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PREFACE

In this edition, Volume 27 No 1, we proudly present articles from animal and veterinary sciences including genetics; reproduction, feed technology, and veterinary from scientist all over the world. The articles published in this edition are:
"Milk Yield of Dairy Buffaloes Supplemented with Yeast Solution and Yeast-fermented Cassava Pulp"; "Growth Performance and Cost Benefits of Broilers Fed Diets Containing *Ipomoea asarifolia* Leaf Meal"; "Effect of *In-ovo* Injection of L-Arginine on Hatchability, Chick Quality, Performances and Muscle Histology of Native Chicken"; "Influence of *Tenebrio molitor* L Supplementation on Egg Quality and Omega-3 Content"; "Chemical Content and *In Vitro* Digestibility of Broiler Litter Fermented at Different Ripen Time"; and "Protective Effects of Gallic acid and Curcumin on Serum Levels of Hepatic Transaminases, Blood Plasma Parameters and Pituitary-testicular Hormones in Rats Treated Nickel Nanoparticles".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, March 2022

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Milk Yield of Dairy Buffaloes Supplemented with Yeast Solution and Yeast-fermented Cassava Pulp

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ABSTRAK

Tapdasan EP, Salces CB, Salces AJ. 2021. Produksi susu kerbau yang disuplemenasi larutan ragi teraktifasi dab ransum dengan bubur singkong fermentasi ragi. JITV 27(1):1-9. DOI: http://dx.doi.org/10.13443.

Ketersediaan dan kualitas sumber pakan adalah dua faktor utama yang membatasi produksi susu di Filipina. Pemanfaatan pakan tambahan berbasis mikroba dan produk samping pertanian seperti ampas singkong dengan bantuan teknologi fermentasi dapat membantu menyediakan sumber daya yang dibutuhkan. Penelitian ini bertujuan untuk mengetahui pengaruh larutan ragi aktif (*Saccharomyces cerevisiae*) (AYS) dan bubur singkong fermentasi ragi (YFCP) terhadap produksi susu dan efisiensi biaya pakan pada kerbau perah. Penelitian mengikuti desain RCBD menggunakan 63 ekor kerbau perah di Pusat Carabao Filipina di Ubay Stock Farm, Bohol. Rata-rata produksi susu harian (ADMY) kerbau yang diberi suplemen 0,5L AYS dan 1L AYS dua kali sehari lebih besar daripada kerbau kontrol masing-masing sebesar 0,67L dan 0,69L (P = 0,0039). Sebaliknya, ADMY kerbau yang diberi pakan YFCP dan YFCP+AYS lebih besar dari kerbau kontrol masing-masing sebesar 0,64 dan 0,68L (P = 0,0320). Suplementasi AYS dan pemberian makan YFCP menghasilkan biaya terendah per liter susu yang diproduksi pada PhP 20,25 dan PhP 16,24, masing-masing. Direkomendasikan untuk melengkapi pemerahan kerbau dengan AYS atau memberi makan YFCP di daerah dengan ampas singkong untuk meningkatkan sumber pakan, meningkatkan produksi susu dan meningkatkan efisiensi biaya pakan sehingga meningkatkan pendapatan peternak secara signifikan.

Kata Kunci: Kerbau, Singkong, Susu, Bubur, *Saccharomyces cerevisiae*

ABSTRACT

Tapdasan EP, Salces CB, Salces AJ. 2021. Milk Yield of dairy buffaloes supplemented with activated yeast solution and fed ration with yeast-fermented cassava pulp. JITV 27(1):1-9. DOI: http://dx.doi.org/10.13443.

Feed resource availability and quality are two of the major factors limiting dairy production in the Philippines. Utilization of microbial-based feed additives and agricultural by-products such as cassava pulp aided by fermentation technology can help provide the needed resource. This study aimed to determine the effect of activated yeast (*Saccharomyces cerevisiae*) solution (AYS) and yeast-fermented cassava pulp (YFCP) on milk production and feed cost-efficiency in dairy buffaloes. The study followed RCBD design using 63 dairy buffaloes at the Philippine Carabao Center in Ubay Stock Farm, Bohol. The average daily milk yield (ADMY) of buffaloes supplemented with 0.5L AYS and 1L AYS twice daily were greater than that of control buffaloes by 0.67L and 0.69L, respectively (P = 0.0039).On the other hand, the ADMY of buffaloes fed with YFCP and YFCP+AYS were greater than that of control buffaloes by 0.64 and 0.68L, respectively (P = 0.0320). Supplementation of AYS and feeding YFCP yielded the lowest cost per liter of milk produced at PhP 20.25 and PhP 16.24, respectively. It is recommended to supplement milking dairy buffaloes with AYS or feeding YFCP in areas with cassava pulp to increase feed resource, increase milk production and improve feed cost-efficiency thereby increasing significantly the farmer's income.

Key Words: Buffaloes, Cassava, Milk, Pulp, *Saccharomyces cerevisiae*

INTRODUCTION

Dairying is one of the opportunities that provides livelihood to farmers in the Philippines including the Visayas region. In the latest dairy industry performance report of the Philippine Statistics Authority (PSA 2016), the total dairy animal population in the country in 2015 stood at 44,432 head. This was 6.34 percent higher than in the previous year (2014). Dairy cattle stocks grew by 8.95 percent, while dairy buffalo and dairy goat population went up by 2.91 percent and 6.75 percent, respectively (PSA 2016).

Despite the growth of the dairy animal population, there are some limitations besetting the dairy industry. FAO (2021) reported that one of the major factors limiting dairy production is the poor-quality of feed

resources particularly low digestibility and nutritive value. In tropical countries, ruminants are mostly grazed on pasture areas with low quality grasses and forages, thereby limiting their potential for growth and milk production (Arowolo & He 2018). With this reality, there is a growing interest to improve the quality and digestibility of these feed resources. In light of this, many studies have reported the positive effects of probiotics (Arowolo & He 2018) as a supplement that is known to improve nutrient availability of poor quality feed resources. Probiotics are also known to improve dry matter feed intake and feed conversion efficiency and enhance nutrient utilization efficiency. Moreover, they stimulate and activate immune cells, reducing methane production thereby minimizing energy loss and generally promote growth and health performance as well as meat and milk production in ruminants (Arowolo & He 2018) Considered as probiotics, yeasts (*Saccharomyces cerevisiae*) are microscopic fungi that reproduce by budding and convert sugar into alcohol and carbon dioxide. In the rumen, live yeast consumes oxygen which is favorable for the growth of anaerobic rumen microbes. Feeding live yeast to ruminants has been empirically known to improve productivity, by optimizing the fermentation conditions of the rumen, stabilizing ruminal pH, improving feed efficiency and increasing fiber digestion (Mazzia & Walker 2008; Fortina et al. 2011). Yeasts can improve rumen function by enhancing feed components, thereby improving milk production while ensuring digestive comfort and health of the animal (Maamouri et al. 2014). In Thailand, Wanapat et al. (2011) reported improved performance in terms of milk production and rumen condition of dairy cattle fed with baker's yeast-fermented cassava which replaced soybean oil meal in the diet. In addition, a local study in goats by Abela & Bestil (2013) reported that laboratory cultured yeast supplementation could enhance rumen pH and bacterial count, increase dry matter intake and digestibility of napier grass-concentrate ration. As a result, there was improved growth rate in newly weaned goats.

On the other hand, cassava pulp, a by-product of cassava starch production can be used as animal feeds. The by-product was abundant in Bohol and was used for feeding to dairy buffaloes of the Philippine Carabao Center in Ubay, Bohol. However, according to Aro (2008), its protein content of approximately 2%, deficiency in carotene and high levels of insoluble fiber makes it unable to support high milk production. The use of cassava pulp was appropriate under Bohol conditions since there were plenty of cassava plantations in the province propelled by the presence of a big starch manufacturing company. On the other hand, yeast (*S. cerevisiae*) was widely available in supermarkets being commonly used in bread making. Feeding dairy buffaloes with yeast-fermented cassava pulp was expected to increase milk production and reduce feed cost while maximizing the utilization of the locally available by-product thereby increasing the dairy farmer's income.

There were no reports concerning the effects on the utilization of direct feed microbe and yeast-fermented cassava pulp for dairy buffaloes under Philippine condition. Generally, the study aimed to increase milk production and profitability by the utilization of baker's yeast (*S. cerevisiae*) as a direct feed microbe and yeast-fermented cassava pulp as feeds in dairy buffaloes. Specifically, it aimed to determine the effect of activated yeast solution (AYS) supplementation in milk yield of dairy buffaloes, determine the effect of yeast-fermented cassava pulp (YFCP) feeding on milk yield of dairy buffaloes, and measure the feed cost-efficiency of supplementing AYS and feeding YFCP in dairy buffaloes. Dairy buffalo was used as the study unit since it was the most common dairy animal raised by small hold farmers in the country.

MATERIALS AND METHODS

Location

The study was conducted at the Institutional Farm of the Philippine Carabao Center at Ubay Stock Farm (PCC-USF) in Ubay, Bohol, Philippines. The farm is located at 9°59'34"N and 124°26'26"E (GPS coordinates).

Materials

Cassava pulp used in this experiment was purchased from a starch producing company in Carmen, Bohol (Liwayway Marketing Corporation). Molasses and commercial lactating feeds (Gromax®, Vitarich Corporation, Iloilo, Philippines) were purchased from the province of Cebu. On the other hand, the commercial baker's yeast (Mauripan®) manufactured by Xinjiang Mauri Food Company Limited in Xinjiang, China were sourced out from a local store in Ubay, Bohol.

Raw materials used in preparing cassava pulp-based feeds were sourced out from an accredited supplier of PCC-USF in Lanao del Norte (RM Longcob Enterprises) and Ubay, Bohol (Bohol Agrivet Supply). These raw materials were rice bran, copra meal, soybean meal, coconut oil, commercial vitamin-mineral premix, and mono-dicalcium phosphate. The whole experiment was performed following all applicable national laws on animal welfare.

Methodology to determine the effect of activated yeast solution on milk yield performance

Preparation of activated yeast solution (AYS)

Based on Boonnop et al. (2010) and preliminary trial results, AYS was prepared by mixing 20kg molasses, 100g baker's yeast and 100L clean drinking water. The solution was then stored in clean drums at room temperature and supplied with oxygen daily for twenty four (24) hours using an electric air pump.

Experimental design and supplementation of buffaloes

Twenty-seven (27) lactating Bulgarian Murrah Buffaloes (BMB) in mid-lactation stage (101-200 days in milk), at second to sixth parity, and having an average daily two (2)-week pretrial milk yield of at least 5 L per day were selected in such a way that the stage of lactation and average milk yield of the three treatment groups were more or less similar at the start of the experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) with three treatments: Treatment 1- Control (No supplement), Treatment 2 – 500 mL AYS and Treatment 3 – 1000 mL AYS. There were three blocks per treatment. The blocking factor was date of the start of feeding experiment (Block 1: March 2017, Block 2: April 2017, Block 3: June 2017). All animals were allowed to graze at daytime and fed with napier silage during the night. Customized concentrate feeds (3 kg twice per day) and AYS were given during the morning and afternoon milking at 4:30 am and 2:00 pm, respectively.

Composition of customized concentrate feeds

To prepare the customized concentrate feeds, the following raw materials and their corresponding volume (kg) were mixed uniformly: copra meal (450), yellow corn grits (300), rice bran (145), commercial vitamin-mineral premix (50), molasses (30), tricalcium phosphate (15) and urea (10). The formulation was provided by the resident animal nutritionist of PCC-USF.

Methodology to determine the effect of YFCP on milk yield performance

Preparation of activated yeast solution (AYS)

The AYS was prepared by mixing 20kg of molasses and 100L of water, and added with 100g baker's yeast. The solution was incubated at room temperature and oxygen was supplied by an electric air pump for 24 hours.

Preparation of yeast fermented cassava pulp (YFCP)-based feeds

Cassava pulp, copra meal and other materials prepared according to formulation were mixed thoroughly using a shovel. The total mixed ration consisted of the following raw materials and their corresponding volume (kg): cassava pulp (771.43), copra meal (375), molasses (110), soybean meal (100), rice bran (75), commercial vitamin-mineral premix (40), coconut oil (20), mono-dicalcium phosphate (20) and urea (15). The mixture was then added with 0.5kg yeast dissolved in 32L molasses and 40L water. After 24 hours, ten (10) liters of Lactic Acid Bacteria Serum (LABS) were added to the feed mixture.

Preparation of LABS

To prepare LABS, the following raw materials and their corresponding volume were mixed thoroughly: clean water (200 L), rice wash (96 L) and molasses (32 L).

Experimental design and supplementation of buffaloes

Thirty six (36) BMBs in mid-lactation stage (101-200 days in milk), at second to sixth parity and having an average daily two (2)-week pretrial milk yield of at least 5 L per day were selected in such a way that the stage of lactation and average milk yield of the three treatment groups were more or less similar at the start of the experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) with three treatments: Treatment 1- Control (3 kg commercial feeds, Gromax® by Vitarich Corporation, Iloilo, Philippines), Treatment 2 – 4 kg YFCP and Treatment 3 – 4 kg YFCP+ 1 L AYS. There were three blocks per treatment. The blocking factor was date of the start of feeding experiment (Block 1: January 15, 2018; Block 2: February 17, 2018; Block 3: February 23, 2018). All animals were allowed to graze at daytime and fed with napier silage during the night. The YFCP was fed during the morning and afternoon milking at 4:30 am and 2:00 pm, respectively.

Composition of commercial vitamin-mineral premix

Each 500 g of the commercial vitamin-mineral premix (Essential Vet Laboratories, Inc., Quezon City, Philippines) contained sodium selenite (100 mg), Vitamin A (150,000 IU), Vitamin D_3 (30,000 IU), Vitamin E (500 IU), potassium iodide (100 mg), cobalt sulfate (3 mg), manganese sulfate (3,700 mg), ferrous sulfate (1,600 mg), copper sulfate (1,500 mg) and zinc sulfate (220 mg).

Data gathering and analysis

Daily milk production records of all experimental animals were collected for eight weeks. Data were entered into Microsoft Excel® and analyzed using Statistical Analysis Software (SAS version 9.4, SAS Institute, Cary, NC, USA). A one-way Analysis of Variance (ANOVA) was performed after the data passed the test of homogeneity of variances. The Least Significant Difference (LSD) was then used as a post-hoc test. Mean difference was considered statistically significant at P <0.05.

RESULTS AND DISCUSSION

Effect of activated yeast solution (AYS) on milk yield performance

Figure 1 shows that dairy buffaloes had higher milk yield at the start of the experiment (week 1) then slowly decreased as they reached the succeeding weeks of lactation. But among the three treatments, Treatment 1 (T1) had the highest drop in milk production when compared to Treatments 2 (T2) and 3 (T3) as the eighth week of data collection had been reached. This trend happened because normally, as the lactation advances to the last stage, milk production gradually drops. However, it is important to note that T2 and T3 buffaloes had higher average weekly milk yield as compared with T1 buffaloes or those without AYS supplementation.

After eight weeks of data collection, results showed that the average daily milk yield of T2 and T3 buffaloes were significantly greater than T1 buffaloes (control) by 0.67 L and 0.69 L, respectively (P = 0.0039) (Table 1). However, the results of post-hoc test (LSD) revealed no significant difference between the milk yields of T2 and T3. The result of the study were in consonance with the study of Anjum et al. (2018) in Pakistan wherein yeast supplementation has been proven to improve the milk yield performance of Nili-Ravi buffaloes. In Egypt, the study of Hansen et al. (2017) concluded that supplementation of yeast in buffaloes may result to increase milk yield during the early stage of lactation and would result to a more consistent milk production during the middle stage. Likewise, several authors claimed that yeast supplementation increases milk yield in dairy cows (Campanile et al. 2008; Yalçın et al. 2011; Ayad & Benallou 2013; AlZahal et al. 2014; Maamouri et al. 2014; Jiang et al. 2017)) including buffalo cows (Azzaz et al. 2015). The mechanism by which yeast increases milk yield were also explained by these authors. Yeast balances rumen ecosystem and increases ruminal cellulolytic bacteria (Jouany & Morgavi 2007; Wadhwa & Bakshi 2013; Jiang et al. 2017) thereby improving fiber digestion (Jouany & Morgavi 2007; AlZahal et al. 2014; Jiang et al. 2017; Bakr et al. 2015). Moreover, yeast increases digestibility of organic matter, crude protein, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) (Jiang et al. 2017; Campanile et al. 2008) and lowers blood ammonia level (Campanile et al. 2008; Ogbuewu et al. 2019).

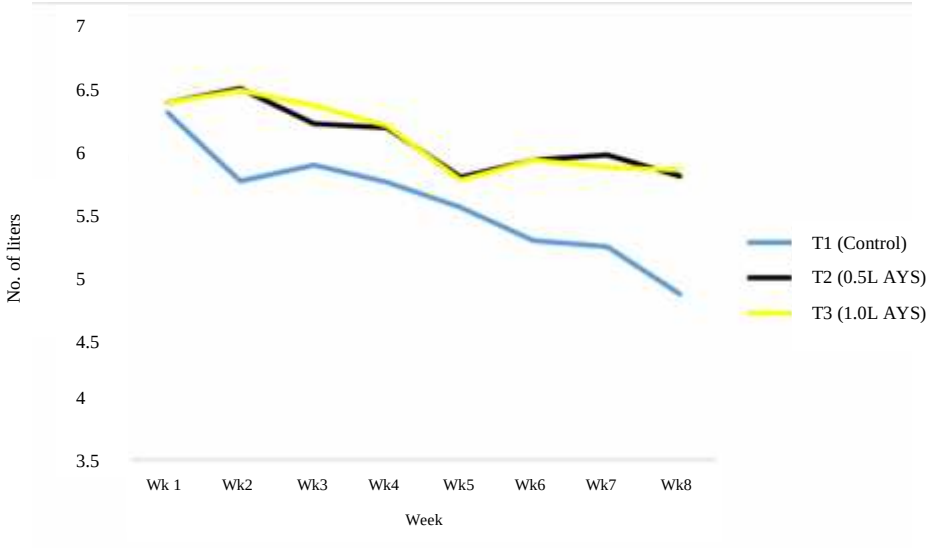

Figure 1. Average weekly milk yield of dairy buffaloes supplemented with 0.5L and 1L of activated yeast solution (AYS)

Aside from being high in crude protein, yeast increases amino acids particularly methionine, phenylalanine, tyrosine, tryptophan and taurine (Yalçın et al. 2011). Yeast is also a source of selenium and Vitamin B-complex (Maamouri et al. 2014) including Vitamin B1 (Ayad & Benallou 2013). A review on the beneficial effects of yeast on the production parameters of livestock including milk yield were compiled by Ogbuewu et al. (2019). Yeast reduces the population of pathogenic microorganisms in the gut, provides direct nutritional benefits and improves intake of feeds and uptake of nutrients from the gut (Ogbuewu et al. 2019).

