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# Nutritive qualities of patties prepared with mixture of meat and oyster mushroom

*Wan Rosli, W. I. and Solihah, M. A.

*School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

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**Abstract**

Nutritive qualities of patties prepared from chicken, beef and oyster mushroom were determined. Three groups of rats were fed with patty diets prepared with either a combination of 75% chicken + 25% oyster mushroom (CMP) or 75% beef + 25% oyster mushroom (BMP) or 100% chicken patty + 0% oyster mushroom (CP). There was no significant difference ($P < 0.05$) in total tryglyceride (0.3-0.5 mmol/L), total cholesterol (1.7-1.9 mmol/L) LDL-cholesterol (0.3-0.4 mmol/L) and HDL-cholesterol (1.2-1.4 mmol/L) for all groups except for protein free. Protein effeciency ratio (PER) values of CMP and BMP groups were significantly lower than casein group but significantly higher than chicken patty (CP) group. Both CMP and BMP fed groups recorded PER values at 1.73 and 1.69 while CP had PER value at 1.52. The AD of rats fed with CMP, BMP and CP diets were closely ranged from 98.3-98.9% but not significant as compared to casein diet group (98.5%). The close AD values between CMP, BMP and CP indicated that the mixture of patty protein from either chicken or beef with protein of oyster mushroom did not affect digestibility aspect. In summary, addition of oyster mushroom into either chicken or beef patties did not changed AD but improved PER value, thus proving that oyster mushroom could be used as an alternative ingredient to replace meat partially in the making of patties.



## Introduction

Globally, meat-based patty has long been reported to be the most popular processed meat product including Malaysia. Generally, processed meat products such as burgers and sausages are often containing high calorie mainly, saturated fats (Baggio and Bragagnolo, 2006) and sodium while low in dietary fibres. The saturated fat can cause high cholesterol levels and may cause health problems such as hypercholesterolemia and hypertension (Jimenez-Colmenero *et al.*, 2001). Both beef and chicken patties are categorized as one of the 10 most frequent consumed foods weekly (Norimah *et al.*, 2012). The increase demand of beef and chicken as main ingredient in the production of burger/patty has increased it price. Rapid increment of beef and chicken meat price has resulted in rising of production cost thus impacting food manufacturer's survivality and profitability. In long run, this situation confers negative impact on quality of products and therefore affecting sustainability of business and entrepreneurial activities. This situation become worsen since most of the main ingredients such as beef and chicken are imported especially from India and other countries (Nam *et al.*, 2010). The dependency of importation of raw materials in long run is not healthy since it cost will be skyrocketing when the shortage of raw materials' supply occured.

Generally, meat provides caloric value in the range from 10 to 20% of total calories in most developed countries (Valsta *et al.*, 2005). In parallel, world food supplies seem to be growing at a slower rate than the needs. Present inadequate food consumption levels are made even more dramatic by rapidly growing populations (Valsta *et al.*, 2005). The pressure of the continuously expanding populations and limited energy supply have led many to search for new and better methods, through which more and higher quality food can be provided.

The linked between health and diet has been comprehensively studied and rising numbers of consumers have been advised to change and improve their eating habits. Energy-dense food utilization, especially saturated fat, is still considered to be surplus. According to the Nutritional guidelines recommended by WHO and other health professional groups such as dietician and medical experts, dietary fat should provide between 15 and 30% of total calories, and that saturated fats should be limited to between 0 and 10% of calorie intake and cholesterol intake below 300 mg/day (Chizzolini *et al.*, 1999).

Edible mushrooms are valued for their various pharma-nutritional functions that offer multiple

*Corresponding author.
Email: *rosliishak@gmail.com / wrosli@usm.my*

benefits to consumers. Besides it pharmacological features, mushrooms are becoming more important due to their nutritional value, related to high protein, low fat and energy contents (Barros *et al*., 2007). Several studies have been carried out on the chemical composition and nutritional quality of edible mushrooms from different countries (Al-Enazi *et al*., 2012; Ibrahim and Hamed, 2002; Manzi *et al*., 2004; Yaovadee *et al*., 2010). However the nutritional quality of patties prepared from chicken or beef and oyster mushroom are never been highlighted. The investigation on nutritive value of patties incorporated with oyster mushroom is also scanty. Thus, this study focuses on the nutritive qualities of chicken and beef patties incorporated with oyster mushroom by evaluating lipid profile, protein efficiency ratio (PER) and apparent digestibility (AD) of Sprague-Dawley rats fed with these diets.

