coli, Pseudomonas sp., Proteus sp., and Enterobacter.
Turtle Egg

higher in turtle egg (13.35%) compared to chicken egg

scarce and limited studies were done on this topic.

about saturated fatty acid related to marine turtle egg
disease significantly. However, there is not much debate
acid has the potential to increase the risk of cardiovascular
It is well known that dietary intake high in saturated fatty

concentrated in the egg yolks compared to the egg white
indicated that most nutrients parameters were found to be

higher egg yolk mass of the chicken eggs in relative to
turtle eggs; 17-20g and 13-14g, respectively.  The study
results reported a lower percentage of crude protein which

highest component followed by moisture 43.4%, fat 3.5%

the other hand, saturated fatty acid data recorded in turtle

great difference with this recent study especially in the
generation. In our ancestral diets, the original ratio of
body perform well in homeostasis and normal
by balancing these two classes of PUFAs, it helps the
Both PUFAs portray distinct physiological functions and
contrast, Omega-6 FAs dietary intake influences Omega-3
response and preventing and giving treatment to coronary
reducing inflammation, supporting healthy immune
human body’s nutrition intake. Previous studies have
appropriate amount should be taken daily to balance the
(1.04%) from the previous study [22]. However, the latter
higher in comparison with wild green turtle egg obtained
fatty acid in this study (5.17%) was almost five times
higher in comparison with wild green turtle egg obtained
(1.04%) from the previous study [22]. However, the latter
one’s data on acids level is quite similar to the results [23]
and suggested that the reptile group might share certain
characteristics in their lipid profile. As they make a
comparison in different dietary intake between green
turtles in the wild and in captivity, they conclude that this
factor does influence the nutritional composition provision
in the egg. Further support comes from a study [24] on
other reptile, Testudo hermanni where different diets may
 affect the lipid profile in the eggs which clearly elucidate
the transfer of dietary fatty acids from mother to egg.
However, little is known that there is a lack of recently
published data for lipid profile contents in the turtle eggs;
therefore, further research is necessary for this topic.

Both Omega-3 and Omega-6 FAs are essential fatty
acids that complete the nutrition of many animal species
including humans. These polyunsaturated fatty acids
(PUFAs) cannot be synthesized by the body; therefore, an
appropriate amount should be taken daily to balance the
human body’s nutrition intake. Previous studies have
shown that Omega-3 FAs benefit human health by
reducing inflammation, supporting healthy immune
response and preventing and giving treatment to coronary
artery disease if taken in the appropriate diet [25,26]. In
contrast, Omega-6 FAs dietary intake influences Omega-3
FAs which likely enhances inflammation in the body.
Both PUFAs portray distinct physiological functions and
by balancing these two classes of PUFAs, it helps the
body perform well in homeostasis and normal
development. In our ancestral diets, the original ratio of

5. Discussion

In this study, the percentage of crude protein in chicken
eggs (11.99%) was higher than turtle eggs (11.58%). Both
results reported a lower percentage of crude protein which
is in contrast with the previous study. According to one
study [8], chicken egg contains only 12.6% of protein and
the rest is made up of 75.8% of water, 9.9% lipid, 1.7%
vitamins, minerals, and a small amount of carbohydrates.
From the results of this current study, it could be
suggested that the higher protein level is related to the
higher egg yolk mass of the chicken eggs in relative to
turtle eggs; 17-20g and 13-14g, respectively. The study
indicated that most nutrients parameters were found to be
concentrated in the egg yolks compared to the egg white
and whole egg except moisture, which in line with
previous study [9]. On the other hand, Olive Ridley sea
turtle eggs’ composition recorded protein 49.2% being the
highest component followed by moisture 43.4%, fat 3.5%
and ash 3.9% [10]. The available literature data shows a
great difference with this recent study especially in the
turtle egg protein content. The lower protein levels in
turtle eggs as relative to protein levels in turtle eggs from
other research could be explained by the disparity in dietary
intake. The previous study shows that the percentage of
crude protein was increased as the dietary crude protein
amounts increased [11]. Besides that, species variation
could also contribute to the results as obtained as it
influenced the nutrient composition of turtle eggs [12]. On
the other hand, saturated fatty acid data recorded in turtle
egg is the second-highest amount (35.27%) compared to other
classification of fatty acids as well as in chicken egg.
It is well known that dietary intake high in saturated fatty
acid has the potential to increase the risk of cardiovascular
disease significantly. However, there is not much debate
about saturated fatty acid related to marine turtle egg
because information on its nutritional composition is
scarce and limited studies were done on this topic.