Table 2 shows the daily total cost of feeding per animal using the three dietary treatments. It was shown that based on the prevailing price in 2018, T1 (Control) was cheaper than T2 by PhP 2.00 and cheaper than T3 by PhP 4.00. However, taking into consideration the cost of feeding to produce one (1) liter of milk per animal, T2 had the lowest cost at PhP 20.25 (Table 3). It was cheaper than T1 and T3 by PhP 2.19 and PhP 0.27, respectively.

To appreciate more the economic benefits of supplementing AYS, Tables 4 and 5 show the total daily cost and daily net income, respectively in a farm with 5 milking cows. Provided that the average daily milk yield (L) per cow in each treatment was the same as in this study, T1 (Control) had the lowest daily cost (Table 4). Treatment 1 was lower than T2 and T3 by PhP 10.02 and PhP 20.10, respectively. However, when the daily net income was considered, T2 generated an income greater than T1 by PhP 207.73 (Table 5). This translates to a higher income for the dairy farmer.

Table 1. Average daily milk yield of dairy buffaloes supplemented with activated yeast (*Saccharomyces cerevisiae*) solution (AYS)

TREATMENT (given twice daily)	N[1]	Initial Milk Yield (L)[2, ns]	Final Milk Yield (L)[3, **]	Standard Deviation
T1- Customized feeds (Control)	9	6.32	5.28[b]	1.06
T2- Customized feeds + 0.5L AYS	9	6.35	5.95[a]	1.06
T3- Customized feeds + 1L AYS	9	6.38	5.97[a]	1.07

ns= *P* value 0.9427, **=*P* value 0.0039, [1]=No. of experimental animals, [2]=Average milk yield a day before the start of the experiment, [3]=Average daily milk yield for eight weeks. Means of the same superscript are not significant from each other

Table 2. Daily cost of feeding for each dietary treatment per animal using customized feeds and activated yeast (*Saccharomyces cerevisiae*) solution (AYS)

Treatment (given twice daily)	Consumed per day[1]	Price per Kilogram or Liter (PhP)[2]	Cost of feeding per day[3]	Total (PhP)[4]
T1- Customized feeds (Control)	6 kg	19.75	118.50	118.50
T2- Customized feeds + 0.5L AYS	6 kg	19.75	118.50	120.50
	1L	2.00	2.00	
T3- Customized feeds + 1L AYS	6 kg	19.75	118.50	122.50
	2 L	2.00	4.00	

[2]=Prevailing market price in 2018, [3]=Product of multiplying item 1 with item 2, [4]=Total cost of feeding per day

Table 3. Cost per liter of milk produced per animal for each dietary treatment using customized feeds and activated yeast (*Saccharomyces cerevisiae*) solution (AYS)

Treatment (given twice daily)	Cost of feeding per day (PhP)[1]	Average daily milk yield (L)[2]	Cost per liter of milk (PhP)[3]
T1- Customized feeds (Control)	118.50	5.28	22.44
T2- Customized feeds + 0.5L AYS	120.50	5.95	20.25
T3- Customized feeds + 1L AYS	122.50	5.97	20.52

[1]=Taken from item 4 of Table 2, [2]=Taken from item 3 of Table 1, [3]=Product of multiplying item 1 with item 2

Table 4. Estimated daily cost per treatment in a farm with five (5) milking cows fed with customized feeds and supplemented with activated yeast (*Saccharomyces cerevisiae*) solution (AYS)

Treatment (given twice daily)	Average daily milk yield (L)[1]	Number of milking cows[2]	Total milk volume (L)[3]	Cost per liter produced (PhP)[4]	Total daily cost (PhP)[5]
T1- Customized feeds (Control)	5.28	5	26.40	22.44	592.416
T2- Customized feeds + 0.5L AYS	5.95	5	29.75	20.25	602.44
T3- Customized feeds + 1L AYS	5.97	5	29.85	20.52	612.52

[1]=Taken from item 3 of Table 1, [2]=Hypothetical no. of cows in a farm, [3]=Product of item 1 multiplied by item 2, [4]=Taken from item 3 of Table 3, [5]=Product of multiplying item 3 with item 4

Table 5. Estimated daily net income per treatment in a farm with five (5) milking cows fed with customized feeds and supplemented with activated yeast (*Saccharomyces cerevisiae*) solution (AYS)

Treatment (given twice daily)	Average daily milk yield (L)[1]	No. of milking cows[2]	Total milk volume (L)[3]	Price per liter of milk (PhP)[4]	Gross income (PhP)[5]	Total daily cost (PhP)[6]	Net income (PhP)[7]
T1- Customized feeds (Control)	5.28	5	26.40	65.00	1,716.00	592.42	1,123.58
T2- Customized feeds + 0.5L AYS	5.95	5	29.75	65.00	1,933.75	602.44	1,331.31
T3- Customized feeds + 1L AYS	5.97	5	29.85	65.00	1,940.25	612.52	1,327.73

[1]= Taken from item 3 of Table 1, [2] = Hypothetical number of cows in farm, [3]= Product of multiplying item 1 with item 2, [4]= Prevailing market price of raw milk in the province of Bohol, [5]= Product of multiplying item 3 with item 4, [6]=Taken from item 5 of Table 4, [7]= Difference of subtracting item 6 from item 5

Effect of yeast-fermented cassava pulp (YFCP) on milk yield performance

After eight (8) weeks of data collection, results showed that the average daily milk yield of T2 and T3 buffaloes were significantly greater than T1 (control) buffaloes by 0.64 L and 0.68 L, respectively (P = 0.0320) (Table 6). However, the results of post-hoc test (LSD) revealed no significant difference between the milk yields of T2 and T3. The addition of yeast as fermentative agent in cassava pulp has numerous beneficial effects in the rumen microbial ecosystem resulting to positive effects in milk production. In the study of Sommai et al. (2020), YFCP increased the population of cellulolytic, amylolytic and proteolytic bacteria. These bacteria are important for the digestion of cellulose and synthesis of protein. Feeding of YFCP could also remarkably enhance Organic Matter (OM) and Crude Protein (CP) resulting to increased dry matter intake (Sommai et al. 2020) Moreover, the use of YFCP at high level could result in highest nitrogen-balance and nitrogen-retention absorption (Sommai et al. 2020). Chuelong et al. (2011) also reported that YFCP could improve ruminal fermentation efficiency as manifested in cross-bred native cattle of Thailand. Moreover, Polyorach et al. (2013) revealed that yeast-fermented cassava chip could contain high protein up to 32.5%. Chuelong et al. (2011) also added that cassava pulp contains high soluble fractions of starch and protein nitrogen and can be added in diets to increase utilization of ruminal ammonia-N for microbial protein synthesis. All these mechanisms contributed significantly to high milk production in dairy animals.

Table 7 shows the daily total cost of feeding per animal using the three dietary treatments. It was shown that based on the prevailing price in 2018, T2 was cheaper than T1 (control) by PhP 42.64 and cheaper than T3 by PhP 14.80. Taking into consideration the cost of feeding to produce one (1) liter of milk per animal, T2 had the lowest cost at PhP 16.24 (Table 8). It was cheaper than T1 and T3 by PhP 10.15 and PhP 2.04, respectively.

To appreciate more the economic benefits of feeding YFCP, Tables 9 and 10 show the total daily cost and daily net income, respectively in a farm with 5 milking cows. Provided that the average daily milk yield (L) per cow in each treatment was the same as in this study, T2 had the lowest daily cost (Table 9). Treatment 2 was lower than T1 (Control) and T3 by PhP 213.46 and PhP 74.17, respectively. If the daily net income was considered, T2 generated an income greater than T1 by PhP 421.46 (Table 10). This translates to a much higher income for the dairy farmers.

Table 6. Average daily milk yield of dairy buffaloes fed ration with commercial feeds and yeast-fermented cassava pulp (YFCP)

Treatment	N[1]	Initial Milk Yield (L)[2, ns]	Mean milk yield (L)[3, *]	Standard Deviation
T1- Commercial Feeds (Control)	12	6.02	5.23[b]	1.40
T2 – YFCP	12	6.79	5.87[a]	1.88
T3 – YFCP+AYS	12	6.83	5.91[a]	1.51

Ns= *P* value 0.239, *= *P* value 0.0320, [2]= Average milk yield a day before the start of the experiment, [3]= Average daily milk yield for eight weeks, means of the same superscript are not significant from each other, AYS = activated yeast (*Saccharomyces cerevisiae*) solution

Table 7. Daily cost of feeding for each dietary treatment per animal using commercial feeds and yeast-fermented cassava pulp (YFCP)

Treatment	Consumed per day[1]	Price per Kilogram or Liter (PhP)[2]	Cost of feeding per day[3]	Total (PhP)[4]
T1- Commercial Feeds (Control)	6 kg	23.00	138.00	138.00
T2- YFCP	8 kg	11.92	95.36	95.36
T3- YFCP + AYS	8 kg	11.92	95.36	110.16
	2 L	7.40	14.8	

[2]= Prevailing market price in 2018, [3]= Product of multiplying item 1 with item 2, [4]= Total cost of feeding per day, AYS = activated yeast (*Saccharomyces cerevisiae*) solution

Table 8. Cost per liter of milk produced per animal for each dietary treatment using commercial feeds and yeast-fermented cassava pulp (YFCP)

Treatment	Cost of feeding per day (PhP)[1]	Average daily milk yield (L)[2]	Cost per liter of milk (PhP)[3]
T1- Commercial Feeds (Control)	138.00	5.23	26.39
T2- YFCP	95.36	5.87	16.24
T3- YFCP + AYS	110.16	5.91	18.64

[1]= Taken from item 4 of Table 7, [2]= Taken from item 3 of Table 6, [3]= Product of multiplying item 1 with item 2.

Table 9. Estimated daily cost per treatment in a farm with five (5) milking cows fed with commercial feeds and yeast-fermented cassava pulp (YFCP)

Treatment	Average daily milk yield (L)[1]	No. of milking cows[2]	Total milk volume (L)[3]	Cost per liter produced (PhP)[4]	Total daily cost (PhP)[5]
T1- Commercial Feeds (Control)	5.23	5	26.15	26.39	690.10
T2- YFCP	5.87	5	29.35	16.24	476.64
T3- YFCP + AYS	5.91	5	29.55	18.64	550.81

[1]= Taken from item 3 of Table 6, [2]= Hypothetical no. of cows in a farm, [3]= Product of item 1 multiplied by item 2, [4]= Taken from item 3 of Table 8, [5]= Product of multiplying item 3 with item 4

Table 10. Estimated daily net income per treatment in a farm with five (5) milking cows fed with commercial feeds and yeast-fermented cassava pulp (YFCP)

Treatment	Average daily milk yield (L)[1]	Number of milking cows[2]	Total milk volume (L)[3]	Price per liter of milk (PhP)[4]	Gross income (PhP)[5]	Total daily cost (PhP)[6]	Net income (PhP)[7]
T1- Commercial Feeds (Control)	5.23	5	26.15	65.00	1,699.75	690.10	1,009.65
T2- YFCP	5.87	5	29.35	65.00	1,907.75	476.64	1,431.11
T3- YFCP + AYS	5.91	5	29.55	65.00	1,920.75	550.81	1,369.94

[1]Taken from item 3 of Table 6, [2]= Hypothetical no. of cows in a farm, [3]= Product of item 1 multiplied by item 2, [4]= Taken from item 3 of Table 9, [5]= Product of multiplying item 3 with item 4

CONCLUSION

Based on the results of the study, it is recommended to supplement milking buffaloes with AYS at a rate of 0.5 L twice a day to increase average daily milk yield by at least 0.67L per cow and improve feed cost efficiency. In areas with abundant cassava pulp, feeding milking dairy buffaloes with YFCP is recommended also to increase feed resource, increase average daily milk yield by at least 0.64L per cow and improve feed cost-efficiency thereby increasing significantly the dairy farmer's income.

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REFERENCES

Abela M, Bestil L. 2013. Effects of cultured yeast supplementation on growing kids fed with napier grass-concentrate ration. Sci Humanit J. 10:1–15.

AlZahal O, Dionissopoulos L, Laarman AH, Walker N, McBride BW. 2014. Active dry *Saccharomyces* cerevisiae can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. J Dairy Sci. 97:7751–7763.

Anjum MI, Javaid S, Ansar MS, Ghaffar A. 2018. Effects of yeast (*Saccharomyces cerevisiae*) supplementation on intake, digestibility, rumen fermentation and milk yield in Nili-Ravi buffaloes. Iran J Vet Res. 19:96–100.

Aro S. 2008. Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. African J Biotechnol. 7:4789–4797.

Arowolo MA, He J. 2018. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. Anim Nutr. 4:241–249.

Ayad M, Benallou B. 2013. Impact of feeding yeast culture on milk yield, milk components, and blood components in Algerian dairy herds. J Vet Sci Technol. 4:1–5.

Azzaz H, Ebeid H, Morsy T, Kholif S. 2015. Impact of feeding yeast culture or yeast culture and propionibacteria 169 on the productive performance of lactating buffaloes. Int J Dairy Sci. 10:107–116.

Bakr HA, Hassan MS, Giadinis ND, Panousis N, Ostojic-Andric D, El-Tawab A, Bojkovski J. 2015. Effect of *Saccharomyces cerevisiae* supplementation on health and performance of dairy cows during transition and early lactation period. Biotechnol Anim Husb. 31:349–364.

Boonnop K, Wanapat M, Navanukraw C. 2010. Replacement of soybean meal by yeast fermented-cassava chip protein (YEFECAP) in concentrate diets fed on rumen fermentation, microbial population and nutrient digestibilities in ruminants. J Anim Vet Adv. 9:1727–1734.

Campanile G, Zicarelli F, Vecchio D, Pacelli C, Neglia G, Balestrieri A, Di Palo R, Infascelli F. 2008. Effects of *Saccharomyces cerevisiae* on in vivo organic matter digestibility and milk yield in buffalo cows. Livest Sci. 114:358–361.

Chuelong S, Siriuthane T, Polsit K, Ittharat S, Koatdoke U, Cherdthong A, Khampa S. 2011. Supplementation levels of palm oil in yeast (*Saccharomyces cerevisiae*) culture fermented cassava pulp on rumen fermentation and average daily gain in crossbred native cattle. Pakistan J Nutr. 10:1115–1120.

[FAO] Food and Agriculture Organization. 2021. Gateway to dairy production and products. Food Agric Organ United Nations. [accessed August 10th

2021]. https://www.fao.org/dairy-production-products/production/feed-resources/en/.

Fortina R, Battaglini LM, Opsi F, Tassone S, Renna M, Mimosi A. 2011. Effects of inactivated yeast culture on rumen fermentation and performance of mid-lactation dairy cows. J Anim Vet Adv. 10:577–580.

Hansen HH, El-Bordeny NE, Ebeid HM. 2017. Response of primiparous and multiparous buffaloes to yeast culture supplementation during early and mid-lactation. Anim Nutr. 3:411–418.

Jiang Y, Ogunade IM, Arriola KG, Qi M, Vyas D, Staples CR, Adesogan AT. 2017. Effects of the dose and viability of *Saccharomyces cerevisiae*. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. J Dairy Sci. 100:8102–8118.

Jouany J-P, Morgavi DP. 2007. Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production. Animal. 1:1443–1466.

Maamouri O, Selmi H, M'hamdi N. 2014. Effects of yeast (*Saccharomyces cerevisiae*) feed supplement on milk production and its composition in Tunisian Holstein Friesian cows. Sci Agric Bohem. 45:170–174.

Mazzia M, Walker N. 2008. Using live yeast to unlock fiber potential. Alberta Beef Magazine Ltd. Nov. Series. France: Lallemand Animal Nutrition. 1.

Ogbuewu IP, Okoro VM, Mbajiorgu EF, Mbajiorgu CA. 2019. Yeast (*Saccharomyces cerevisiae*) and its effect on production indices of livestock and poultry—a review. Comp Clin Path. 28:669–677. DOI: 10.1007/s00580-018-2862-7.

Polyorach S, Wanapat M, Wanapat S. 2013. Enrichment of protein content in cassava (*Manihot esculenta* Crantz)by supplementing with yeast for use as animal feed. Emirates Journal of Food and Agriculture. 142-149.

[PSA] Philippine Statistics Authority. 2016. Dairy Industry Performance Report, January – December 2015. Quezon City (Philiphine): Philippine Statistics Authority. 1-11

Sommai S, Ampapon T, Mapato C, Totakul P, Viennasay B, Matra M, Wanapat M. 2020. Replacing soybean meal with yeast-fermented cassava pulp (YFCP) on feed intake, nutrient digestibilities, rumen microorganism, fermentation, and N-balance in Thai native beef cattle. Trop Anim Health Prod. 52:2035–2041. DOI: 10.1007/s11250-020-02228-3.

Wadhwa M, Bakshi MPS. 2013. Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. Food and Agriculture Organization of the United Nations–Regional Office for Asia and the Pacific (FAO-RAP) Publication. 4:1-67.

Wanapat M, Polyorach S, Chanthakhoun V, Sornsongnern N. 2011. Yeast-fermented cassava chip protein (YEFECAP) concentrate for lactating dairy cows fed on urea–lime treated rice straw. Livest Sci. 139:258–263.

Yalçın Sakine, Yalçın Suzan, Can P, Gürdal AO, Bağcı C, Eltan Ö. 2011. The nutritive value of live yeast culture (*Saccharomyces cerevisiae*) and its effect on milk yield, milk composition and some blood parameters of dairy cows. Asian-Australasian J Anim Sci. 24:1377–1385. DOI: 10.5713/ajas.2011.11060.

Growth Performance and Cost Benefits of Broilers Fed Diets Containing *Ipomoea asarifolia* Leaf Meal

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ABSTRAK

Leonard UC, Charles OO, Caleb EI, Esther CC. 2022. Performa pertumbuhan dan keuntungan ayam broiler yang diberi pakan mengandung tepung daun *Ipomoea asarifolia*. JITV 27(1):10-17. DOI: http//dx.doi.org/10.14334/jitv.v27i1.2944.

Percobaan dilakukan untuk melihat performa pertumbuhan ayam pedaging dan keuntungan dari pemberian pakan dengan penambahan tepung daun *Ipomoea asarifolia* (CIALM). Tepung daun *Ipomoea asarifolia* sebanyak 0; 2,5; 5 dan 7,5% secara berurutan dimasukkan ke dalam 8 pakan untuk membentuk perlakuan T1, T2, T3 dan T4 untuk fase *starter* dan *finisher*. Seratus dua puluh ekor ayam pedaging digunakan dalam percobaan selama delapan minggu dan dirancang dengan Rancangan Acak Lengkap. Dilakukan penghitungan untuk konsumsi pakan harian, pertambahan berat badan mingguan, rata-rata konsumsi pakan harian, rata-rata pertambahan berat badan harian, total pakan yang dikonsumsi, rasio konversi pakan dan parameter *cost benefit*. Pada fase *starter* menunjukkan perbedaan (P<0,05) antar rerata perlakuan pada semua parameter kecuali bobot awal. Terdapat perbedaan (p<0,05) antara rata-rata perlakuan dalam semua parameter yang diukur selama fase *finisher* termasuk parameter analisis biaya. Berbeda dengan biaya pakan yang menunjukan penurunan dengan meningkatnya kadar CIALM. Keuntungan bersih menurun pada perlakuan T1 dan T4 yang disebabkan oleh penurunan total pertambahan berat badan. Pertambahan berat akhir, pertambahan berat total, rata-rata konsumsi pakan harian, konsumsi pakan total, rata-rata pertambahan bobot harian, rasio konversi pakan, biaya pakan dan biaya pakan per kg kenaikan berat badan menurun dengan meningkatnya kadar CIALM. Hasil yang diperoleh dalam percobaan ini menunjukkan bahwa pakan kontrol lebih baik daripada pakan dengan perlakuan. Hasil yang positif diakibatkan oleh tingkat inklusi yang lebih rendah yaitu kurang dari 2,5%.