## Materials and Methods

### *Preparation of oyster mushroom*

Oyster mushroom (*Pleurotus sajor caju*) was purchased from local wet market in Kota Bharu city, Kelantan state of Malaysia. Fully-grown mushrooms with the pileus cap diameters between 9 to11 cm were used throughout the study. The mushroom was prepared by rinsing with clean water and ground for 30 seconds. The prepared mushroom was then incorporated partially to replace beef and chicken in patty formulations.

### *Beef burger formulation*

The patties were prepared according to formulations described by Wan Rosli *et al.* (2011) with slight modification. Three patty formulations were prepared. The patties were prepared with either a combination of 75% chicken + 25% oyster mushroom (CMP) or 75% beef + 25% oyster mushroom (BMP) or 100% chicken + 0% oyster mushroom patty (CP). Addition of 25% oyster mushroom in both chicken and beef patty formulations was based on the significant nutritional values and palatability characteristics as reported in our earlier works (Wan Rosli and Solihah, 2012; Wan Rosli *et al*., 2011). The mushroom used in the present study was grey oyster mushroom (*Pleurotus sajor-caju*), the species that most commonly consumed by Malaysian. After preparation, the patties were stored in a freezer at -18°C before further analysis.

### *Rat diet preparation*

The frozen raw patties were manually cut into small sizes of approximately 0.5 cm$^2$ before drying in an oven (854 Schwabach Mermert, Germany) at 60°C for 24 hours. The dried patties were then ground using a food grinder (Waring, New Harford, Connecticut) to form fine powder. Rat diet formulations were prepared using the procedure for protein efficiency ratio (PER) as outlined by AOAC (2000), with casein as the reference protein and protein-free. The patties containing oyster mushroom were dried and used for protein source in rat diets at the level of 10%. Other ingredients included in the diet were vitamin mixture (AIN 76), mineral mixture (AIN 76), cellulose-celufil nonnutritive bulk, corn starch (Stanley, USA) and corn oil (Mazola). Casein, mineral mixture, vitamin mixture and cellulose-celufil were purchased from MP Biochemicals Company (USA). After diet preparation for each type of dried patties and the reference protein (casein), proximate analysis was carried out to ensure the diet formulation was prepared properly according to the AOAC (2000) method.

### *Rat feeding protocol*

Fifty male weanling Sprague-Dawley rats between 27-28 days old weighing between 85-100 g were obtained from the Animal Research And Service Centre (ARASC) Universiti Sains Malaysia. The rats were placed in individual cages and distributed into five treatment groups namely casein, protein free, CMP, BMP and CP. After 4 days of adaptation, the rats were subjected to a feeding trial for 28 days. During the feeding period, water was provided ad libitum and the diets were restricted to 15 g/day. The diet was replaced daily, while the spilled and leftover food was collected and weighed to determine total food intake. The food intake was recorded daily and the weight of the rats was recorded individually every two days. Faeces were collected from the 10$^{th}$ day to the 18th day of the experiment, and kept in an open container after which they were dried overnight at 100°C and analysed for nitrogen content (AOAC, 2000).

### *Nutritive value calculation*

*The apparent digestibility (AD)*

The AD was determined using a formula (Acton and Ruud, 1987). Food consumption and fecal output data were recorded daily for eight days (10-18) of the 28-day study to determine the in vivo apparent protein digestibility.

$$AD = (N \text{ in diet (g)} - N \text{ in feces (g)}) \times 100$$

*Protein effeciency ratio (PER)*

The PER was also determined using the method established by Acton and Ruud (1987). The PER was calculated using a formula:

$$\text{PER} = \frac{\text{Increase in body weight (gram)}}{\text{Weight of protein consumed (gram)}}$$

*Lipid profile of rats*

Total, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein–cholesterol (HDL-C) and triglycerides (TG) of rat blood serum were measured enzymatically according to Artiss and Zak (1997). Cholesterol is measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, $H_2O_2$ is measured quantitatively in a peroxidase catalyzed reaction that produces a color. At the end of the experimental period, rats were weighed and deeply sedated with a barbiturate. Blood samples were obtained from the abdominal aorta and centrifuged at 1500 rpm for 15 min. Rats blood serum were then stored at -80$^o$C before analysis. Serum samples were sent to BP Laboratory Sdn Bhd for analyses of total, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein–cholesterol (HDL-C) and triglycerides (TG).