In the present study, Omega-3 FAs obtained were much
higher in turtle egg (13.35%) compared to chicken egg
(6.44%). This could be due to different classes of animals
that lead to differences in dietary intake [13]. Reference
[14] found that seagrass is a vital diet especially for green
turtles which are categorized as herbivorous species. They
also feed on marine algae and seafood which high in
Omega-3 FAs [15]. Marine algae are considered as a
source of Omega-3 FAs other than fish and seafood. In
fact, it can be extracted and purified for the oil as an end-
product linked to human health benefits [16]. The algal oil
can be replaced with fish oil as a plant-based supplement
as an alternative especially for those who preferred a
vegetarian diet [17,18]. On the contrary, the other
essential fatty acids, Omega-6 were higher in chicken eggs
(16.27%) than turtle eggs (2.58%). This is highly related
to the composition of chicken’s diet as egg’s nutritional
content is determined by the hen’s diet [8]. The diets are
mainly made up of grains, nuts, and vegetable oils which
is an abundance of Omega-6 FAs. Sufficient essential
proteins, fats, vitamins, minerals and the compositions and
net amount in chicken eggs may be affected by hen diet,
strain, age and environmental conditions [19]. Additionally,
chicken eggs are also accessible and inexpensive but
highly nutritious food that provides balanced nutrients for
human health [20]. It is relevant to see a worldwide egg
consumption and production because the demand for
protein sources is rising especially in developing countries
as there is a third of the population is undernourished [21].

A major difference of lipid contents in the amount of
Omega-3 FAs and Omega-6 FAs are recorded in the green
turtle sample. Surprisingly, the present data somewhat is
highly distinctive whereby the ratio of Omega-3/Omega-6
fatty acid in this study (5.17%) was almost five times
higher in comparison with wild green turtle egg obtained
(1.04%) from the previous study [22]. However, the latter
one’s data on acids level is quite similar to the results [23]
and suggested that the reptile group might share certain
characteristics in their lipid profile. As they make a
comparison in different dietary intake between green
turtles in the wild and in captivity, they conclude that this
factor does influence the nutritional composition provision
in the egg. Further support comes from a study [24] on
other reptile, Testudo hermanni where different diets may
affect the lipid profile in the eggs which clearly elucidate
the transfer of dietary fatty acids from mother to egg.

Figure 4. Bacterial isolated from turtle egg content

Bacterial isolation from egg content
Omega-6/Omega-3 PUFA was 1:1~2:1. However, in the present-day, the proportion has risen around 20:1 due to the practice of Western diets especially in developing countries [27]. Unfortunately, the imbalance ratio of Omega-6/Omega-3 dietary intake would increase the risk of getting many diseases and disrupt the body’s physiological and biochemical process [28,29]. Therefore, it is recommended to have a lower proportion of Omega-6/Omega-3 PUFA, 4:1 in the human diet as suggested by [30].

In this study, seven out of ten turtle eggs shell isolated on XLD Agar resulted black-centred colonies and assumed to be presumptively positive for Salmonella sp. The same result was observed on the turtle egg content isolated on XLD Agar. For chicken eggs shell and their content, only one out of 20 isolated resulted in a black centres colonies. From the BGA cultures, only one out of ten turtle eggs shell isolates gave a negative result for red colonies, which presumptively assumed as Salmonella sp. On the other hand, only three out of ten chicken eggs content isolates produced red colonies and two out of ten chicken eggs content isolates resulted positive for red colonies. Subcultures on blood agar were done prior to biochemical testing to confirm the presence of Salmonella sp. From the biochemical test for both turtle and chicken eggs shell and their content, only one out of 40 isolates gave out positive for Salmonella sp. which is from a chicken eggshell sample. This could be due to Proteus strains that also give black-centred colonies on XLD Agar. Proven by the biochemical test results, the majority of the isolated bacteria were Proteus sp. especially Proteus mirabilis. According to a study [31], they discovered that the predominant bacterial phylum in the microbiota of nesting sea turtle was proteobacteria. Proteus mirabilis isolated from most of the samples is widely distributed in the environment, occurring in polluted water, manure, soil and exist predominantly in the human gastrointestinal tract of humans and animals [32].

P. mirabilis is a common cause of urinary tract infection in humans characterized by these symptoms. In more severe cases, pyelonephritis may result from an ascending infection. Other than that, it can also enter the bloodstream through wounds causing sepsis and triggers systemic inflammatory response syndrome (SIRS). Less commonly, P. mirabilis can cause pneumonia. The concerns are related to turtle eggs as they are commonly consumed raw.

6. Conclusion

In conclusion, although turtle eggs contain a high amount of Omega-3 which is considered good fatty acids, it also contains high saturated fatty acids which in excess is a vicious threat towards public health. The turtle eggs also have an imbalance Omega-6 and Omega-3 ratio and low in protein. Although turtle egg is edible for humans, they might have a bacterial infection if consumed raw. Thus, the choice of chicken eggs consumption is always right and cause no harms to consumer as compared to turtle eggs consumption. In addition, in terms of price, turtle eggs are more expensive than chicken eggs, thus, it is not practical to buy the turtle eggs when it also poses health risks to the consumer. Indeed, the current study displayed the comparative data of nutritional properties and possible pathogenic microorganism exist between turtle and chicken egg. This kind of data is mainly important in order to prevent the consumption of turtle eggs by humans and illustrate the conservation effort to protect the species from extinction.

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