Kata Kunci; Ayam Pedaging, Keuntungan, Performa Pertumbuhan, *Ipomoea asarifolia*

ABSTRACT

Leonard UC, Charles OO, Caleb EI, Esther CC. 2022. Growth performance and cost benefits of broilers fed diets containing *Ipomoea asarifolia* leaf meal. JITV 27(1):10-17. DOI: http//dx.doi.org/10.14334/jitv.v27i1.2944.

This experiment was conducted to determine growth performance and cost benefit of including cooked *Ipomoea asarifolia* leaf meal (CIALM) in broiler diets. Eight diets were compounded by including *Ipomoea asarifolia* leaf meal at 0, 2.5, 5 and 7.5% to form T1, T2, T3 and T4 respectively of both starter and finisher diets. One hundred and twenty broiler birds were used in a Complete Randomized Design experiment that lasted for eight weeks. Daily feed intake and weekly weight gain were measured. Average daily feed intake, average daily weight gain, total feed consumed, feed conversion ratio and cost benefit parameters were calculated. In starter phase results showed that there were significant differences (P<0.05) between treatment means in all the parameters except initial weight of the birds. There were significant differences (p<0.05) between treatment means in all the parameters measured during the finisher phase including the cost analysis parameters except that feed cost decreasing with increasing levels of CIALM and net profit decreased from T1 to T4 because of the decrease in total weight gain from T1 to T4. Final weight gain, total weight gain, average daily feed intake, total feed intake, average daily weight gain, feed conversion ratio, feed cost and feed cost per kg weight gain all decreased with increasing levels of CIALM. Results control diet performed better than other treatment diets which suggest that lower levels of inclusion that is less than 2.5% may give positive results.

Key Words; Broiler Chicken, Cost Benefit, Growth Performance, *Ipomoea asarifolia*

INTRODUCTION

The science of nutrition involves providing a balance of nutrients that best meets the need of an animal for optimal growth and effective metabolic activities. For economic reasons, the supply of nutrients should be at the least possible cost. Therefore, other feed alternatives that could possibly meet the nutrient requirement at a cheaper rate should be opted for (Ranjhan 2001).

Food and Agricultural organization (FAO 2010) estimated that the average Nigerian consumes 51g of

protein per day which is less than the recommended 86g per day. The animal protein shortage in the diets of Nigerians and people from developing countries is now a matter of urgent concern and measures to save people from imminent protein malnutrition are imperative. It therefore becomes essential to increase animal production in order to meet the recommended daily protein need.

In broiler production, the cost of getting the normal conventional feed sources is so expensive and keeps rising by the day. This has become more uneconomical for the farmer as this alters the main aim of the farmer which is profit maximization, by using readily and cheap available feed sources during production. Studies have shown that providing a balanced broiler feed is one of the challenges of the farmer. This has contributed to the recent decline in poultry production. (Esonu et al. 2001), stated that more than 50% of Nigerian farmers have closed down and another 30% forced to reduce production capacity due to shortage of feed.

The present shortage of animal feed have been blamed on the ever increasing cost of feed ingredients which (Esonu et al. 2001), and Madubuike & Ekenyem (2001) have rated at 70% to 80% arising from protein sources. This is as a result of increasing competition between man and animal for available grains (Ahitey & Flake 2008; Aviagen 2019). Poultry feed diets consist of carbohydrate and protein of both plant and animal protein sources, mineral, vitamins and fats and these are also required in the human diet.

The protein deficit in livestock production in Nigeria and most developing countries of the world is now a matter of urgent concern and measures are being put in place to save people from imminent protein malnutrition. Nwakpu et al. (2010); Ekenyem (2002) and Esonu et al. (2003), have suggested that immediate search for cheap and readily available protein and energy sources particularly those that are not competed for between man and animal is important.

The recommended policy is to identify and use locally available feed resources to formulate diets that are as balanced as possible (Kakengi et al. 2007). There is the need, therefore, to explore the use of non-conventional feed sources like *Ipomoea asarifolia* leaf meal (CIALM) that have the capacity to yield the same output as conventional feeds, and perhaps at cheaper cost.

The economization of feed cost using cheaper and unconventional feed resources is an important aspect of commercial poultry production (Muriu et al. 2002). This strategy could help to reduce the cost of production, and ensure cheaper meat production thereby freeing up the major crops for human consumption (Olugbemi et al. 2010).

These Non-conventional feed sources can be put to use for animal feed formulation as there is no competition between animals and humans for these feed stuffs. In this direction, Esonu et al. (2003) have suggested that leaf meal of tropical legumes and browse plants should be used as potential sources of cheap animal feed source. Leaf meals do not only provide protein, but also some essential vitamins such as vitamins A and C, minerals, etc. (Gadzirayi et al. 2011).

This trial seeks to assess the effects of varying dietary inclusion levels of cooked *Ipomoea asarifolia* leaf meal (CIALM) in the growth performance and carcass quality of broiler. In Nigeria, the leaf of *Ipomoea asarifolia* has no known food value and thus popularly used as compost material, mulch, as well as constituting weed in farms. Thus with a crude protein level of 32%, metabolizable energy of 2760kcal/kg and good mineral profile, *Ipomoea asarifolia* leaf has the potential as a cheap feed ingredient for broilers. However, this great potential which could transform our poultry production industry has hardly been utilized. Cooking has also been seen as a way of reducing the level of anti-growth factors as reported by (El-Adawy 2002). There seems to be lack of information regarding its usage and possible effects on poultry production. There is therefore a high and urgent need to carry out a comprehensive study on this regard. This study would enlighten poultry producers on the potentials of adding *Ipomoea asarifolia* leaf meal in the diets of broiler.

MATERIALS AND METHODS

Location and duration of experiment

The experiment was carried out at the teaching and research Poultry farm of the Department of Animal Science and technology, Faculty of Agriculture, Nnamdi Azikiwe University Awka, Anambra State Nigeria. The experiment lasted for a period of 2 months. The location lies between latitude 6.24˚N and 6.28˚N and Longitude 7.00˚E and 7.08˚E on the South Eastern part of Nigeria. The climate is the tropical wet and dry type with clearly distinguished seasons. The mean daily maximum temperature is usually 27˚c all year round although it could get to as high as 34˚C in March and lowest during the harmattan season between December and January. The mean annual rainfall according to the Local Meteorological station which has maintained records since 1978 is about 1600mm with a relative humidity of about 80% at dawn.

Preparation of cooked *Ipomoea asarifolia* leaf meal (CIALM)

Fresh *Ipomoea asarifolia* leaves were harvested green from the bush prior to flowering. The leaves were washed, chopped to facilitate easy boiling and then it

Table 1. Composition of broiler starter diets

Ingredients	T1	T2	T3	T4
Maize, (%)	44	44	44	44
Wheat offal, (%)	8.3	7.3	5.8	4.3
Brewers dry grain, (%)	4.0	4.0	4.0	4.0
Soya bean meal, (%)	21.2	20	19.2	19.0
Leaf meal (CIALM), (%)	0.00	2.50	5.00	7.50
Groundnut cake, (%)	11.5	11.2	11	10.2
Fish meal, (%)	4	4	4	4.0
Bone meal, (%)	2	2	2	2
Oyster shell, (%)	3	3	3	3
Iodized salt, (%)	0.25	0.25	0.25	0.25
Lysine, (%)	0.25	0.25	0.25	0.25
Methionine, (%)	0.25	0.25	0.25	0.25
Premix, (%)	0.25	0.25	0.25	0.25
Mycotoxin binder, (%)	1	1	1	1
Calculated chemical composition (DM)				
Total, (%)	100	100	100	100
Crude protein, (%)	22.5	22.214	22.034	21.835
Crude fiber, (%)	4.39	5.34	6.28	7.41

T1= 0% tepung daun *Ipomoea asarifolia*, T2= 2.5% tepung daun *Ipomoea asarifolia*, T3= 5% tepung daun *Ipomoea asarifolia*, T4= 7.5% tepung daun *Ipomoea asarifolia*

was boiled at 100^{o}C for 2 minutes and immediately sun-dried by spreading under the sun (Ncube et al. 2015). The dried leaves were then milled, sieved and incorporated into the treatment diets.

Experimental diets

Eight experimental diets were formulated by including CIALM at 0, 2.5, 5, and 7.5% levels to form T1, T2, T3, and T4 for both starter and finisher diets respectively (Table 1 and Table 2).

Experimental design and experimental birds

Complete Randomized Design (CRD) was adopted for this study. A total of 120 broiler chicks were used for this experiment. The birds were grouped into four treatments with 30 birds per treatment and three replications of 10 birds each. The four treatments were tagged T1, T2, T3 and T4 containing cooked *Ipomea asarifolia* leaf meal (CIALM) at 0, 2.5, 5, and 7.5% at dry matter bases, respectively. The experiment was conducted in two phases; phase one and phase two. The phase one experiment lasted for a period of 0 to 4 weeks and the broilers were fed with broiler starter while the phase two was between 4 weeks to 8 weeks and the broilers were fed with the broiler finisher diet.

Vaccination and medication

A vaccination program was planned and followed carefully. The procedure for vaccination was recommended by the Veterinary Services and the dosage was according to the manufacturer's specifications.

Housing and management

The birds were housed in deep litter pens. Wood shavings were used as litter material for the birds. To ensure a clean bedding material at all times, the litter material was changed fortnightly. Each bird had an average floor space of 1.3sq ft. (0.397 m^2). Heating system for the brooding periods was charcoal pots.

Feeding and Watering

The birds were given a weighed quantity of feed every morning but were having an ad libitum access to both feed and water.

Parameters that were measured

The parameters that were measured include: daily feed consumption (feed intake) and weekly body weight.

Table 2. Composition of broiler finisher diet

Ingredients	T1	T2	T3	T4
Maize, (%)	50	49	48	47
Full fat soya, (%)	5	5	5	5
Brewers dry grain, (%)	10	9.5	9	8.5
Soya bean meal, (%)	16	15	14	13
Leaf meal (CIALM), (%)	0	2.5	5	7.5
Groundnut cake, (%)	7.0	7.0	7.0	7.0
Fish meal, (%)	3	3	3	3
Bone meal, (%)	3	3	3	3
Limestone, (%)	3	3	3	3
Iodized salt, (%)	0.5	0.5	0.5	0.5
Lysine, (%)	0.5	0.5	0.5	0.5
Methionine, (%)	0.5	0.5	0.5	0.5
Premix, (%)	0.5	0.5	0.5	0.5
Mycotoxin binder, (%)	1	1	1	1
Calculated chemical composition (DM)				
Total, (%)	100	100	100	100
Crude protein, (%)	19.93	19.8	19.67	19.54
Crude fiber, (%)	4.73	5.67	6.64	7.925

T1= 0% tepung daun *Ipomoea asarifolia*, T2= 2.5% tepung daun *Ipomoea asarifolia*, T3= 5% tepung daun *Ipomoea asarifolia*, T4= 7.5% tepung daun *Ipomoea asarifolia*

Calculated parameters

The calculated parameters were: average daily feed intake, average daily weight gain, total feed consumed, feed conversion ratio, feed cost, cost of feed per kg weight gain, total cost of production, gross profit, net profit, economic efficiency and return on investment

Statistical analysis

Data collected was computed and subjected to analysis of variance (ANOVA) using SPSS software and differences between means were separated using the Duncan's (1955) multiple range test at significance level of 5%.

Animal ethics and welfare

The experiment was performed according to animal ethics and welfare of Nnamdi Azikiwe University Awka.

RESULTS AND DISCUSSION

Proximate and phytochemical composition of cooked *Ipomoea asarifolia* leaf meal

The proximate and phytochemical composition of cooked *Ipomoea asarifolia* leaf meal is presented in Table 3 and Table 4 respectively. The proximate analysis of the cooked *Ipomoea asarifolia* leaf meal (CIALM) showed that the crude protein reduced from 32% to 18.8% thus disagreeing with Frias et al. (2000) who reported that Cooking pods in water, with or without pressure increases the protein quality. It can also be seen from the results that there was reduction in the ash, fat and carbohydrate levels of the cooked *Ipomoea asarifolia* leaf meal (CIALM) when compared to the uncooked one and this is in agreement with El-El-Adawy (2002) who reported that cooking caused significant decreases in protein, fat, total ash, carbohydrate fractions and anti-nutritional factors.

The result of the phytochemical analysis of cooked *Ipomoea asarifolia* leaf meal also showed a considerable decrease in the anti-nutritional factors and this is in agreement with El-Adawy (2002) who reported that cooking caused a significant decrease in anti-nutritional factors.

Growth performance of birds fed diets containing cooked *Ipomoea asarifolia* leaf meal during the starter phase

Table 5 shows the effect of cooked *Ipomoea asarifolia* leaf meal (CALM) on the growth performance of broiler during the starter phase. The result of the starter phase shows that there was no significant difference ($p>0.05$) in the initial weight of the birds. However significant differences ($p<0.05$)

Table 3. Proximate analysis of cooked *Ipomoea asarifolia* leaf meal (CIALM)

Nutrient	(%)
Moisture content	12.5
Ash	9.25
Fat	1.0
Fiber	45
Protein	18.33
Carbohydrate	13.67

Table 4. Phytochemical analysis of cooked *Ipomoea asarifolia* leaf meal (CIALM)

Phytochemical	(%)
Flavonoid	4.70
Alkaloid	4.50
Pytate	5.0
Tannin	0.87
Saponin	1.60

existed among treatment means on the final weight, total weight gain, average daily weight gain, total feed intake, average daily feed intake, feed conversion ratio, feed cost and feed cost per kg weight gain. The final weight, total weight gain, average daily weight gain, total feed intake, feed cost and average daily feed intake decreased with increasing levels of cooked *Ipomoea asarifolia* leaf meal. However, no significant difference ($p>0.05$) existed between treatment means of T1 (0% CIALM) and T2 (2.5% CIALM) but significant differences ($p<0.05$) existed between treatment means of T3(5%) and T4(7.5% CIALM). The results of feed conversion ratio and feed cost per kg weight gain showed that T3 (5% CIALM) was significantly different ($p<0.05$) and better than other treatments means while T1 (0% CIALM), T2 (2.5% CIALM) and T4 (7.5% CIALM) showed no significant difference ($p>0.05$) between treatment means of the feed conversion ratio and feed cost per kg weight gain. This means that T3 (5% CIALM) was able to utilize feed efficiently when compared to T1 (0% CIALM), T2 (2.5% CIALM) and T4 (7.5% CIALM). As a result of this, the feed cost per kg weight gain of T3 (5% CIALM) was lower than T1 (0% CIALM), T2 (2.5% CIALM), and T4 (7.5% CIALM) thus implying that it cost less to produce a kg of feed in T3 (5% CIALM). This means that even though T3 (5% CIALM) consumed less when compared to T1 (0% CIALM) and T2 (2.5% CIALM) and weighed less than them, they were able to utilize their feed more efficiently and thus cost less. This agreed with the work of (Ferket & Gernat 2006), who reported that feed intake influences final body weight and feed conversion.

Growth performance of birds fed diets containing cooked *Ipomoea asarifolia* leaf meal (CIALM) during the finisher phase

Table 6 shows the growth performance of broiler fed diets containing cooked *Ipomoea asarifolia* leaf meal (CALM) at the finisher phase.

There was no significant difference ($p>0.05$) in the feed cost per kg weight gain among treatment means but significant differences ($p<0.05$) existed between treatment means of the final weight gain, the total weight gain, the average daily feed intake, the total feed intake, feed conversion ratio and feed cost with T1(0% CIALM) being significantly higher than other treatments, followed by T2(2.5% CIALM), T3(5% CIALM) and T4(7.5% CIALM) respectively. This means that there was a decrease in final weight gain, the total weight gain, the average daily feed intake, the total feed intake, feed conversion ratio and feed cost as the level of inclusion increased. The poor performances of the birds fed cooked *Ipomoea asarifolia* leaf meal can be attributed to the high crude fiber content in the feed. Generally, the use of leaf meals in broiler diets may be limited by their high content of crude fiber (Santoso & Sartini 2001; Ubua et al. 2019) but cooking it seemed to increase the crude fiber content even more which is in agreement with Emenike et al. (2016), Rehinan et al. (2004) who reported that cooking increases dietary fiber content and also induces losses of vitamins and minerals. These poor performances can be attributed to the fiber content of the feed, increased crude fiber in the cooked *Ipomoea asarifolia* leaf meals which increased the fiber content of the feed. Tesfaye et al. (2013) suggested that the increased fiber content in rations with the increased levels of leaf meal may consequently depress feed intake and thus growth performance of broilers. The fiber may increase the bulkiness in the gastrointestinal tract and thereby reduce feed consumption (Buragohain 2016).