## Results and Discussion

*Proximate composition of dried patties used for rat diets*

The proximate content of different formulations of dried patties used for rat diets is shown in Table 1. Dried CP contained 44.8% protein followed by CMP (38.0%) and BMP (34.8%). Although these levels were vary but the final protein and fat content of all rat diets were kept at 10 and 8% (AOAC 2000). Higher level of protein content in CP dried patties than other patties required lower amount of its source (200 g) used for rat diet formulation (Table 2). Contrarily, lower concentration of protein content in dried BMP than other patties required higher amount to be added in rat diet formulation (292g). The final protein content in all rat diets except for protein free was achieved 10% (Table 3) and comply with requirement set by AOAC (2000). On the other nutrient, fat content of dried CMP (29.2%) was significantly higher ($P < 0.05$) than BMP (26.6%) and CP (24.6%). There was significant difference in moisture and ash content between all dried patties (Table 1).

All patties recorded moisture content ranging from 41.0-56.7%. In addition, all patties prepared with chicken, beef and mushroom recorded ash content ranging from 1.9-2.3%. However, after all ingredients being mixed for rat diets it proximate composition was comply with requirement set by AOCS (1992). Both fat and ash content in the diet for protein quality study must be prepared isocaloric at the levels of 8.0% and 5%, respectively (AOAC, 2000). There was no significant difference in proximate composition between all rat diets (Table 3). The level of protein found in the present study was comparable to Johnsy *et al*. (2011). They reported that the highest protein contents (39.1%) was obtained from *Pleurotus sajor-caju*. The concentration of dried patty containing chicken was in accord to our previous study (Wan Rosli *et al*., 2007).

Table 1. Proximate analyses of different formulations of dried patties used for rat diets

| Treatment | Protein (%) | Fat (%) | Moisture (%) | Ash (%) |
|---|---|---|---|---|
| CP | 44.8 ± 0.1[a] | 24.6 ± 0.2[c] | 41.0 ± 0.2[c] | 2.0 ± 0.1[b] |
| CMP | 38.0 ± 1.7[b] | 29.2 ± 03[a] | 43.1 ± 0.4[b] | 1.9 ± 0.1[c] |
| BMP | 34.8 ± 1.3[c] | 26.6 ± 0.8[b] | 56.7 ± 0.4[a] | 2.3 ± 0.2[a] |

[a] Mean values within the same column bearing different superscripts differ significantly P < 0.05)
Note:
CP: 100% chicken patty + 0% oyster mushroom
CMP: 75% chicken + 25% oyster mushroom
BMP: 75% beef + 25% oyster mushroom

Table 2. Formulation of Diets from different Protein Sources

| Ingredient | Casein (g) | Protein free (g) | CP (g) | CMP (g) | BMP (g) |
|---|---|---|---|---|---|
| Casein | 135 | | | | |
| CP | | | 200 | | |
| CMP | | | | 280 | |
| BMP | | | | | 292 |
| Corn oil (8 %) | 76 | 78 | | | |
| Starch + sucrose (1:1) | 719 | 852 | 730 | 650 | 638 |
| Mineral (5 %) | 50 | 50 | 50 | 50 | 50 |
| Vitamin (1 %) | 10 | 10 | 10 | 10 | 10 |
| Cellulose (1 %) | 10 | 10 | 10 | 10 | 10 |

CP: 100% chicken patty + 0% oyster mushroom
CMP: 75% chicken + 25% oyster mushroom
BMP: 75% beef + 25% oyster mushroom