This experiment of adding cooked *Ipomoea asarifolia* leaf meal can be compared with uncooked *Ipomoea asarifolia* leaf meal with reference to the work done by Ekenyem & Madubuike (2005). It can be seen that the uncooked *Ipomoea asarifolia* leaf meal though containing high level of phytochemical seemed to perform better than the cooked *Ipomoea asarifolia* leaf meal at inclusion levels of 0%, 5%, 10% and 15%. This was even at higher treatment levels than the cooked *Ipomoea asarifolia* leaf meal of inclusion levels of 0%, 2.5%, 5% and 7.5%, and this can particularly be attributed to the high fiber content of the cooked *Ipomoea asarifolia* leaf meal which made up almost 50 percent of the leaf meal and this produced a harmful

Table 5. Growth performance of birds fed diets containing cooked *Ipomoea asarifolia* leaf meal (CIALM) during the starter phase

Parameters	T1	T2	T3	T4	SEM	Pvalue
Initial weight, (g/bird)	38.733	38.60	38.57	38.87	0.29	0.988
Final weight gain,(g/bird)	606.63[c]	579.46[c]	482.07[b]	377.97[a]	27.59	0.000
Total weight gain,(g/bird)	568.07[c]	540.86[c]	443.50[b]	339.60[a]	27.53	0.000
Average daily weight gain, (g/bird)	19.58[c]	18.65[c]	15.47[b]	11.85[a]	0.93	0.000
Total feed intake, (g/bird)	1175.67[c]	1171.27[c]	840.25[b]	759.07[a]	57.80	0.000
Average daily feed intake, (g/bird)	40.53[c]	40.39[c]	28.97[b]	26.18[a]	1.99	0.000
Feed conversion ratio	2.06[b]	2.16[b]	1.87[a]	2.21[b]	0.04	0.003
Feed cost,(₦)	180.26[c]	176.54[c]	125.18[b]	111.90[a]	9.26	0.000
Feed cost per kg weight gain, (₦)	317.39[b]	326.06[b]	279.09[a]	326.29[b]	6.52	0.003

abc means on the same row with different superscripts are significantly different at 5%. T1= 0% tepung daun *Ipomoea asarifolia*, T2= 2.5% tepung daun *Ipomoea asarifolia*, T3= 5% tepung daun *Ipomoea asarifolia*, T4= 7.5% tepung daun *Ipomoea asarifolia*

Table 6. Growth performance of birds fed diets containing cooked *Ipomoea asarifolia* leaf meal during the finisher phase

Parameters	T1	T2	T3	T4	SEM	P value
Final weight gain, (g/bird)	2271.63[d]	2010.82[c]	1646.00[b]	1339.77[a]	106.93	0.000
Total weight gain, (g/bird)	1680.20[d]	1431.51[c]	1160.73[b]	961.80[a]	82.34	0.000
Average daily feed intake, (g/bird)	60.02[d]	51.12[c]	40.14[b]	34.35[a]	3.00	0.000
Total feed intake, (g/bird)	3517.27[d]	3059.08[c]	2588.03[b]	2406.43[a]	130.41	0.000
Feed conversion ratio	2.09[a]	2.13[a]	2.30[b]	2.50[c]	0.52	0.000
Total Feed cost, (₦)	533.39[d]	453.66[c]	370.80[b]	326.31[a]	23.84	0.000
Feed cost per kg weight gain, (₦)	317.22[a]	316.39[a]	331.81[ab]	339.45[b]	3.81	0.051
Total weight gain overall, (₦)	2233.90[d]	1972.20[c]	1607.87[b]	1300.90[a]	106.93	0.000

abc means on the same row with different superscripts are significantly different at 5%. T1= 0% tepung daun *Ipomoea asarifolia*, T2= 2.5% tepung daun *Ipomoea asarifolia*, T3= 5% tepung daun *Ipomoea asarifolia*, T4= 7.5% tepung daun *Ipomoea asarifolia*

Table 7. cost analysis of broiler birds fed cooked *Ipomoea asarifolia* leaf meal(CIALM)

Parameters	T1	T2	T3	T4	SEM	P value
Gross profit, (₦)	2725.92[d]	2412.99[c]	1975.20[b]	1607.72[a]	128.31	0.000
Net profit, (₦)	1509.27[d]	1282.69[c]	977.23[b]	669.50[a]	95.84	0.000
Return on investment, (₦)	1.25[d]	1.14[c]	0.98[b]	0.71[a]	0.06	0.000
Economic efficiency	2.25[d]	2.14[c]	1.98[b]	1.71[a]	0.06	0.000
Feed cost, (₦)	713.65[d]	630.20[c]	497.98[b]	438.22[a]	438.22	0.000

abc means on the same row with different superscripts are significantly different at 5%. T1= 0% tepung daun *Ipomoea asarifolia*, T2= 2.5% tepung daun *Ipomoea asarifolia*, T3= 5% tepung daun *Ipomoea asarifolia*, T4= 7.5% tepung daun *Ipomoea asarifolia*

effect on the performance of broiler although their control was better. In the case of uncooked *Ipomoea asarifolia* leaf meal, inclusion levels of 5 percent and 10 percent seemed to have performed better whereas in this case, inclusion of 5 percent and 7.5 percent was highly deleterious and negatively affected their performance.

Cost analysis of broiler birds fed cooked *Ipomoea asarifolia* leaf meal (CALM)

Table 7 shows the cost analysis and economic evaluation of the dietary inclusion of cooked *Ipomoea asarifolia* leaf meal (CIALM) on broiler diets. The results of the economic evaluation of birds fed cooked *Ipomoea asarifolia* leaf meal showed significant differences ($p<0.05$) in the gross profit, net profit, return on investment, economic efficiency and total cost of feed consumed. The gross profit, net profit, return on investment, economic efficiency and feed cost decreased with increasing levels of cooked *Ipomoea asarifolia* leaf meal. The total feed cost for T1 (0%) was higher while the total feed cost for T4 (7.5%) was lowest which implies that the leaf meal was a cheaper feed source. This is in agreement with Esonu et al. (2003) who suggested that leaf meal of tropical legumes and browse plants should be used as potential sources of cheap animal feed source. However, T1 (0%) was better in terms of the gross profit, net profit, return on investment and economic efficiency and T4 (7.5%) was the lowest thus showing that even though the feed cost was cheaper with higher levels of inclusion, the farmer made more profit with reduced inclusion levels because the ones with higher inclusion levels showed high feed conversion ratio values. This implies that for them to add more weight they need to eat more feed which cannot be efficiently converted to meat as a result of the high fiber content thus making the control diet more profitable. This is in agreement with the work of Ugwuowo et al. (2019) which reported that there was significant differences between treatment means on gross profit, net profit, economic efficiency and return on investment of birds fed *Moringa oliefera* leaf meal and the economic indices reduced as the inclusion levels increased. The economic indices of broilers fed varying dietary levels of sun dried neem leaf meal (NLM) Which was investigated by Onyimonyi & Ernest (2009) was also in agreement with this trend as there was decrease in profit as the leaf meal inclusion level increased.

CONCLUSION

The results show that broiler birds in T1 (0% CIALM) performed better than the birds in the other treatments. Cooking affected the nutrient composition of *Ipomoea asarifolia* and reduced the quality of the feed as the level of inclusion of cooked *Ipomoea asarifolia* leaf meal (CIALM) increased. Therefore, uncooked *Ipomoea asarifolia* leaf meal is better than the cooked one (CIALM) in broiler diets.

REFRENCES

Ahitey E, Flake L. 2008. Ghana poultry and products annual report. Washington DC (USA).

Aviagen. 2019. Ross Nutrition Supplement. Huntsville (USA): Aviagen.

Buragohain R. 2016. Growth performance, nutrient utilization, and feed efficiency in broilers fed *Tithonia diversifolia* leaf meal as substitute of conventional feed ingredients in Mizoram. Vet World. 9:444–449. DOI: 10.14202/vetworld.2016.444-449.

Duncan DB. 1955. Multiple Range and Multiple F Tests. Biometrics. 11:1–42.

Ekenyem B. 2002. The growth responses of weaner pigs fed varying levels of palm kernel cake. In: Proceeding Prod. Akure (Niger): Federal University of Technology.

Ekenyem B, Madubuike F. 2005. An assessment of *Ipomoea asarifolia* leaf meal as feed ingredient in broiler chick production. Pakistan J Nutr. 5:46–50. DOI: 10.3923/pjn.2006.46.50.

El-Adawy T. 2002. Nutritional composition and anti-nutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods Hum Nutr. 57:83–97. DOI: 10.1023/a:1013189620528.

Emenike H, Udedibie A, Emenalom O. 2016. Effects of differently processed Jackbean (*Canavalia ensiformis*) meals on the performance of grower pigs. Br J Appl Sci Technol. 13:1–8. DOI: 10.9734/BJAST/2016/18996.

Esonu B, Emenalom O, Udedibie A, Herbert U, Ekpor C, Okoli I, Iheukwumere F. 2001. Performance and blood chemistry of weaner pigs feed raw mucuna bean (velvet bean) meal. Trop Anim Prod Invt. 4:49–54.

Esonu BO, Iheukwumere FC, Iwuji TC, Akanu N, Nwugo OH. 2003. Evaluation of microdesmis puberula leaf meal as feed ingredient in broiler starter diets. Niger J Anim Prod. 30:3–8. DOI: 10.4314/njap.v30i1.3306.

[FAO] Food and Agriculture Organization. 2010. Agribusiness Handbook- Poultry meat and eggs. Rome (Italy): Food and Agriculture Organization of the United Nations.

Ferket PR, Gernat AG. 2006. Factors that affect feed intake of meat birds: A review. Int J Poult Sci. 5:905-911. DOI: 10.3923/ijps.2006.905.911.

Frias J, Vidal-Valverde C, Sotomayor C, Diaz-Pollan C, Urbano G. 2000. Influence of processing on available

carbohydrate content and antinutritional factors of chickpeas. Eur Food Res Technol. 210:340–345. DOI: 10.1007/s002170050560.

Gadzirayi CT, Masamha B, Mupangwa JF, Washaya S. 2011. Performance of broiler chickens Fed on Mature *Moringa oleifera* Leaf meal as a protein supplement to soyabean meal. Int J Poult Sci. 11:5–10. DOI: 10.3923 /ijps.2012.5.10.

Kakengi AM V, Kaijage JT, Sarwatt S V, Mutayoba SK, Shem MN, Fujihara T. 2007. Effect of *Moringa oleifera* leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. Livest Res Rural Dev. 19.

Madubuike F, Ekenyem B. 2001. Non-ruminant livestock production in the tropics: Gust-Chuks. Owerri (Niger): Graphic Centre.

Muriu JI, Njoka-Njiru EN, Tuitoek JK, Nanua JN. 2002. Evaluation of sorghum (*Sorghum bicolor*) as replacement of maize in the diet of growing rabbit (*Oryctolagus cuniculus*). Asian-Australasian J Anim Sci. 15:565–569. DOI: 10.5713/ajas.2002.565.

Ncube S, Saidi PT, Halimani TE. 2015. Effect of stage of growth and processing methods on the nutritional content of Acacia angustissima leaf meal harvested for broiler feeding. Livest Res Rural Dev. 27.

Nwakpu P, Omeje J, Alaku S. 2010. The response of weaner pigs to diets containing fishmeal and bloodmeal as separate sources of animal protein. Trop J Anim Sci. 3:45–51. DOI: 10.4314/tjas.v3i1.49716.

Olugbemi TS, Mutayoba SK, Lekule FP. 2010. Moringa oleifera leaf meal as a hypocholesterolemic agent in laying hen diets. Livest Res Rural Dev. 22.

Onyimonyi AE, Ernest O. 2009. An assessment of pawpaw leaf meal as protein ingredient for finishing broiler. Int J Poult Sci. 8:995–998. DOI: 10.3923/ijps.2009.995.998.

Ranjhan S. 2001. Animal Nutrition in the tropics. 5th ed. New Delhi (IN): Vikas Publishing House Pvt. Ltd.

Rehinan Z, Rashid M, Shah WH. 2004. Insoluble dietary fibre components of food legumes as affected by soaking and cooking processes. Food Chem. 85:245–249. DOI: 10.1016/j.foodchem.2003.07.005.

Santoso U, Sartini. 2001. Reduction of fat accumulation in broiler chickens by *Sauropus androgynus* (Katuk) leaf meal supplementation. Asian-Australasian J Anim Sci. 14:346–350. DOI: 10.5713/ajas.2001.346.

Tesfaye E, Animut G, Urge M, Dessie T. 2013. Moringa olifera Leaf meal as an alternative protein feed ingredient in broiler ration. Int J Poult Sci. 12:289–297. DOI: 10.3923/ijps.2013.289.297.

Ubua JA, Ozung PO, Inagu PG. 2019. Dietary inclusion of neem (*Azadirachta indica*) leaf meal can influence growth performance and carcass characteristics of broiler chickens. Asian J Biol Sci. 12:180–186. DOI: 10.3923/ajbs.2019.180.186.

Ugwuowo L, Ezeano C, Olabode A, Anikwue C. 2019. Performance and cost evaluation of broilers fed diets containing soyabean meal replaced with varying levels of *Moringa olifera* leaf meal. J Anim Res Nutr. 4:1–7. DOI: 10.21767/2572-5459.100052.

Effect of *In-ovo* Injection of L-Arginine on Hatchability, Chick Quality, Performances and Muscle Histology of Native Chicken

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ABSTRAK

Azhar M, Sara U, Wahyuni S. 2022. Pengaruh *in-ovo* injeksi l-arginine terhadap daya tetas, kualitas anak ayam, performan dan histologi otot ayam lokal. JITV 27(1):18-27. DOI:http://dx.doi.org/14334/jitv.v27i1.2987.

Penelitian ini bertujuan untuk mengetahui pengaruh *in-ovo* injeksi menggunakan *L-arginine* terhadap daya tetas, kualitas *day old chick*, performan dan histologi otot ayam lokal. *In-ovo injeksi* dilakukan pada hari ke-10. Sebanyak 375 butir telur fertil dengan ranges bobot rata-rata 39-43 g dikelompokkan menjadi 5 perlakuan. Perlakuan pertama tanpa injeksi (kontrol negatif), Perlakuan ke-2 injeksi larutan NaCl fisiologis 0,9% (kontrol positif), Perlakuan ke-3 injeksi larutan *L-arginine* 0,5 g per 100 ml NaCl fisiologis 0,9% (0,5%, m/v), Perlakuan ke-4 injeksi larutan *L-arginine* 1,0 g per 100 ml NaCl fisiologis 0,9% (1,0%, m/v), dan Perlakuan ke-5 injeksi larutan *L-arginine* 1,5 g per 100 ml NaCl fisiologis 0,9% (1,5%, m/v). Injeksi dilakukan pada area runcing telur dengan posisi vertikal (runcing dibawah, tumpul diatas). Injeksi dilakukan dengan kedalaman 10 mm dari cangkang telur menggunakan injektor otomatis. Hasil penelitian menunjukkan bahwa semua perlakuan menghasilkan daya tetas yang sama. *In-ovo* injeksi *L-arginine* memberikan pengaruh yang menguntungkan terhadap kualitas anak ayam dan perfoma pasca tetas, konsentrasi larutan *L-arginine* yang digunakan tidak menyebabkan pengaruh negatif terhadap kematian embryo. *In-ovo* injeksi of 0,5% *L-arginine* meningkatkan bobot tetas, bobot badan mingguan, massa otot, dan ukuran *myofiber*.

Kata Kunci: *In-ovo*, *L-arginine*, Perfoman, *Myofiber*

ABSTRACT

Azhar M, Sara U, Wahyuni S. 2022. Effect of *in-ovo* injection of l-arginine on hatchability, chick quality, performances and muscle histology of native chicken. JITV 27(1):18-27. DOI:http://dx.doi.org/14334/jitv.v27i1.2987.

This study aimed to determine the effect of *in-ovo* injection of L-arginine on hatchability, chick quality, performances, and muscle histology of native chicken. *In-ovo* injection was carried out on the 10th day. A total of 375 fertile eggs with an average weight ranged 39-43 g were grouped into 5 treatments. The first treatment was without injection (negative control), the second treatment was injection of NaCl solution 0.9% (positive control), the third treatment was injection of L-arginine solution 0.5 g per 100 ml of NaCl 0.9% (0.5%, m/v) The fourth treatment was injection of 1.0 g L-arginine solution per 100 ml of NaCl 0.9% (1.0%, m/v), and the fifth treatment was injection of 1.5 g L-arginine solution per 100 ml of NaCl 0.9% (1.5%, m/v). The injection was carried out at the pointed area of the egg in a vertical position (pointed below, blunt above). The Injection was carried out with a depth of 10 mm from the eggshell using an automatic injector. The results showed that all treatments produced the same hatchability. *In-ovo* injection of L-arginine has a beneficial effect on chick quality and post-hatch performance, the concentration of L-arginine solution used did not cause embryo death. *In-ovo* injection of 0.5% L-arginine increased hatching weight, weekly body weight, muscle mass, and myofiber size.

Key Words: *In-ovo*, L-arginine, Performances, Myofiber

INTRODUCTION

In-ovo injection technology is a technique of adding nutritional substances directly into eggs during the incubation period. *In-ovo* feeding aims to stimulate the growth and development of the embryo and the performance of the lifelong effect after hatching. The results of previous studies showed that *in-ovo* injection can increase embryo weight and hatching weight (Zhao et al. 2017; Abousaad et al. 2017; Araújo et al. 2019), hatchability (Zhu et al. 2020; Oke et al. 2021), post-hatch performance (Zhu et al. 2019; Wang et al. 2020; Slawinska et al. 2020), and tissue histology (Zhao et al. 2017b; Fatemi et al. 2021). However, *in-ovo* injection technology has also been reported (Zhang et al. 2018; Araújo et al. 2020) to have a negative impact on embryo mortality. The report showed that *in-ovo* injection method can be a solution to increase the productivity of native chickens.

Embryo death caused by the *in-ovo* injection method can occur due to the concentration of the solution injected into the hatching eggs (Zhang et al.

2018; Kop-Bozbay and Ocak 2019). The *in-ovo* concentration of the solution that does not match the embryonic fluid causes differences in osmolarity. This condition causes embryonic fluid cannot be absorbed by the embryo and results in the death of the embryo. The results of research Omede et al. (2017) showed the osmolarity of the amniotic fluid from late-incubation (day 17) broiler eggs was 278.8 mmol/kg. Furthermore Vassallo et al. (2019) reported that differences in osmolarity would impair absorption of extraembryonic fluid nutrients.

L-arginine has been widely reported as an *in-ovo* substance that has an important role in stimulating embryo growth and development (Fouad et al. 2012; Yu et al. 2018; Omidi et al. 2020). L-arginine can stimulate IGF-1 which plays an important role in embryonic muscle cell division (Chen et al. 2013). It is assumed that muscles with a greater number of cells in the embryonic phase will grow and develop more rapidly after hatching. It was observed Subramaniyan et al. (2019) that L-arginine was also able to increase the activity of muscle cell division. In addition, Azhar et al (2016) also reported that *in-ovo* injection of L-arginine was able to improve post-hatch performance.

Investigation of the optimum concentration of L-arginine in a solution injected by the *in-ovo* method has become one of the goals of many types of research. The best L-arginine concentrations reported by previous researchers vary widely. 0.5% or 5 mg L-arginine per ml sterile water in the broiler (Omidi et al. 2020), 2% or 2 g L-arginine per 100 mL sterile distilled water in quail (Al-Daraji et al. 2012). 10 g L-arginine per 1 L solvent in the broiler (L.L. Yu et al. 2018b). L-arginine 100 g/µL/egg (Subramaniyan et al. 2019). 1.% L-arginine solution per broiler embryo (Gao et al. 2018). *In-ovo* injection of L-arginine has been carried out by Azhar et al (2016) on native chickens. This study refers to that research with refines the incubation management and more complex observation parameters. Reports on the concentration of L-arginine through *in-ovo* injection technology on native chickens are still very limited, so it is necessary to make observations to determine the effect of *in-ovo* injection of L-arginine on hatchability, chick quality, performances, and muscle histology of native chicken.

MATERIALS AND METHODS

Bird and incubation

The eggs used came from native chicken breeding in Pinrang Regency, South Sulawesi. Parent chickens from eggs have an age ranged of 55-70 weeks which were reared with an intensive system. Collected eggs have been stored for 2-4 days before incubation period. All incubation, *in-ovo* injection, and rearing activities were carried out at the Poultry Production Technology Laboratory, Hasanuddin University.

The incubator used has a semi-automatic type with separate setter and hatcher models. Before use, the incubator was sterilized with 40% formalin and 70% alcohol. The egg incubation process lasts for 21.5 days. The incubator from the first to the 17th day was maintained at a temperature of 37-38 ^{o}C with a humidity of 55-60%. The temperature from day 18 to 21 was raised to 38-39 ^{o}C with a humidity of 65-70%. Egg turning was done 3 times a day (7 am, 3 pm, and 11 pm) from the 4th to the 18th day. On the 7th day, the observation was carried out to determine the fertile eggs. Meanwhile, eggs that were not fertile or experience death of the embryo will be removed. The hatching vent was gradually opened on the 4th day of the incubation period and fully opened on the 7th day.

In-ovo procedure

In-ovo injection was carried out on the day tenth. A total of 375 fertile eggs with an average weight ranged of 39-43 g were grouped into 5 treatments and arranged according to a completely randomized design (CRD). The first treatment was without injection (negative control), the second treatment was an injection of NaCl solution 0.9% (positive control), the third treatment was an injection of L-arginine solution 0.5 g per 100 ml of NaCl 0.9% (0.5%, m/v), the fourth treatment was injection of 1.0 g L-arginine solution per 100 ml of NaCl 0.9% (1.0%, m/v), and the fifth treatment was injection of 1.5 g L-arginine solution per 100 ml of NaCl 0.9% (1.5%, m/v). The injection was carried out in the pointed area of the egg in a vertical position (pointed below, blunt above). The injection was carried out with a depth of 10 mm from the eggshell using an automatic injector with a needle size of 26 G x 0.5 (0.45 x 13 mm). The target of *in-ovo* solution deposition is albumen. Each egg was injected 0.5 ml of *in-ovo* injection solution. The hole in the eggshell after the injection process is sealed using silicone. All *in-ovo* injection processes were carried out under aseptic conditions.

Hatching and animal husbandry

On day 21.5 of incubation, all hatched chicks were weighed. Meanwhile, eggs that did not hatch were observed to determine the age of embryonic death based on the morphology of embryonic development (Tong et al. 2013) regarding the stages of development of chicken embryos during incubation. The calculation of hatchability is based on method Zhu et al. (2019), namely the number of chick hatch/number of fertilized eggs x 100.

After hatching, 45 DOCs were placed randomly based on each treatment on a pen measuring height x length x width (0.5 x 1 x 0.5 m). The pen has walls made of bamboo insulated and lined with sawdust litter with a thickness of 3 cm. Before use, the pens were sterilized with a disinfectant. Each pen was filled with 3 chicks, 1 male and 2 female. The rearing process was carried out based on commercial chicken management standards. The feed used was commercial feed (crumble BP 11) with nutritional content of 13% water content, 21-23% protein, 5% fat, 5% fiber, 7% ash, 0.9% calcium, and 0.6% phosphorus. Feed and drinking water were provided without restrictions.

Sample collection and muscle histology

Collection of muscle samples begins with slaughtering chickens at the age of 42 days, the slaughtering process is carried out in an Islamic way. Muscle samples collected were from 2 chickens (1 male and 1 female) per treatment unit. The muscle samples collected were right pectoralis major and pectoralis minor muscles which had been separated from other bones, organs, and muscles using a scalpel. The muscle samples obtained were immediately weighed. After weighing the Pectoralis minor muscle was separated from the Pectoralis major muscle. The pectoralis major muscle was then put into a sample pot containing 10% formalin solution for further histological preparations.

Histology samples were made based on Chen et al. (2012), muscle samples from formalin solution were hydrated through a series of alcohols with increasing concentrations. The samples were transferred one by one into each alcohol concentration and allowed to submerge in each alcohol concentration for approximately 15 seconds. The sample was then put into xytol and finally immersed in paraffin. Using a microtome, the sample was thinly sliced and then stained with haemotoxylin-eosin on a glass object and covered with a cover glass.

Observation of histological images

Histological images were obtained from the results of shooting using a microscope with an OptiLab device connected to a computer. The results of the histology images obtained were trnsfered into the Axio Visio Rail program. 4.8.2. Observation of histology images program Axio Visio Rel. 4.8.2. was adjusted to the magnification scale of the microscope used when taking pictures. Observation of the number of myofibers was carried out at 10x magnification, while the diameter and surface area of myofiber were measured using 40x magnification.

Observation parameters

Observations on the incubation process were egg weight, fertility, embryonic mortality (early, middle, late), albumen weight, yolk weight, and Ratio hatching weight/Egg weight. The post-hatching parameters were hatchability, age embryo died, hatching weight, performance (feed intake, body weight gain, body weight, feed conversion ratio), muscle mass, and muscle histology (number of myofiber, myofiber surface area) of male and female native chickens.

Statistical analysis

Analysis of variance for data collected was done based on Completely Randomized Design (CRD). The treatment which showed a significant effect was then continued with Duncan's test using IBM SPSS 20.0 software. Data from the analysis of each treatment is presented in the form of mean ± standard deviation.

RESULTS AND DISCUSSION

Hatchability, embryonic mortality, and age embryo died

Table 1 shows that there is no effect of treatment ($P>0.05$) on hatchability, embryonic age died, and embryonic mortality (early, middle, and late embryo died). All treatments produced the same hatchability. Similar results were also reported by (Al-Daraji et al. 2012) in quail, (Gao et al. 2018) in broilers, and (Zhu et al. 2019) in domestic pigeons. However, in contrast to reports (Araújo et al. 2020) on broilers, (Abousaad et al. 2017b) on turkeys, (Zhang et al. 2018) on domestic pigeons, and (Silva et al. 2012) on broiler breeders. The absence of differences in hatchability indicated that the *in-ovo* injection technique did not damage the extraembryonic sac and the concentration used was following the osmolarity of the egg albumen, so it did not cause embryo death. Embryo mortality data support this argument, with no differences in embryo mortality across all treatments. However, further observations need to be made regarding the osmolarity value of each treatment to ensure that the *in-ovo* solution osmolarity is following the albumen osmolarity on the 10th day of incubation.

Embryo mortality is the main factor that determines high or low hatchability. The more embryos died, the lower the hatchability value and vice versa. In general, embryonic death during incubation can be caused by many factors such as outside epigenetic temperature (Loyau et al. 2014), low humidity (El-Hanoun et al. 2012), egg nutrition (Oladokun & Adewole 2020),

Table 1. Effect of *in-ovo* injection of L-arginine on hatchability, embryonic mortality, and age embryo died of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Fertility (%)	74.29±4.95	75.24±6.60	73.33±3.30	74.29±2.86	65.71±9.90
Hatchability (%)	52.38±4.58	51.91±4.00	52.05±3.93	51.95±4.00	50.88±2.51
Embryonic mortality (% of fertile eggs)					
Early *	0.09±0.03	0.08±0.04	0.06±0.02	0.06±0.04	0.08±0.04
Middle *	0.08±0.03	0.09±0.05	0.08±0.04	0.06±0.02	0.05±0.01
Late *	0.31±0.06	0.33±0.04	0.35±0.06	0.36±0.03	0.38±0.02
Age embryo died (day)	19.55±1.26	18.84±1.31	19.42±1.34	19.26±1.35	18.78±1.22

*early (0-7 days of incubation), middle (8-14 days of incubation), late (15-21 days of incubation)

Table 2. Effect of *in-ovo* injection of L-arginine on extraembryonic weight and chick quality of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Egg weight (g)	42.37±0.45	42.82±0.74	42.59±0.31	42.69±0.40	42.20±0.10
Albumen weight (g)	5.21±0.31	5.25±0.31	5.04±0.65	5.06±0.48	5.13±0.77
Yolk weight (g)	7.36±0.48^{b}	7.83±0.50^{b}	6.27±0.32^{a}	6.38±0.31^{a}	6.29±0.39^{a}
Hatching weight (g)	26.68±0.34^{a}	27.66±1.11^{a}	29.39±0.79^{b}	29.83±1.31^{b}	29.22±0.23^{b}
Ratio hatching weight / Egg weight (%)	62.97±0.15^{a}	64.57±1.48^{a}	69.00±1.36^{b}	69.89±3.25^{b}	69.25±0.71^{b}

Means ± SD in the same rows with different superscripts (a,b) differ significantly (P<0.05). Albumen weight and yolk weight were collected on the day 17 of incubation

eggabnormalities (Onbaşilar et al. 2014). The age of death of chicken embryos was divided into 3 main groups (Al-Shamery and Al-Shuhaib 2015). The first group, namely early-embryo dead, are embryos that die in the first stage of embryogenesis (1-7 days). The second group, namely middle-embryo dead, are embryos that die in the second stage of embryogenesis (8-14 days). The third group, namely late-embryo dead, is embryos that die in the last stage of embryogenesis (15-21 days). The average age of embryonic death occurs at the late stage of embryogenesis (late-embryo dead). This indicates that the embryonic death that occurs is assumed not to be caused by the *in-ovo* injection technique and the concentration of L-arginine because *in-ovo* injection was carried out on the 10th day of the incubation period (second stage of embryogenesis).

Extraembryonic and chick quality

The effect of *in-ovo* injection of L-arginine on extraembryonic and hatchability characteristics of native chicken is presented in Table 2. *In-ovo* injection of L-arginine significantly affected (P<0.05) yolk weight, hatching weight, and hatching weight ratio/ egg weight, while egg weight and albumen weight did not show any effect (P>0.05). Yolk weight decreased with *in-ovo* injection of L-arginine, but there was no difference between *in-ovo* treatments. All *in-ovo* treatments of L-arginine increased hatching weight and hatching weight/egg weight ratio.

Albumen weight did not show any difference for all treatments. This indicates that the concentration of the *in-ovo* injection solution does not interfere with albumen absorption activity. Changes in the osmolarity of the embryonic environment with *in-ovo* injection, as a cause of impaired nutrient absorption by the embryo. Nutrient absorption can occur because the osmolarity of the injected solution is different from the osmolarity of the embryonic environment (Oladokun & Adewole 2020). The albumen weight that did not differ also indicated that the embryos treated with *in-ovo* injection absorbed more L-Arginine than the control. Absorption of albumen by the embryo through the *sero-amniotic connection* pathway, albumen then enters the amniotic fluid and is utilized by the embryo. The egg turning process is the main factor determining albumen content changes. Willems et al. (2014) reported that the turning process would assist albumen through the sero-amniotic connection.

In-ovo injection of L-arginine produced lighter yolks than the control treatment. This may be due to *in-ovo* injection of L-Arginine increase the metabolic rate of the embryo, so that absorption of the yolk is more than the control treatment. A high metabolic rate will require more energy. The increased energy requirements of the embryo are required to support organogenesis activities (Hu et al. 2013). During incubation, the yolk is the most important source of energy for the embryo. The limited amount of egg carbohydrates (1%) causes fat to become the main energy source for the embryo through the process of gluconeogenesis (Nangsuay et al. 2015). Alshamy et al. (2018) reported that the transport of yolk fat to the embryo via blood vessels is endocytosis, after hatching absorption of nutrients from the yolk through *Meckel's diverticulum*.

L-arginine injection via the *in-ovo* technique resulted in a higher hatching weight than control. The increase in hatching weight may be due to the increase in embryo weight produced by the administration of L-arginine. Onbaşilar et al. (2014) showed that heavier embryos would produce higher hatching weights than lighter embryos. Increased liver and muscle glycogen concentrations may also be responsible for the increased hatching weight (Hu et al. 2013). The increase in glycogen is believed to occur due to the low weight of the yolk with the administration of L-arginine.

At the end of the incubation period, the energy needs of the embryo will increase to carry out the hatching process. During this process, most of the embryo's energy is obtained from the breakdown of liver and muscle glycogen (Yang et al. 2019). The greater the number of energy reserves (glycogen) needed to support hatchery activities. When the energy reserve status of the embryo is low during the hatching process, there will be an overhaul of protein tissue, especially muscle (Dong et al. 2013). The reshuffle of muscle protein tissue causes a decrease in muscle mass so that it will have an impact on low hatching weight. The results of the study Shafey et al. (2012) showed that an increase in glycogen of 1.5 mg/g of organs would increase the hatching weight of 1.9 g. Meanwhile Al-Daraji et al. (2012) reported that the increase in hatching weight with *in-ovo* injection of L-arginine occurred due to the increase in muscle mass during the myogenesis process.

Parameter of hatching weight to egg weight ratio was observed to avoid the effect of egg weight on hatching weight. The eggs that were sampled had relatively the same weight. Heavier eggs were reported to produce higher hatching weights than lighter eggs (Dymond et al. 2013). The higher the ratio of hatching weight to egg weight, the higher the growth rate of the embryo, even though it comes from eggs with smaller sizes. The ratio of hatching weight to egg weight was higher in the L-arginine treatment than in the control. This happened because the hatching weight obtained in the treatment with L-arginine was also higher. Results obtained indicated that the embryonic growth rate was higher with *in-ovo* injection of L-arginine.

Post-hatch performance

As shown in Table 3, feed consumption, weight gain, body weight, and feed conversion were significantly affected ($P<0.05$) by *in-ovo* injection treatment on the 7th week of rearing day. On day 14, *in-ovo* injection of L-arginine showed an effect ($P<0.05$) on feed consumption, body weight, and feed conversion, but not on weight gain. Bodyweight gain, body weight, and feed conversion on day 21 were significantly affected ($P<0.05$) by *in-ovo* treatment, while feed consumption did not show any difference between treatments. Effect of treatment on post-hatch performance observation on day 28 was significant ($P<0.05$) on weight gain and body weight, but feed consumption and feed conversion did not show any different ($P>0.05$).

Feed consumption on the 7th day of observation showed a decrease with the injection of L-arginine 0.5% and 1.5%, while on the 14th day the lowest feed consumption was in the control + treatment. Observations on the 21st and 28th days did not show any difference in feed consumption between treatments. Weight gain on the 7th day increased with the injection of 0.5% and 1.5%, the same results also occurred on the 21st day of observation. However, on day 28, the highest weight gain was with the injection of 1.5% L-arginine. Observations on the 7th, 14th, and 21st days showed that all the treatments could increase body weight, while at week 28, the highest body weight was with the injection of 1.5%. *In-ovo* injection of L-arginine 0.5% and 1.5% showed lower feed conversion values on the 7th and 21st-day observations. However, the feed conversion did not show any difference between the treatments on the 14th and 28th-day observations.

Results of the analysis showed that there was no difference in feed consumption from all treatments on the 14th, 21st, and 28th days of observation. However, on the 7th day of observation, showed that the injection of 0.5% and 1.5% resulted in lower feed consumption compared to the other treatments. These results indicated that the metabolic energy requirements of chickens are the same whether injected with L-arginine or not on days 8 to 28. However, before the 8th day, the energy requirements of *in-ovo* injection chickens were higher than control. In the growth phase, metabolic energy was focused on supporting hypertrophiccell activity (Chen et al. 2013). The more cells that

Table 3. Effect of *in-ovo* injection of L-arginine on post-hatch performances of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
day 0 to day 7th					
Feed intake (g/bird)	47.88±0.52^{c}	47.69±0.53^{bc}	46.85±0.39^{a}	46.93±0.26^{bc}	46.71±0.70^{a}
Body weight gain (g/bird)	39.46±0.10^{b}	37.37±1.56^{a}	41.52±2.12^{c}	40.45±0.28^{bc}	41.80±0.14^{c}
Bodyweight (g/bird)	66.17±0.34^{b}	64.98±0.76^{a}	70.45±1.06^{c}	70.47±0.83^{c}	70.99±0.06^{c}
Feed conversion ratio	1.21±0.01^{b}	1.27±0.06^{c}	1.13±0.05^{a}	1.16±0.01^{ab}	1.11±0.02^{a}
day 7st to day 14th					
Feed intake (g/bird)	91.80±1.75^{b}	86.63±4.38^{a}	92.98±1.67^{b}	92.43±1.74^{b}	91.08±1.35^{b}
Body weight gain (g/bird)	58.88±3.90	58.09±3.27	60.52±3.47	64.18±3.56	63.87±2.05
Bodyweight (g/bird)	125.05±3.87^{a}	123.07±3.92^{a}	130.97±4.22^{b}	134.65±3.83^{b}	134.87±2.10^{b}
Feed conversion ratio	1.56±0.12	1.50±0.14	1.54±0.06	1.44±0.06	1.43±0.07
day 14st to day 21th					
Feed intake (g/bird)	156.26±5.04	153.42±7.37	155.84±7.08	155.43±4.07	155.47±4.19
Body weight gain (g/bird)	77.05±2.78^{a}	76.96±6.36^{a}	88.99±0.97^{c}	81.52±3.83^{ab}	87.26±5.57^{bc}
Bodyweight (g/bird)	202.11±2.26^{a}	200.04±2.78^{a}	219.96±5.11^{b}	216.18±7.07^{b}	222.13±5.15^{b}
Feed conversion ratio	2.03±0.04^{b}	2.02±0.26^{b}	1.75±0.06^{a}	1.91±0.10^{ab}	1.78±0.06^{a}
day 21st to day 28th					
Feed intake (g/bird)	212.80±7.75	232.49±1.48	232.68±2.01	232.69±1.22	231.51±1.51
Body weight gain (g/bird)	84.73±3.09^{a}	90.64±5.20^{ab}	89.30±5.89^{a}	97.12±6.07^{bc}	103.09±3.42^{c}
Bodyweight (g/bird)	286.84±2.87^{a}	290.69±3.37^{a}	309.27±10.85^{b}	313.32±1.59^{b}	325.26±6.88^{c}
Feed conversion ratio	2.52±0.36	2.57±0.12	2.61±0.14	2.40±0.16	2.24±0.06

Means ± SD in the same rows with different superscripts (a,b,c) differ significantly (P<0.05). Bodyweight was measured on day 7, 14, 21, and 28

Table 4. Effect of *in-ovo* injection of L-arginine on muscle histology of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Male					
Muscle mass (g)	29.026±3.172^{a}	29.748±2.654^{a}	35.385±2.945^{b}	37.490±3.530^{b}	38.195±3.085^{b}
Bodyweight Ratio (%)	5.396±0.256^{a}	5.407±0.427^{a}	5.408±0.075^{a}	6.147±0.304^{b}	6.195±0.403^{b}
Number of Myofiber (n/mm^2)	91.00±4.00	81.67±10.50	97.67±1.52	95.00±6.55	92.00±7.93
Myofiber (µm)	47.73±3.02^{a}	48.36±1.42^{a}	60.11±4.05^{b}	62.49±3.95^{b}	62.19±3.92^{b}
Myofiber Surface Area (µm^2)	1874.8±323.9^{a}	1627.6±233.8^{a}	2488.8±81.7^{b}	3118.7±130.1^{c}	2820.9±194.7^{bc}
Female					
Muscle mass (g)	24.281±2.379^{a}	24.571±2.378^{a}	29.490±2.950^{b}	30.758±3.048^{b}	30.686±1.587^{b}
Bodyweight Ratio (%)	5.170±0.133	5.030±0.674	5.230±0.103	5.729±0.701	5.783±0.299
Number of Myofiber (n/mm^2)	91.33±6.50	77.00±4.58	88.67±6.65	93.00±7.21	93.33±8.08
Myofiber (µm)	38.07±4.76^{a}	45.66±2.30^{a}	59.68±5.31^{b}	57.60±6.81^{b}	58.31±9.24^{b}
Myofiber Surface Area (µm^2)	1227.0±118.8^{a}	1735.8±321.1^{a}	2813.5±445.3^{b}	2343.9±145.6^{b}	2591.3±417.8^{b}

Note: Means ± SD in the same rows with different superscripts (a,b,c) differ significantly (P<0.05)

experience hypertrophy, the higher the amount of energy needed (Yu et al. 2018). Based on this, it is assumed that chickens that received L-arginine treatment had more cell numbers than other treatments because they had a high metabolic energy requirement. Although, it is still necessary to do further observations to prove this.