Table 3. Proximate composition of rat diet formulations

| Rat Diets | Treatment | Protein (%) | Fat (%) | Moisture (%) | Ash (%) |
|---|---|---|---|---|---|
| 1 | Casein | 10.0 ± 0.7[a] | 8.1 ± 0.4[a] | 5.0 ± 0.1[a] | 4.9 ± 0.3[a] |
| 2 | Protein free | 2.3 ± 0.1[b] | 7.8 ± 0.5[a] | 4.6 ± 0.5[a] | 4.9 ± 0.5[a] |
| 3 | CP | 10.1 ± 0.4[a] | 8.1 ± 0.1[a] | 4.9 ± 0.3[a] | 4.8 ± 0.8[a] |
| 4 | CMP | 10.1 ± 0.8[a] | 8.2 ± 0.2[a] | 5.1 ± 0.3[a] | 4.9 ± 0.6[a] |
| 5 | BMP | 9.9 ± 0.9[a] | 8.3 ± 0.1[a] | 5.3 ± 0.4[a] | 4.9 ± 0.9[a] |

[a] Mean values within the same column bearing different superscripts differ significantly P < 0.05)
CP: 100% chicken patty + 0% oyster mushroom
CMP: 75% chicken + 25% oyster mushroom
BMP: 75% beef + 25% oyster mushroom

*Serum lipid profile of rats*

The results of serum lipid profile are summarized in Table 4. Rat groups fed with either CMP or BMP or CP diets resulted in no significant difference in triglyceride (TG) value ranging from 0.3-0.5 mmol/L compared to control (casein) group (0.4 mmol/L). There was also no significant difference in total cholesterol content between all groups including casein with values ranging from 1.7-1.9 mmol/L. This indicated that addition of mushroom into chicken and beef patty formulations did not affect total cholesterol content in rats' blood serum. In addition, there was no significant difference ($P < 0.05$) in HDL-cholesterol content between all treatments (1.2-1.4 mmol/L).

Both groups fed with CMP and BMP diets recorded LDL-cholesterol content ranging from 0.3-0.4 mmol/L and not significantly difference compared

Table 4. Triacylglycerol and Cholesterol Frofile in Rat Blood Serum Fed with Different Diets

| Rat diets | Triacylglycerol (mmol/l) | Total cholesterol (mmol/l) | HDL-cholesterol (mmol/l) | LDL-cholesterol (mmol/l) |
|---|---|---|---|---|
| Casein (R) | 0.4 ± 0.2[a] | 1.8 ± 0.2[a] | 1.2 ± 0.2[a] | 0.4 ± 0.1[ab] |
| Protein free | 0.5 ± 0.1[a] | 1.9 ± 0.2[a] | 1.2 ± 0.3[a] | 0.5 ± 0.1[a] |
| CP | 0.5 ± 0.2[a] | 1.7 ± 0.1[a] | 1.2 ± 0.2[a] | 0.3 ± 0.1[b] |
| CMP | 0.4 ± 0.1[a] | 1.7 ± 0.2[a] | 1.3 ± 0.1[a] | 0.3 ± 0.1[b] |
| BMP | 0.3 ± 0.1[a] | 1.9 ± 0.1[a] | 1.4 ± 0.1[a] | 0.4 ± 0.1[ab] |

[a-c]Mean values within the same column bearing different superscripts differ significantly P < 0.05)
CP: 100% chicken patty + 0% oyster mushroom
CMP: 75% chicken + 25% oyster mushroom
BMP: 75% beef + 25% oyster mushroom

Table 5. PER Values of Patties Containing Oyster Mushroom (OM) and Casein Reference

| Rat diet | Diet intake (g/rat/28 days) | Weight gain (g) | % Protein in diet (N x 6.25) | Protein consumed (g/rat/28 days) | PER[1] | Corrected PER[2] |
|---|---|---|---|---|---|---|
| Casein | 390.7±17.7[b] | 103.0 ± 14.9[a] | 10.0 ± 0.1[a] | 40.3 ± 0.2[a] | 2.05±0.04[a] | 2.50[a] |
| Protein free | 242.3±10.6[b] | -32.2 ± 9.1[c] | 2.3 ± 0.1[b] | 5.7 ± 0.1[b] | - | - |
| CP | 405.3±13.5[b] | 80.0 ± 5.3[b] | 10.1 ± 0.1[a] | 41.5 ± 0.2[a] | 1.52±0.02[d] | 1.85[d] |
| CMP | 404.9±13.4[b] | 91.0 ± 6.6[ab] | 10.1 ± 0.2[a] | 40.8 ± 0.2[a] | 1.73±0.03[b] | 2.11[b] |
| BMP | 405.3±12.0[b] | 87.4 ± 5.2[ab] | 9.9 ± 0.1[a] | 40.0 ± 0.3[a] | 1.69±0.04[c] | 2.06[c] |