L-arginine injection resulted in higher final body weight than without L-arginine administration at all measurement times. The same results were also shown in weight gain. Optimal final body weight was obtained from injection of L-arginine with a concentration of 0.5%. The final weight gain occurs because L-arginine injection can increase muscle mass. Muscle mass was reported by Yang et al. (2019) as an important component in the chicken body that will determine body weight. Muscle weight has a positive correlation with body weight (Chen et al. 2013). The report explains that giving L-arginine can increase muscle mass, resulting in high body weight. It was also reported by Al-Daraji et al. (2012) that administration of L-arginine could increase muscle mass.

The injection of L-arginine 1.0% and 1.5% on the 7th and 21st-day of observations resulted in lower feed conversion values compared to the other treatments. The low feed conversion value may occur due to the increased performance of the digestive tract with the administration of L-arginine. Similar results were obtained by Al-Daraji et al. (2012) in quail. In general, the digestive tract (small intestine) that can work optimally has a large surface (Proszkowiec-Weglarz et al. 2020). It also can release digestive enzymes at maximum levels (Castro et al. 2020). Fouad et al. (2012) reported that L-arginine stimulate small intestinal cell proliferation. Thus, an increase in L-arginine levels in the embryonic phase will cause an increase in the number of small intestinal cells (Castro et al. 2020). Proszkowiec-Weglarz et al. (2020) explained that the increase in the size of the villi and crypts was the result of the high proliferative activity of small intestinal cells at the beginning of growth. This causes the amount of nutrients absorbed is also increasing.

Massa and histology of muscle

In the male sex, muscle mass, muscle mass/body weight ratio, myofiber diameter, and myofiber surface area were significantly affected ($P<0.05$) by the treatment, but the number of myofibers did not show any change (Table 4). The ratio of muscle mass/body weight and the number of muscles myofibers with female sex showed no difference between treatments ($P>0.05$). Meanwhile, muscle mass, the diameter of myofiber, and surface area of myofiber were significantly affected by treatment ($P<0.05$) (Table 4). Muscle mass with the injection was higher than control, both male and female. The ratio of muscle mass/bodyweight of male chickens increased with *in-ovo* injection of L-arginine 1.0% and 1.5%, respectively. *In-ovo* injection of L-arginine caused an increase in myofiber diameter, in both male and female sexes and optimally at a concentration of 0.5%. Maximum myofiber surface area occurred with the injection of 1.0% in the male sex, whereas in female sex optimal myofiber surface area with the injection of 0.5%.

L-arginine injection resulted in higher muscle mass than without L-arginine injection, in both male and female sexes. The increase in muscle mass was caused by an increase in myofiber diameter with the administration of L-Arginine compared to the control. Muscle size is determined by the size of myofibers (Chen et al. 2013). The wider the size of the myofibers, the higher the muscle mass. The larger myofiber size with L-arginine treatment may also be due to the higher number of myoblast cells during myogenesis as reported Xiao et al. (2017) in broiler. The increase in myofiber size was due to L-arginine being an IGF-1 stimulator. Increased concentrations of IGF-1 using L-arginine were also reported by (Yu et al. 2018) in layer chickens and (Castro et al. 2020) in broiler. Chen et al. (2013) suggested that L-arginine can increase IGF-1 gene expression by increasing IGF-1 RNA transcription activity. The L-arginine stimulant process on IGF-1 production is believed to occur through the L-arginine/IGF-1R mechanism (Chen et al. 2013). Another report explains that the increase in IGF-1 concentration occurs because L-arginine and amino acids produced by L-arginine (proline and glutamine) are important amino acids in the chemical structure of IGF-1 (Fouad et al. 2012). In this study, IGF-1 levels were not measured. Thus, the assumption of an increase in IGF-1 levels with *in-ovo* injection of L-arginine needs to be investigated further.

IGF-1 has a very important role during the myogenesis process. In the early phase of myogenesis, IGF-1 plays a role in stimulating the proliferative activity of myoblast cells (Musumeci et al. 2015). The results of the study Xiao et al. (2017) showed that an increase in the concentration of IGF-1 at the beginning of the myogenesis phase caused an increase in the number of myoblast cells. The effect of IGF-1 on myoblast proliferation activity begins with a signal from IGF-1 that activates Mitogen-Activated Protein Kinase (MAPK) pathway (Endo 2015). MAPK pathway is an activity that occurs in the cell nucleus with the IGFr-Ras-Raf-MEK-ERK mechanism (Hu et al. 2016).

CONCLUSION

In-ovo injection of L-arginine has a beneficial effect on chick quality and post-hatch performance, the concentration of L-arginine solution used does not

cause a negative effect on embryo death. *In-ovo* injection of 0.5% L-arginine increased hatching weight, weekly body weight, muscle mass, and myofiber size.

REFERENCES

Abousaad S, Lassiter K, Piekarski A, Chary P, Striplin K, Christensen K, Bielke LR, Hargis BM, Dridi S, Bottje WG. 2017a. Effect of in ovo feeding of dextrin-iodinated casein in broilers: II. Hatch window and growth performance. Poult Sci. 96:1478–1484. DOI: 10.3382/ps/pew439.

Abousaad S, Lassiter K, Piekarski A, Chary P, Striplin K, Christensen K, Bielke LR, Hargis BM, Dridi S, Bottje WG. 2017b. Effects of in Ovo feeding of dextrin-iodinated casein in broilers: I. Hatch weights and early growth performance. Poult Sci. 96:1473–1477. DOI: 10.3382/ps/pew438.

Al-Daraji HJ, Al-Mashadani AA, Al-Hayani WK, Al-Hassani AS, Mirza HA. 2012. Effect of in ovo injection with L-arginine on productive and physiological traits of Japanese quail. South African J Anim Sci. 42:139–145. DOI:10.4314/sajas.v42i2.6.

Alshamy Z, Richardson KC, Hünigen H, Hafez HM, Plendl J, Al Masri S. 2018. Comparison of the gastrointestinal tract of a dual-purpose to a broiler chicken line: A qualitative and quantitative macroscopic and microscopic study. PLoS One. 13:1–22. DOI:10.1371 /journal.pone.0204921.

Araújo ICS, Café MB, Mesquita MA, Caiado BN, Faria AM, Mello HHC, Stringhini JH, Leandro NSM. 2020. Effect of a commercial product containing canthaxanthin for in ovo feeding to broiler embryos on hatchability, chick quality, oxidation status, and performance. Poult Sci. 99:5598–5606. DOI:10.1016/j.psj.2020.08.044.

Araújo ICS, Café MB, Noleto RA, Martins JMS, Ulhoa CJ, Guareshi GC, Reis MM, Leandro NSM. 2019. Effect of vitamin E in ovo feeding to broiler embryos on hatchability, chick quality, oxidative state, and performance. Poult Sci. 98:3652–3661. DOI: 10.3382/ps/pey439.

Azhar M, Rahardja DP, Pakiding W. 2016. Embryo development and post-hatch performances of kampung chicken by in ovo feeding of L-arginine. Media Peternak. 39:168–172. DOI:10.5398/medpet.2016.39. 3.168.

Castro FLS, Teng PY, Yadav S, Gould RL, Craig S, Pazdro R, Kim WK. 2020. The effects of L-Arginine supplementation on growth performance and intestinal health of broiler chickens challenged with Eimeria spp. Poult Sci. 99:5844–5857. DOI:10.1016/j.psj.2020. 08.017.

Chen R. 2013. Dietary arginine supplementation altered expression of IGFs and IGF receptors in weaning piglets. J Cell Anim Biol. 7:44–50. DOI:10.5897 /jcab12.053.

Chen W, Lv YT, Zhang HX, Ruan D, Wang S, Lin YC. 2013. Developmental specificity in skeletal muscle of late-term avian embryos and its potential manipulation. Poult Sci. 92:2754–2764. DOI:10.3382/ps.2013-03099.

Chen W, Tangara M, Xu J, Peng J. 2012. Developmental transition of pectoral muscle from atrophy in late-term duck embryos to hypertrophy in neonates. Exp Physiol. 7:861–872. DOI: 10.1113/expphysiol.2011.061234.

Dong XY, Jiang YJ, Wang MQ, Wang YM, Zou XT. 2013. Effects of in ovo feeding of carbohydrates on hatchability, body weight, and energy status in domestic pigeons (*Columba livia*). Poult Sci. 92:2118–2123. DOI:10.3382/ps.2013-03091.

Dymond J, Vinyard B, Nicholson AD, French NA, Bakst MR. 2013. Short periods of incubation during egg storage increase hatchability and chick quality in long-stored broiler eggs. Poult Sci. 92:2977–2987. DOI:10.3382 /ps.2012-02816.

El-Hanoun AM, Rizk RE, Shahein EHA, Hassan NS, Brake J. 2012. Effect of incubation humidity and flock age on hatchability traits and posthatch growth in Pekin ducks. Poult Sci. 91:2390–2397. DOI:10.3382/ps.2011-02075.

Endo T. 2015. Molecular mechanisms of skeletal muscle development, regeneration, and osteogenic conversion. Bone. 80:2–13. DOI:10.1016/j.bone.2015.02.028.

Fatemi SA, Alqhtani A, Elliott KEC, Bello A, Zhang H, Peebles ED. 2021. Effects of the in ovo injection of vitamin D3 and 25-hydroxyvitamin D3 in Ross 708 broilers subsequently fed commercial or calcium and phosphorus-restricted diets. I. Performance, carcass characteristics, and incidence of woody breast myopathy1,2,3. Poult Sci. 100:101220. DOI:10.1016/ j.psj.2021.101220.

Fouad AM, El-Senousey HK, Yang XJ, Yao JH. 2012. Role of dietary L-arginine in poultry production. Int J Poult Sci. 11:718–729. DOI:10.3923/ijps.2012.718.729.

Gao T, Zhao MM, Li YJ, Zhang L, Li JL, Yu LL, Gao F, Zhou GH. 2018. Effects of in ovo feeding of L-arginine on the development of digestive organs, intestinal function and post-hatch performance of broiler embryos and hatchlings. J Anim Physiol Anim Nutr (Berl). 102:e166–e175. DOI:10.1111/jpn.12724.

Hu Q, Agarwal U, Bequette BJ. 2013. Energy sensing in developing chicken embryos and posthatch chicks from different size eggs. Poult Sci. 92:1650–1654. DOI: 10.3382/ps.2013-03047.

Hu X, Liu L, Song Z, Sheikhahmadi A, Wang Y, Buyse J. 2016. Effects of feed deprivation on the AMPK signaling pathway in skeletal muscle of broiler chickens. Comp Biochem Physiol Part - B Biochem Mol Biol. 191:146–154. DOI:10.1016/j.cbpb.2015. 10.007.

Kop-Bozbay C, Ocak N. 2019. In ovo injection of branched-chain amino acids: Embryonic development, hatchability and hatching quality of turkey poults. J

Anim Physiol Anim Nutr (Berl). 103:1135–1142. DOI: 10.1111/jpn.13111..

Loyau T, Collin A, Yenisey Ç, Crochet S, Siegel PB, Akşit M, Yalçin S. 2014. Exposure of embryos to cyclically cold incubation temperatures durably affects energy metabolism and antioxidant pathways in broiler chickens. Poult Sci. 93:2078–2086. DOI:10.3382 /ps.2014-03881.

Musumeci G, Castrogiovanni P, Coleman R, Szychlinska MA, Salvatorelli L, Parenti R, Magro G, Imbesi R. 2015. Somitogenesis: From somite to skeletal muscle. Acta Histochem. 117:313–328. DOI:10.1016/j.acthis. 2015.02.011.

Najih Jabir Al-Shamery, Mohammed Baqur S. Al-Shuhaib. 2015. Effect of in ovo injection of various nutrients on the hatchability, mortality ratio and weight of the broiler chickens. IOSR J Agric Vet Sci. 8:30–33. DOI: 10.9790/2380-08123033.

Nangsuay A, Molenaar R, Meijerhof R, Van Den Anker I, Heetkamp MJW, Kemp B, Van Den Brand H. 2015. Differences in egg nutrient availability, development, and nutrient metabolism of broiler and layer embryos. Poult Sci. 94:415–423. DOI:10.3382/ps/pev007.

Oke OE, Oyelola OB, Iyasere OS, Njoku CP, Oso AO, Oso OM, Fatoki ST, Bankole KO, Jimoh IO, Sybill NI, et al. 2021. In ovo injection of black cumin (Nigella sativa) extract on hatching and post hatch performance of thermally challenged broiler chickens during incubation. Poult Sci. 100:100831. DOI:10.1016/j.psj. 2020.10.072.

Oladokun S, Adewole DI. 2020. In ovo delivery of bioactive substances: an alternative to the use of antibiotic growth promoters in poultry production—a review. J Appl Poult Res. 29:744–763. DOI:10.1016/j.japr.2020. 06.002.

Omede A, Bhuiyan MM, Lslam AF, Iji PA. 2017. Physico-chemical properties of late-incubation egg amniotic fluid and a potential in ovo feed supplement. Asian-Australasian J Anim Sci. 30:1124–1134. DOI: 10.5713/ajas.16.0677.

Omidi S, Ebrahimi M, Janmohammadi H, Moghaddam G, Rajabi Z, Hosseintabar-Ghasemabad B. 2020. The impact of in ovo injection of l-arginine on hatchability, immune system and caecum microflora of broiler chickens. J Anim Physiol Anim Nutr (Berl). 104:178–185. DOI:10.1111/jpn.13222.

Onbaşilar EE, Erdem E, Hacan Ö, Yalçin S. 2014. Effects of breeder age on mineral contents and weight of yolk sac, embryo development, and hatchability in Pekin ducks. Poult Sci. 93:473–478. DOI:10.3382/ps.2013-03355.

Proszkowiec-Weglarz M, Schreier LL, Kahl S, Miska KB, Russell B, Elsasser TH. 2020. Effect of delayed feeding post-hatch on expression of tight junction– and gut barrier–related genes in the small intestine of broiler chickens during neonatal development. Poult Sci. 99:4714–4729. DOI:10.1016/j.psj.2020.06.023.

Subramaniyan SA, Kang DR, Park JR, Siddiqui SH, Ravichandiran P, Yoo DJ, Na CS, Shim KS. 2019. Effect of in ovo injection of L-Arginine in different chicken embryonic development stages on post hatchability, immune response, and Myo-D and myogenin proteins. Animals. 9:1–8. DOI:10.3390/ani9 060357.

Shafey TM, Alodan MA, Al-Ruqaie IM, Abouheif MA. 2012. In ovo feeding of carbohydrates and incubated at a high incubation temperature on hatchability and glycogen status of chicks. South African J Anim Sci. 42:210–220. DOI:10.4314/sajas.v42i3.2.

Silva LMGS, Murakami AE, Fernandes JIM, Dalla RD, Urgnani JF. 2012. Effects of dietary arginine supplementation on broiler breeder egg production and hatchability. Rev Bras Cienc Avic. 14:267–273. DOI: 10.1590/S1516-635X2012000400006.

Slawinska A, Zampiga M, Sirri F, Meluzzi A, Bertocchi M, Tavaniello S, Maiorano G. 2020. Impact of galactooligosaccharides delivered in ovo on mitigating negative effects of heat stress on performance and welfare of broilers. Poult Sci. 99:407–415. DOI 10.3382/ps/pez512.

Tong Q, Romanini CE, Exadaktylos V, Bahr C, Berckmans D, Bergoug H, Eterradossi N, Roulston N, Verhelst R, McGonnell IM, Demmers T. 2013. Embryonic development and the physiological factors that coordinate hatching in domestic chickens. Poult Sci. 92:620–628. DOI:10.3382/ps.2012-02509.

Vassallo BG, Litwa HP, Haussmann MF, Paitz RT. 2019. In ovo metabolism and yolk glucocorticoid concentration interact to influence embryonic glucocorticoid exposure patterns. Gen Comp Endocrinol. 272:57–62. DOI:10. 1016/j.ygcen.2018.11.013.

Wang Jiguang, Lin J, Wang Jing, Wu S, Qi G, Zhang H, Song Z. 2020. Effects of in ovo feeding of N-acetyl-L-glutamate on early intestinal development and growth performance in broiler chickens. Poult Sci. 99:3583–3593. DOI:10.1016/j.psj.2020.04.003.

Willems E, Decuypere E, Buyse J, Everaert N. 2014. Importance of albumen during embryonic development in avian species, with emphasis on domestic chicken. Worlds Poult Sci J. 70:503–518. DOI: 10.1017/S0043933914000567.

Xiao Y, Wu C, Li K, Gui G, Zhang G, Yang H. 2017. Association of growth rate with hormone levels and myogenic gene expression profile in broilers. J Anim Sci Biotechnol. 8:1–7. DOI:10.1186/s40104-017-0170-8.

Yang T, Zhao M, Li J, Zhang L, Jiang Y, Zhou G, Gao F. 2019. In ovo feeding of creatine pyruvate alters energy metabolism in muscle of embryos and post-hatch broilers. Asian-Australasian J Anim Sci. 32:834–841. DOI:10.5713/ajas.18.0588.

Yu J, Yang H, Wang Z, Dai H, Xu L, Ling C. 2018. Effects of arginine on the growth performance, hormones,

digestive organ development and intestinal morphology in the early growth stage of layer chickens. Ital J Anim Sci. 17:1077–1082. DOI:10.1080/1828051X.2018. 1434692.

Yu LL, Gao T, Zhao MM, Lv PA, Zhang L, Li JL, Jiang Y, Gao F, Zhou GH. 2018a. In ovo feeding of L-arginine alters energy metabolism in post-hatch broilers. Poult Sci. 97:140–148. DOI:10.1017/S1751731118000241.

Yu LL, Gao T, Zhao MM, Lv PA, Zhang L, Li JL, Jiang Y, Gao F, Zhou GH. 2018b. Effects of in ovo feeding of l-arginine on breast muscle growth and protein deposition in post-hatch broilers. Animal. 12:2256–2263.

Zhang H, Elliott KEC, Durojaye OA, Fatemi SA, Peebles ED. 2018. Effects of in ovo administration of L-ascorbic acid on broiler hatchability and its influence on the effects of pre-placement holding time on broiler quality characteristics. Poult Sci. 97:1941–1947. DOI:10.3382 /ps/pey040

Zhang XY, Li LL, Miao LP, Zhang NN, Zou XT. 2018. Effects of in ovo feeding of cationic amino acids on hatchability, hatch weights, and organ developments in domestic pigeon squabs (*Columba livia*). Poult Sci. 97:110–117. DOI:10.3382/ps/pex260.

Zhao MM, Gao T, Zhang L, Li JL, Lv PA, Yu LL, Gao F, Zhou GH. 2017a. Effects of in ovo feeding of creatine pyruvate on the hatchability, growth performance and energy status in embryos and broiler chickens. Animal. 11:1689–1697. DOI:10.1017/S1751731117000374.

Zhao MM, Gao T, Zhang L, Li JL, Lv PA, Yu LL, Gao F, Zhou GH. 2017b. In ovo feeding of creatine pyruvate alters energy reserves, satellite cell mitotic activity and myogenic gene expression of breast muscle in embryos and neonatal broilers. Poult Sci. 96:3314–3323. DOI: 10.3382/ps/pex150.

Zhu MK, Zhang XY, Dong XY, Zou XT. 2019. Effects of in ovo feeding of L-lysine on hatchability, hatching time, and early post-hatch development in domestic pigeons (*Columba livia*). Poult Sci. 98:5533–5540. DOI: 10.3382/ps/pez300.

Zhu YF, Bodinga MB, Zhou JH, Zhu LQ, Cao YL, Ren ZZ, Yang XJ. 2020. Effects of in ovo injection of vitamin C on heat shock protein and metabolic genes expression. Animal. 14:360–367. DOI: 10.1017/S175173111900 2088.

Zhu YF, Li SZ, Sun QZ, Yang XJ. 2019. Effect of in ovo feeding of Vitamin C on antioxidation and immune function of broiler chickens. Animal. 13:1927–1933. DOI: 10.1017/S1751731118003531.

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Influence of *Tenebrio molitor* L Supplementation on Egg Quality and Omega-3 Content

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ABSTRAK

Rahmawati T, Fuah AM, Arifin HS, Syukur M, D Salundik. 2022. Pemanfaatan *Tenebrio molitor* L dalam pakan terhadap kualitas dan kandungan omega-3 telur. JITV 27(1): 28-34. DOI: http://dx.doi.org/10.14334/jitv.v27i1.2995

Tenebrio molitor L merupakan salah satu bahan pakan alternative karena kaya dengan kandungan nutrien, yaitu protein, vitamin, mineral (kalsium), energi dan lemak. Tenebrio molitor L juga mengandung omega-3 sebesar 33.64±0.22%, sehingga diharapkan telur yang dihasilkan mengandung omega-3. Penelitian ini bertujuan untuk mengetahui pengaruh Tenebrio molitor L sebagai bahan pakan sumber protein pengganti meat bone meal (MBM) terhadap kadar omega-3 dan kualitas telur. Dalam penelitian ini digunakan 300 ekor ayam petelur strain Lohman Brown umur 20 minggu. Rancangan acak lengkap (RAL) digunakan dalam penelitian ini dengan 3 perlakuan dan 10 ulangan, masing-masing ulangan 10 ekor. Perlakuan adalah P0= Pakan mengandung 5% MBM, P1= Pakan mengandung 2.5% MBM + 2.5% *Tenebrio molitor* L, dan P2= Pakan mengandung 5% *Tenebrio molitor* L. Perlakuan pakan selama 6 bulan. Peubah yang diamati adalah produksi telur, berat telur, indeks telur, berat kerabang, tebal kerabang, haugh unit, skor yolk, dan omega-3. Perlakuan tidak mempengaruhi kualitas fisik telur, namun berpengaruh nyata terhadap berat telur. Perlakuan P0 menghasilkan berat telur yang paling rendah yaitu 59.02±0.53 g. Perlakuan P2 mengandung omega-3 lebih banyak daripada P0 dan P1 yaitu 88.18±0.12mg 100 g^{-1}. Kesimpulan penelitian ini bahwa *Tenebrio molitor* L dapat menggantikan MBM hingga 5% dalam pakan ayam petelur, meningkatkan kualitas telur, dan menghasilkan telur omega-3.

Kata Kunci: *Tenebrio molitor* L, Kualitas Telur, Omega-3

ABSTRACT

Rahmawati T, Fuah AM, Arifin HS, Syukur M, D Salundik. 2022. The influence of *Tenebrio molitor* L supplementation on egg quality and omega-3 content. JITV 27(1): 28-34. DOI: http://dx.doi.org/10.14334/jitv.v27i1.2995

Tenebrio molitor L is one of the alternative feed ingredients because it is rich in nutrients, namely protein, vitamins, minerals (calcium), energy, and fat. *Tenebrio molitor L* also contains 33.64±0.22% omega-3, so it is hoped that the eggs produced contain omega-3. In this study 300 Lohman Brown laying hens of 20-week-old were used. Completely randomized design (CRD) was applied in this study with 3 treatments and 10 replications, each replication contained 10 laying hens. Treatments were: P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*. This research was conducted for 6 months. The variables observed were egg production, egg weight, egg shape index, shell weight, shell thickness, Haugh unit, yolk index, and omega-3. Treatment had no influence on egg physical quality but had a significant influence on egg weight. Treatment P0 prodeced the lowest egg weight that was 59.02±0.53 g. Treatment P2 had higher omega-3 contents than P0 and P1 that was 88±0.12 mg 100 g^{-1}. It was concluded that *Tenebrio molitor* L could replace MBM up to 5% in laying hens feed, improve eggs quality, and omega-3 content in eggs.

Key Words: *Tenebrio molitor* L, Egg Quality, Omega-3

INTRODUCTION

The production of laying hens has only met the needs of the domestic market by 65%, the rest is met by kampong chicken, ducks, and quail. In the livestock business, laying hens need expensive protein feed raw materials such as meat bone meal (MBM). The feed in the poultry business accounts for 70% of the production cost. Therefore, alternative steps are needed to reduce feed costs but still maintain feed quality and livestock production. An alternative source of protein to be used as feed is insects.

Edible insects such as mealworms (*Tenebrio molitor* L) have potential as feed ingredients. This is because, first, *Tenebrio molitor* L is rich in nutrients, namely protein (Makkar et al. 2014), vitamins, minerals (calcium), energy, fat (Van Huis 2013; Bosch *et al.* 2014), and can be readily available sustainable (Veldkamp et al. 2012). Second, *Tenebrio molitor L* can utilize organic waste as a breeding substrate, so that two benefits are obtained at the same time, namely environmental cleanliness and production of *Tenebrio molitor* L as feed material (Mutafela 2015). Third,

chickens naturally consume insects at various insect life cycles from the wild (Bovera et al. 2016). Fourth, *Tenebrio molitor* L is fast-growing and easy to reproduce, as well as high feed efficiency (Józefiak et al. 2016). Fifth, the need for animal protein source feed ingredients in the chicken feed will always exist, because the amino acid composition of animal protein is better than that of vegetable protein source feed ingredients, especially the content of essential amino acids containing sulfur (Bovera et al. 2016).

Tenebrio molitor L farm is a promising business opportunity considering the very conducive market share in Indonesia. The need for *Tenebrio molitor* L in Jakarta, Bogor, Depok, Tangerang, and Bekasi (Jabodetabek) is around 73 tons per month, where Bogor needs 12 tons per month, Jakarta needs 20 tons per month, Bekasi needs 15 tons per month, Depok and Tangerang each need 13 tons per month (Hapsari et al. 2018). *Tenebrio molitor* L are an interesting protein source in feeding fish, poultry, and pig (Veldkamp et al. 2012). The larva and puppa *Tenebrio molitor* are rich in protein (46 to 60%) and easy to breed (Bovera *et al.* 2016). One adult female beetle aged 2-3 months can produce 200-300 eggs or can reach 100 kg in one harvest (22-137 days) (Manullang et al. 2018).

Tenebrio molitor L is a mealworm larva belonging to the genera Tenebrio and Tribolium (Ordo Coleoptera) experiencing 15 molts. *Tenebrio molitor* L larva stage has a positive impact because it can be bred and used as a food source for fish, reptiles, amphibians, and birds. Adult Tenebrio molitor has a negative impact because it spoils grain and stored food (Hapsari et al. 2018). The life phase starts from the egg, then hatches into a larva, pupa, or cocoon, and the last phase becomes an adult *Tenebrio molitor* L (Hartiningsih & Sari EF 2014). The potential of larvae *Tenebrio molitor* L for livestock, especially as animal feed is quite good, because cultivation is easy, has a short life cycle, high production and contains high nutrients needed by livestock.

A study on the use of larvae *Tenebrio molitor L* as a protein source substitute for Meat Bone Meal (MBM) in broiler farms showed that *Tenebrio molitor* L could replace MBM up to 50% without affecting the performance of broilers reared for 35 days (Purnamawati 2015). This will be beneficial for broiler chicken farms because most of the protein source feed for poultry in Indonesia are imported such as soybean meal, fish meal, and MBM. The high price of MBM has an impact on the use of MBM so that the use of larvae *Tenebrio molitor* L can reduce feed costs (Purnamawati 2015).

Larvae *Tenebrio molitor* L contains 33.64±0.22 % omega-3 so it is expected that the eggs produced contain omega-3. The addition of omega-3 fatty acids in poultry feed is becoming increasingly important because it can affect the immunity of chickens, improve the quality of poultry products and benefit human health (Lee et al. 2019). The strategy in preparing feed formulations for poultry can increase the nutritional content of the eggs produced (Dhama *et al.* 2014), can increase the quality and quantity of eggs (Sihvo et al. 2014), and can increase the use-value of eggs for humans (Sujatha dan Narahari 2011). Eggs do not naturally contain omega-3, so omega-3 supplementation in poultry feeds is necessary to obtain omega-3 eggs (Chen et al. 2012); (Maroufyan et al. 2012). Omega-3 eggs are beneficial for health and fulfill human nutrition (Kassis et al. 2012).

MATERIALS AND METHODS

This study was carried out at the Field Laboratory of the Faculty of Animal Husbandry, Institut Pertanian Bogor University, West Java. The use of experimental animals has obtained approval from Komisi Kesejahteraan Hewan Coba Balitbangtan (KKHB), Ministry of Agriculture with registration number: Balitbangtan/BBP2TP/A/01/2021. In this study 300 heads of 20-week-old Lohman Brown laying hens were used with 3 treatments and 10 replications, each replication with 10 laying hens. The study was carried out for 6 months. Before treatment, laying hens were adapted to feed contain *Tenebriomolitor* L (P1 and P2) for 7 days.

Laying hens were placed in individual battery cages and randomized to 3 treatments × 10 replications × 10 individuals. Feeding was carried out at 07.00 WIB and 15.00 WIB. Vitamin was also given in drinking water to reduce stress in laying hens. Feed treatment for 6 months was carried out after laying hens was adapted to the cage for 2 weeks and adapted to feed contain *Tenebriomolitor* L (P1 and P2) for 7 days. During rearing, laying hens were vaccinated with ND, IB, and AI vaccines. ND and IB vaccinations were carried out every 3 months through clean and chlorine-free drinking water following procedure or SOP described by Center of Veterinary Research, Ministry of Agriculture. Before being given the vaccine, the laying hens fasted for 2 hours so that the vaccine in drinking water could be consumped immediately and evenly distributed throughout the laying hens. In addition, drinking water must also be protected from the sun (Wiyono et al. 2019).

The feed formulation was prepared based on the nutritional needs of laying hens with crude protein content of 22% each treatment (Table 1). The feed formulation was prepared using the Pearson Square method (Table 2) with 3 treatments are P0: Feed contain 5% meat bone meal, P1: Feed contain 2.5% meat bone meal + 2.5% *Tenebrio molitor* L, P2: Feed contain 5% *Tenebrio molitor* L. A hundred fourty gram

Table 1. Nutritional content of feed for each treatment

Nutrional Content	P0 (%)	P1 (%)	P2 (%)
Crude protein	22	22	22
Metabolic energy	3.08	3.21	3.35
Calcium	0.1	0.1	0.1
Phospor	0.5	0.5	0.5
Methionine	0.3	0.4	0.3
Lysine	1.1	1.2	1.1
Omega-3	0	0.84	1.68

P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

Table 2. Feed Composition of each treatment based on NRC (1994)

Feed Material	P0	P1	P2
Corn	52	52	52
Fine barn	10	10	10
Soybean meal	31	31	31
Meat bone meal	5	2.5	0
Tenebrio molitor L	0	2.5	5
Coconut oil	1	1	1
Salt	0.3	0.3	0.3
Premix	0.5	0.5	0.5
Total	100	100	100

P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

feed per head of laying hens per day and drinking water was provided ad libitum. Variables observed included (1) egg production, eggs were collected every day at 09.00 WIB, then weighed and counted for 6 months, (2) egg weight (g hen-1), Eggs were weighed digitally with an accuracy of 0.1 g then calculated the average of each treatment and replication, (3) egg shape index. The egg shape index is the quotient between the width and length of the egg and then multiplied by 100 (Ledvinka et al. 2012). The measurement of the egg length and width used a caliper with an accuracy of 0.05 m, (4) eggshell weight, Eggshell was weighed with a digital scale, (5) eggshell thickness (mm), eggshell thickness was measured using a caliper at the center (equator), the blunt end, and the pointed end of the egg to then calculate the average, (6) Haugh Unit (HU), the eggs were cracked on a smooth and flat surface (glass), then the egg white height was measured using a caliper. According to (Rahmawati & Irawan 2021), (7) egg yolk color, egg yolk color was tested using a yolk color fan by comparing the yolk color with a standard egg yolk fan, on a scale of 1 - 15 (light yellow to dark yellow), (8) omega-3, the omega-3 fatty acids content in egg yolk was analyzed by the Park and Goins method (1992). The Haugh Unit formula is:

$$HU = Log\ 100\ (H - 1.7\ W0.37 + 7.57)$$

where: H = Egg white height (mm), W = egg weight (g egg-1). HU >72 is grouped to grade AA, HU= 60-72 grouped to grade A, and HU= 31-60 grouped to grade B. The data were analyzed using variance (ANOVA) and if they were significantly different (P<0.05) followed by the Duncan Multiple Range Test (Steel and Torrie 1993). The descriptive analysis results are presented in tabular form.

RESULTS AND DISCUSSION

Effect of *Tenebrio molitor* L supplementation in feed on average egg production, egg weight, physical quality, and external quality are presented in Table 3.

Egg production

Tenebrio molitor L supplementation into feed did not significantly influence (P < 0.05) egg production (Table 2). This is because the crude protein content in all treatments was not different (22%), although the omega-3 content was different. Fatty acid saturation was significantly influence production of laying hens at the end of production period (58–74 weeksold), on egg production performance, egg weight, egg production, feed efficiency, and body weight (Buitendach et al. 2014). Egg production is influenced by linoleic fatty acid and amino acid methionine. Linoleic fatty acids control proteins and lipids needed for follicle development which directly control egg production (Mulyadi 2013). Since the crude protein level was the same in the three treatments resulted in non significant influence on egg production. Egg production in this study was around 83.78%-85.40% or in normal conditions. This is because the feed was sufficient for basic living needs and egg production. Egg production is expressed as Hen Day Egg Production (HDP), influenced by the fulfillment of basic living needs (Ledvinka et al. 2012; Kingori et al. 2014; Setiawati et al. 2016; Berenjian et al. 2021).

Egg weight

Tenebrio molitor L supplementation into feed significantly influenced (P<0.05) egg weight (Table 2). The average egg weight in P1 and P2 treatments were significantly higher (P<0.05) than P0. It was suspected that the amino acid content in *Tenebrio molitor* L and MBM proteins was different, although the protein

Table 3. Average production, weight, physical quality, and external quality with *Tenebrio molitor* L supplementation in feed

Variable	Treatment		
	P0	P1	P2
Egg production (%)	83.78±0.04	84.77±0.05	85.40±0.05
Egg weight (g)	59.02±0.53[a]	60.21±0.60[b]	60.26±0.61[b]
Egg shape index	76.57±0.02	78.39±0.01	77.28±0.02
Shell weight	4.56±0.06	4.75±0.05	4.61±0.05
Shell thickness	0.39±0.01	0.33±0.01	0.38±0.01
Haugh unit	78.56±0.56	78.53±0.58	79.13±0.49
Yolk index	7.20±0.21	7.40±0.11	7.32±0.20

[ab] denotes that same line with different letters shows significant difference in $P < 0.05$. P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

content of the feed was adjusted to the need for feed per laying hens namely 22%. The feed quality will affect the egg weight, a good feed will produce large eggs. Egg weight is influenced by protein content, genetic, environment, and age of chickens (Rahmawati & Irawan 2021). Protein and amino acids are the most important food substances in controlling egg size. *Tenebrio molitor* L crude protein was little bit higher than (45.87%) than crude protein of MBM (42.4%). This caused eggs P1 and P2 to be heavier than P0.

Egg weight between P1 and P2 was not different because both of treatment P1 and P2 contented *Tenebrio molitor* L. In addition to protein, the Ca content in the feed also affects egg weight, the higher the percentage of calcium in the feed, the heavier the eggs will be. Good quality feed have good protein, amino acids, and linoleic acid so that it affects product output (Sjofjan et al. 2020). Egg weight is influenced by several factors such as seasonal changes, parent body weight, feed given, storage time, lineage, heredity, room temeperature, hen age, and sanitary (Ledvinka et al. 2012; Kingori et al. 2014; Kasmiati et al. 2019; Viana et al. 2020). Egg weight is a price-determining factor in the marketing aspect. Egg weight based on SNI 01-3926-2006 is divided into 5 groups, namely extra-large (> 60 g), large (56–60 g), medium (51–55 g), small (46–50 g), and extra small (< 46 g). Table 2 shows that the average egg weight P0 was in the large group while egg weight P1 and P2 was in the extra group.

Egg shape index

Tenebrio molitor L supplementation into feed was not significantly influence ($P>0.05$) egg shape index. This is because the protein feed content in the three treatments was not different. Protein will affect the viscosity reflecting the internal quality of the egg, thus affecting the egg shape index. The more protein content in the feed, the thicker the albumen, so the higher the egg shape index (Christina et al. 2020; Elem 2021).

The egg shape index was in the range of 76.57–78.39 or in good category (70-79) (Widyantara et al. 2017). Egg shape index can be used to physically determine egg quality because egg shape index will affect egg shape and reproductive function. The decrease in the egg shape index was caused by the evaporation of CO_2 gas and water in the egg so that the alkaline nature of the albumen increased and caused the ovomucin fibers to be damaged. The egg shape is affected by the isthmus diameter. If the diameter is wide, the egg shape produced tends to be round, and conversely, the egg shape tends to be oval if the isthmus diameter is not wide (Ledvinka et al. 2012). Besides being influenced by the isthmus diameter, the egg shape index is also influenced by the age of the parent. Young parents tend to produce small and oval eggs, while older parents tend to produce round eggs (Kasmiati et al. 2019).

Yolk index

Tenebrio molitor L supplementation into feed significantly influenced yolk index. This is because the content of β-carotene or xanthophyll pigments between MBM and *Tenebrio molitor L* was significantly different. Yolk color is affected by feed. If the feed contains more carotenoids, namely xanthophylls, the yolk index will be more reddish-orange in color (Amo et al. 2013; Kotrbáček et al. 2013).

The Yolk index was of good quality or ranged from 7.2–7.4. Poultry consuming higher carotenoid pigments will produce a higher intensity of egg yolk color. The type and amount of carotenoid pigment consumed by laying hens is a major factor in egg yolk pigmentation. Yolk index is influenced by the content of carotenoid

compounds found in plants and pigments affecting egg yolk color namely carotene pigments (Kotrbáček et al. 2013; Christina et al. 2020). The Yolk index was in a good category or 7–12 and had a positive effect on consumer tastes (Mulyadi 2013).