[a-c]Mean values within the same column bearing different superscripts differ significantly P < 0.05)
[1]PER = protein efficiency ratio = (increase in body weight, g / weight of protein consumed, g)
[2]Corrected PER = (2.50/2.05) x PER
CP: 100% chicken patty + 0% oyster mushroom
CMP: 75% chicken + 25% oyster mushroom
BMP: 75% beef + 25% oyster mushroom

Table 6. Percent *In vivo* Apparent Digestibility (AD) of Beef Burgers Containing Palm Based Fats and Casein Reference

| Rat diet | DI (g/rat/8 days) | TNC (g/rat/8 days) | NC (%) | DF (g/rat/8 days) | NCF (%) | TNF (g/rat/8 days) | AD | TD |
|---|---|---|---|---|---|---|---|---|
| Casein | 111.4 ± 9.0[a] | 1.6± 0.1[a] | 1.8[a] | 12.3±2.2[a] | 2.0±0.6[b] | 0.35±0.0[b] | 98.53 | 86.52 |
| Protein free | 69.3 ± 8.6[b] | 0.4± 0.1[b] | 0.3[b] | 3.5±0.4[b] | 0.3±0.1[b] | 0.01±0.0[b] | - | - |
| CP | 119.3 ± 2.4[a] | 1.6± 0.1[a] | 1.9[a] | 12.9±0.9[a] | 2.1±0.2[b] | 0.27±0.0[b] | 98.94 | 86.39 |
| CMP | 117.7 ± 3.5[a] | 1.6± 0.1[a] | 1.9[a] | 14.9±1.9[a] | 2.8±0.1[b] | 0.41±0.0[b] | 98.26 | 78.72 |
| BMP | 117.3 ± 3.9[a] | 1.6± 0.1[a] | 1.9[a] | 16.2±2.6[a] | 2.0±0.4[b] | 0.52±0.0[b] | 98.42 | 72.87 |

[a-c]Mean values within the same column bearing different superscripts differ significantly (P < 0.05)
DI = diet intake for 8 days
NC = nitrogen content in diet
TNC = total nitrogen consumed in diets for 8 days
DF = weight of dried faecal for 8 days
NCF = nitrogen content in feces
TNF = total nitrogen content in feces for 8 days
AD = apparent digestibility = (NCD-NCF) /NCD X 100
TD = true digestibility = Protein intake – [fecal protein-(metabolic fecal protein/protein intake)] x 100
CP: 100% chicken patty + 0% oyster mushroom
CMP: 75% chicken + 25% oyster mushroom
BMP: 75% beef + 25% oyster mushroom

to casein group (0.4 mmol/L). The dietary fiber (14 g/1000 kcal) has shown remedial effects on serum lipid levels, reducing total cholesterol and LDL-cholesterol amounts (Erkkila and Lichtenstein, 2006; Anderson *et al*., 2009). The formation of viscous gels from soluble dietary fiber such as glucans contribute to inhibition of cholesterol and triglycerol absorption (Marlett *et al*., 2002). The viscous properties of gels are related to an increase in the fecal excretion of bile acids and short-chain fatty acids (Marlett *et al*., 2002). Results of some reports suggest that the hypocholesterolemic effects of some fruiting bodies of edible mushroom could be attributed to the dietary fiber (Guillamón *et al*., 2010). Besides, mushrooms are also containing large amounts of β-glucans polysaccharides, which exhibit hypocholesterolemic and anticoagulant functions (Vetvicka and Vetvickov, 2009).

*Protein efficiency ratio (PER)*

PER bioassay reflects the capacity of a protein to uphold body weight gain of animals. Results of PER are shown in Table 5. PER values of CMP and BMP fed groups were significantly lower than casein group but significantly higher than CP group. Both CMP and BMP fed groups recorded PER values of 1.73 and 1.69 while casein and CM had PER values of 2.05 and 1.52, respectively. This finding is in close agreement with results of Al-Enazi *et al.* (2012), which concluded that diets containing 10% mushroom protein resulted in Protein Efficiency Ratio (PER) of 1.50 compared with value for casein of 2.40. There was no significant difference (P < 0.05) in diet intake and body weight gain between rats fed with either CMP or BMP or CP. However, both diet intake and body weight gain of these groups were significantly lower than Casein group.

Higher amounts of diet intake which recorded during the 28 days of study has increased rat body weight gain. All groups except casein and protein free groups recorded higher total diet intake ranging from 404.9-405.3 g compared to casein (390.7 g) and protein free (242.3 g) during the 28 days of study. These results indicate that protein present in chicken, beef and mushroom play an important roles in enhancing the satiety and growth of rats. Significant intake of isocaloric protein sources from CMP, BMP and CP diets over 28 days have resulted in increment of rats' body weight thus promoted their growth. Edible mushroom has been reported rich in glutamic acid, aspartic acid and arginine but deficient in methionine and lysine (Guillamon *et al*., 2010). In addition, two uncommon amino acids namely γ-amino butyric acid (GABA) and ornithine found in edible mushroom have shown important physiological activities as reported by Guillamon *et al*. (2010). Eventhough the group of rats treated with CMP (91.0 g) and BMP (87.4 g) recorded higher weight gain values than CP diet (80.0 g) but not significant. The present result also shows that the weight gain of rats fed the non-protein diet (protein free) decreased during the entire feeding period, resulting in average weight loss of -32.0 g. This finding accords with those of Al-Enazi *et al.* (2012) who found lower body weight gain (45.1 g) and PER value (1.5) for casein group. The decrease in body weight gain was also reported by other study. Al-Enazi *et al.* (2012) and (Huda *et al.*, 2000) who decomented that the weight gain of rats fed with non-protein diet was -17.1(g) and -17.7(g), respectively. The reduction in body weight non-protein diet (protein free) may due to the insufficient essential amino acids to promote weight gain and rat growth. The growth of the animals recorded in the present study was proportional to the intake of diet. As the results indicate, the oyster mushroom protein combined with both chicken and beef protein was able to promote growth in the rats comparable to the growth in the rats fed with chicken protein alone.

*Apparent digestibility*

Apparent digestibility (AD) was calculated based on the amount of diet intake and nitrogen content from rat faeces accumulated from day 10 until day 28. The results show that AD of rats fed with CMP, BMP and CP diets were closely ranged from 98.3-98.9% (Table 6) but not significant as compared to casein diet group (98.5%). The close AD values between CMP, BMP and CP indicated that the mixture of protein from either chicken or beef with protein of oyster mushroom did not affect digestibility aspect. However, the AD values determined from the present study were slighlty higher than the study done by Hernandez *et al.* (1996). They reported that AD of rats fed with casein diet was the highest (91.4%) compared to the group fed with the other sources of beef cuts (sirloin, liver, round and mixture of round cut with rice, corn, wheat and beans) (Hernandez *et al.*, 1996).

In addition, true digestibility value (TD) of rats fed with CMP, BMP and CP diets were ranging from 72.9-86.4% slightly lower than TD of casein (86.5%). These values are considered high as compared to other study done previously. The values of TD of different mushroom species such as *Pleurotus sajor caju*, *Pleurotus ostreatus*, *Lentinus edodes* and *Lentinus lepidus* were recorded ranging from 47.32-52.16% (Yaovadee *et al.*, 2010). The result of TD from the present study indicated that diet in the form of patty prepared from mixture of protein of either chicken or beef with protein from oyster mushroom were better than diet prepared from mushroom protein alone. The higher levels of TD values determined from the present study might be due to the positive integration between protein from oyster mushroom with either chicken and beef developed into patty formulations. The high percentage of digestibility may be due to the increase in solubility and the denaturation of protein molecules making them more accessible to proteolytic enzymes.

## Conclusions

Diets prepared with patties containing mixture of either chicken or beef with oyster mushroom did not affect the total cholesterol concentrations including HDL-cholesterol concentrations in rat blood serum. In summary, addition of oyster mushroom into either chicken or beef patties did not changed AD but improved PER value, thus proving that oyster mushroom could be used as an alternative ingredient to replace chicken of beef partially in the making of patties. Therefore, the dietary importance of mushrooms is expected to grow in the future years due to the gradual demand of the increasing world population and the interest of reducing the risks related to the consumption of animal foods sources.

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