The yolk color was influenced by xanthophyll dyes with the most abundant in the hydrolytic carotenoid group (Kotrbáček et al. 2013), and also due to the relationship between nutritional content feed or is almost the same in each treatment (Suryana et al. 2020). The high and low yolk index is influenced by a number of color pigment content in the feed called carotenoids. Carotene is contained in xanthophyll pigments, while xanthophyll pigments are abundant in corn (Christina et al. 2020).

Shell thickness

Shell thickness was measured at the blunt, middle, and taper sections, then averaged. *Tenebrio molitor L* did not significantly influence (P>0.05) influence shell thickness. This is because the Ca and P in the feed in the 3 treatments was significantly different. Shell thickness and quality depend on the calcium content (Christina et al. 2020). Shell thickness is influenced by the absorption of calcium in the small intestine. If calcium is well absorbed in the small intestine, egg weight and thickness increase in the uterus (Suryana et al. 2020). The higher the calcium quality, the better the eggshell quality. Eggshell quality is influenced by the nutritional quality of livestock, livestock health, maintenance management, and environmental conditions.

Shell is influenced by light use and laying hens weight (Mulyadi 2013), and vitamin D (Świątkiewicz et al. 2010; Rahardja et al. 2015). Adequate levels of vitamin D are needed to absorb calcium (Kannan & Lim 2014) in the shell formation process (Amo *et al.* 2013)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014). The eggshell is the outermost part of the egg and it is important to pay attention to its quality because the eggshell serves to protect egg content from bacteria causing damage to egg content resulting in decreased egg quality (Amo *et al.* 2013). Eggshell has an important role to protect eggs from microorganisms thereby reducing spoilage (Kingori *et al.* 2014; Vakili dan R 2016). Lack of calcium and phosphorus in the feed produces a thin shell so that the eggs are easy to crack and bacteria can easily enter the eggs (Hanusová *et al.* 2015).

Shell thickness was in the range of 0.33–0.39 mm or normal and of good quality. Good shell thickness for the market is 0.3–0.33 mm so it is not easily broken (Suryana *et al.* 2020). Shell thickness is influenced by several factors, namely age, type of chicken, nutrients, organ function, stress, and components of the eggshell layer (Mulyadi 2013). Thin eggshells are relatively more porous and large (Kasmiati *et al.* 2019), thereby accelerating the decline in egg quality due to evaporation and faster spoilage (Widyantara *et al.* 2017).

Haugh unit (HU)

Haugh unit reflects albumen condition to determine egg quality. The HU value was determined based on albumen conditions or the ratio between albumen height and egg weight. Haugh unit is used to determine egg freshness, especially in the albumen (Rahmawati & Irawan 2021).

Tenebrio molitor L did no significantly influence (P > 0.05) HU value (Table 2). The resulting Haugh unit was included in quality I with a HU > 72 (SNI, 2006). This means that the eggs are fresh and have thick albumen, so the correlation between albumen and egg weight is high. The higher the HU, the better the egg quality (Rahardja et al. 2015). The best egg quality is still new and fresh (Suryana et al. 2020). HU is influenced by ovomucin found in albumen. The higher the albumen, the higher the HU obtained. Some of the indicators determining HU in laying hens are shelf life, age, nutrients, egg storage, strain, vitamin C and or E supplementation (Ledvinka et al. 2012; Rahmawati & Irawan 2021). The albumen height was maximum at the time the eggs were released and decreased with increasing storage time. The decrease in quality is caused by long storage time, handling factors, and environmental conditions (Rahardja et al. 2015; Feddern et al. 2017). Egg quality is affected by season, ambient temperature, and age (Hanusová et al. 2015; Christina et al. 2020).

Omega-3 content

Tenebrio molitor L supplementation into feed influenced Omega-3 content.

Table 4. The influence of *Tenebrio molitor L* supplementation into feed on Omega-3 content

Treatment	Omega-3 content (mg/100 g)
P0	37.08±0.23[a]
P1	72.42±0.43[b]
P2	88.18±0.12[c]

[abc] denotes that same line with different letters shows significant difference in P < 0.05. P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

Tenebrio molitor L supplementation into feed significantly influence (P<0.05) omega-3 content. *Tenebrio molitor* L in feed serves as an energy source to

produce eggs containing omega-3 fatty acids. *Tenebrio molitor L* contains 33.64±0.22 % of omega-3. Treatment P2 contains more omega-3 than P0 and P1. This is because *Tenebrio molitor L* contains omega-3 fatty acids, so the more *Tenebrio molitor L* supplementation in the feed, the higher the omega content in eggs produced. The increase in omega-3 in eggs can be manipulated by providing feeds containing omega-3 (Kassis et al. 2012; Hasyim et al. 2016; Lee et al. 2019; Berenjian et al. 2021). Egg yolks from laying hens given omega-3 supplements contain higher levels of Omega-3 than chickens not given supplements (Konieczka et al. 2016).

CONCLUSION

As much as 5% *Tenebrio molitor* L (P2) supplementation produced eggs with more omega-3 than P0 and P1 namely 88±0.12 mg. *Tenebrio molitor* L supplementation into feed did not significantly influence ($P<0.05$) egg production, egg shape index, yolk index, shell thickness, and HU, but significantly influence ($P<0.05$) egg weight. Egg production was ranged from 85.40±0.05%, egg weight 60.26±0.16 g; egg shape index was ranged from 77.28±0.02 or in good value, yolk index was of good quality or ranged from 7.32±0.204. *Tenebrio molitor* L could replace MBM up to 5% in laying hens feed, it improved eggs quality, and omega-3 content of eggs.

REFERENCES

Ahmadi F, Rahimi F. 2011. Factors Affecting Quality and Quantity of Egg Production in Laying Hens : A Review. World Appl Sci J. 12:372–384.

Amo M, Saerang JLP, Najoan M, Keintjem J. 2013. Pengaruh penambahan tepung kunyit (*Curcuma domestica* val) dalam ransum terhadap kualitas telur puyuh. Zootec. 33:48. DOI:10.35792/zot.33.1.2013.3335.

Berenjian A, Sharifi SD, Mohammadi-Sangcheshmeh A, Bakhtiarizadeh MR. 2021. Omega-3 fatty acids reduce the negative effects of dexamethasone-induced physiological stress in laying hens by acting through the nutrient digestibility and gut morphometry. Poult Sci. 100:1–11. DOI:10.1016/j.psj.2020.12.002.

Bosch G, Zhang S, Oonincx D, Hendriks H. 2014. Protein quality of insects as potential ingredients for dog and cat foods. J Nutr Sci. 3:1–4. DOI:10.1017/jns.2014.23.

Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi V, Gasco L, Nizza A. 2016. Use of *Tenebrio molitor* larvae meal as protein source in broiler diet : Effect on growth performance , nutrient digestibility , and carcass and meat trait use. J Anim Sci. 94:639-647. DOI:10.2527/jas2015-9201.

Buitendach G, De Witt F, Hugo A, Van der Merwe H, Fair M. 2014. Effect of dietary fatty acid saturation on egg production at end-of-lay. S Afr J Anim Sci. 43:131. DOI:10.4314/sajas.v43i5.24.

Ceylan N, Ciftci I, Mizrak C, Kahraman Z, Efil H. 2011. Influence of different dietary oil sources on performance and fatty acid profile of egg yolk in laying hens. J Anim Feed Sci. 20:71–83. DOI:10.22358/jafs/66159/2011.

Chen W, Wang JP, Huang YQ. 2012. Effects of dietary n-6:n-3 polyunsaturated fatty acid ratio on cardiac antioxidative status, T-cell and cytokine mRNA expression in the thymus, and blood T lymphocyte subsets of broilers. Livest Sci. 150:114–120. DOI:10.1016/j.livsci.2012.08.008.

Christina C, Hidayat H, Warnoto W. 2020. Pengaruh penambahan roti afkir dalam pakan terhadap kualitas telur ayam ras. Bul Peternak Trop. 1:54–63. DOI:10.31186/bpt.1.2.54-63.

Dhama K, Tiwari R, Khan RU, Chakraborty S, Gopi M, Karthik K, Saminathan M, Desingu PA, Sunkara LT. 2014. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: The trends and advances-a review. Int J Pharmacol. 10:129–159. DOI:10.3923/ijp.2014.129.159.

Elem O. 2021. Feed Conversion Ratio Calculator. Omni Calculatoir. https://www.omnicalculator.com/biology/fcr

Feddern V, Coldebella A, Abreu PG De. 2017. Egg quality assessment at different storage conditions , seasons and laying hen strains. 41:322–333. DOI: 10.1590/1413-705420174130023 17.

Hanusová E, Hrnčár C, Hanus A, Oravcová M. 2015. Effect of breed on some parameters of egg quality in laying hens. 2015:20–24. DOI:10.15414/afz.2015.18.01.20.

Hapsari D, Fuah A, Endrawati Y. 2018. Produktifitas Ulat Hongkong (*Tenebrio molitor*) pada media pakan yang berbeda. J Ilmu Produksi Teknol Has Peternak. 6:53–59. DOI: 10.29244/jipthp.6.2.53-59.

Hartiningsih, Sari EF. 2014. Peningkatan bobot panen ulat hongkong akibat aplikasi limbah sayur dan buah pada media pakan berbeda. 14:55-64. DOI:10.33366/bs.v14i1.81.

Hasyim Z, Soekendarsi E, S MA. 2016. Efektivitas alga Eucheuma cottonii dan cacing tanah lumbricus rubellus dalam peningkatan kandungan omega-3 pada telur. Pros Semin Nas from Basic Sci to Compr Educ. 2:134–138.

Van Huis A. 2013. Potential of insects as food and feed in assuring food security. Annu Rev Entomol. 58:563–583. DOI:10.1146/annurev-ento-120811-153704.

Kannan S, Lim HW. 2014. Photoprotection and vitamin D: A review. Photodermatol Photoimmunol Photomed. 30:137–145. DOI:10.1111/phpp.12096.

Kasmiati, Lumatauw S, Sumpe I. 2019. Uji kualitas telur ayam ras di Kota Manokwari. JITPVET. 8:9-18. DOI:10.30862/jipvet.v8i1.28.

Kassis NM, Gigliotti JC, Beamer SK, Tou JC, Jaczynski J. 2012. Characterization of lipids and antioxidant

capacity of novel nutraceutical egg products developed with omega-3-rich oils. J Sci Food Agric. 92:66–73. DOI:10.1002/jsfa.4542.

Kingori AM, Wachira AM, Tuitoek JK. 2014. Influence of Energy Intake on Egg Production and Weight in Indigenous Chickens of Kenya. Int J Pountry Sci. 13:151–155.

Konieczka P, Czauderna M, Smulikowska S. 2016. The enrichment of chicken meat with omega-3 fatty acids by dietary fish oil or its mixture with rapeseed or flaxseed − effect of feeding duration. Anim Feed Sci Technol. 223. DOI:10.1016/j.anifeedsci.2016.10.023

Kotrbáček V, Skřivan M, Kopecký J, Pěnkava O, Hudečková P, Uhríková I, Doubek J. 2013. Retention of carotenoids in egg yolks of laying hens supplemented with heterotrophic *Chlorella*. 2013(5):193–200.

Ledvinka Z, Eva T, Englmaierová M, Podsední M. 2012. Egg quality of three laying hen genotypes kept in conventional cages and on litter Eiqualität bei drei Legehennenherkünften in konventionellen Käfigen und in Bodenhaltung. 76:38–43.

Lee SA, Whenham N, Bedford MR. 2019. Review on docosahexaenoic acid in poultry and swine nutrition: Consequence of enriched animal products on performance and health characteristics. Anim Nutr. 5:11–21. DOI:10.1016/j.aninu.2018.09.001.

Makkar HPS, Tran G, Heuzé V, Ankers P. 2014. State-of-the-art on use of insects as animal feed. Anim Feed Sci Technol. 197:1–33. DOI:10.1016/j.anifeedsci.2014.07.008.

Manullang DVC, Nukmal N, Umar S. 2018. Kemampuan Berbagai Tingkatan StadiumLarva Kumbang T*enebrio molitor* L. (Coleoptera : Tenebrionidae) dalam mengkonsumsi styrofoam (polystyrene). J-BEKH. 5:83–88. DOI:10.23960/jbekh.v5i1.56.

Maroufyan E, Kasim A, Ebrahimi M, Loh TC, Bejo MH, Zerihun H, Hosseni F, Goh YM, Farjam AS. 2012. Omega-3 polyunsaturated fatty acids enrichment alters performance and immune response in infectious bursal disease challenged broilers. Lipids Health Dis. 11:1–10. DOI:10.1186/1476-511X-11-15.

Mulyadi Y. 2013. Penggunaan pakan fungsional terhadap performan produksi dan kualitas telur ayam Arab. JITPVT. 13:27-33. DOI:10.24198/jit.v13i2.5104.

Mutafela RN. 2015. High value organic waste treatment via black soldier fly bioconversion. Stokholm(SW): Royal Institute of Technology.

NRC. 1994. Nutrient Requirements of Poultry — ninth revised edition. J Appl Poult Res. 3:101. DOI:10.1093/japr/3.1.101.

Purnamawati Y. 2015. Performa ayam broiler yang diberi tepung ulat Hongkong (*Tenebrio molitor* L.) sebagai pengganti meat and bone meal. Bogor (Indones): IPB University.

Rahardja DP, Hakim MR, Lestari VS. 2015. Egg production performance of old laying hen fed dietary turmeric powder. Int J Anim Vet Sci. 9:748–752.

Rahmawati N, Irawan AC. 2021. Pengaruh penambahan herbafit dalam pakan terhadap kualitas fisik telur ayam ras petelur. J Nutr Ternak Trop. 4:1–14. DOI:10.21776/ub.jnt.2021.004.01.1.

Józefiak D, Józefiak A, Kierończyk B, Rawski M, Świątkiewicz S, Długosz J, Engberg RM. 2016. Insects – a natural nutrient source for poultry – a review. Ann Anim Sci. 16:297-313. DOI:10.1515/aoas-2016-0010.

Setiawati T, Afnan R, Ulupi N. 2016. Performa produksi dan kualitas telur ayam petelur pada sistem *litter* dan *cage* dengan suhu kandang berbeda. JIPTHP. 4:197–203. DOI:10.29244/4.1.197-203.

Sihvo HK, Immonen K, Puolanne E. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet Pathol. 51:619–623. DOI:10.1177/0300985813497488.

Sjofjan O, Adli DN, Djunaidi IH, Kuncoro AS. 2020. Utilization of biogas liquid waste for starter in the fermentation of rice husk as a potential feedstuff. 22:24–30.

Sujatha T, Narahari D. 2011. Effect of designer diets on egg yolk composition of 'White Leghorn' hens. 48:494–497. DOI:10.1007/s13197-010-0132-z.

Suryana IK, Mastika IM, Puger AW. 2020. Kualitas telur ayam Isa Brown umur 100-104 minggu yang diberi ransum komersial dengan tambahan tepung kulit kerang. J Peternak Trop. 8:181–188.

Świątkiewicz S, Koreleski J, Arczewska A. 2010. Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. 2010:294–306.

Vakili R, Heravi RM. 2016. Performance and egg quality of laying hens fed diets supplemented with herbal extracts and flaxseed. 6604:107–116. DOI:10.22069/PSJ.2016.9833.1156.

Veldkamp T, van Duinkerken G, van Huis A, Ottevanger E, Bosch G, van Boekel T. 2012. Insects as a sustainable feed ingredient in pig and poultry diets : a feasibility study = Insecten als duurzame diervoedergrondstof in varkens- en pluimveevoeders : een haalbaarheidsstudie. Food Chem. 50:192–195.

Viana E de F, de Souza WJ, da Costa MA, Arnhold E, de Carvalho FB, de Carvalho Mello HH, Café MB, Stringhini JH. 2020. Performance of brown layers fed reduced dietary protein levels in two rearing systems. Rev Bras Zootec. 49. DOI:10.37496/RBZ4920200063.

Widyantara PR., Dewi GAM., Ariana IN. 2017. Pengaruh lama penyimpanan terhadap kualitas telur konsumsi ayam kampung dan ayam Lohman Brown. 20:5–11. DOI: 10.24843/MIP.2017.v20.i01.p02.

Wiyono A, Adjid RA, Suhardono, Widiastuti R, Noor SM, Nuradji H, Susanti, Dharmayanti IN. 2019. Petunjuk teknis aspek kesehatan hewan ternak ayam mendukung program perbibitan ternak Balitbangtan dan Program Bedah Kemiskinan Rakyat Sejahtera ("Bekerja") di Balitbangtan. Bogor (Indones): Balai Besar penelitian Veterine.

Chemical Content and *In Vitro* Digestibility of Broiler Litter Fermented at Different Ripen Time

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ABSTRAK

Christiyanto M, Utama CS. 2022. Kandungan kimia dan kecernaan *in vitro litter* ayam broiler yang difermentasi dengan waktu pemeraman yang berbeda. JITV 27(1): 35-44. DOI: http://dx.doi.org/10.14334/jitv.v27i1.2750

Penelitian bertujuan untuk mengkaji pengaruh lama fermentasi litter ayam terhadap kandungan kimia dan nilai kecernaan secara *in vitro*. Penelitian menggunakan Rancangan Acak Lengkap dengan 4 perlakuan dan 4 ulangan, perlakuan tersebut adalah T0 = tanpa fermentasi; T1 = litter ayam fermentasi selama 3 minggu; T2 = litter ayam fermentasi selama 6 minggu; dan T3 = litter ayam fermentasi selama 9 minggu. Parameter yang diamati yaitu kandungan kimia dan nilai kecernaan litter ayam fermentasi. Lama fermentasi yang berbeda mempengaruhi kandungan kimia litter ayam fermentasi yaitu kadar air, lemak, BETN dan TDN, namun tidak mempengaruhi kadar abu dan kadar serat. Lama fermentasi yang berbeda mempengaruhi nilai kecernaan bahan kering, kecernaan protein, kecernaan fraksi serat (ADF, NDF, Hemiselulosa), namun tidak mempengaruhi nilai kecernaan bahan organik, konstentrasi VFA, konsentrasi NH3 dan prouksi protein total litter ayam. Berdasarkan hasil penelitian dari parameter kecernaan bahan kering, VFA, kecernaan ADF, NDF dan hemiselulosa direkomendasikan fermentasi litter ayam selama 6 minggu.

Kata Kunci: Litter Ayam Broiler, Kandungan Kimia, Kecernaan, Fermentasi, *In vitro*

ABSTRACT

Christiyanto M, Utama CS. 2022. Chemical content and *in vitro* digestibility of broiler litter fermented at different ripen time. JITV 27(1): 35-44. DOI: http://dx.doi.org/10.14334/jitv.v27i1.2750

The aim of this study was to examine effect of length of chicken litter fermentation on chemical content and *in vitro* digestibility. Completely randomized design was applied in this study with 4 treatments and 4 replications. The treatments were T0 = no fermentation; T1 = fermentation of chicken litter for 3 weeks; T2 = fermentation of chicken litter for 6 weeks; and T3 = fermentation of chicken litter for 9 weeks. Parameters observed were chemical content and digestibility value of fermented chicken litter. Different fermentation time affected the chemical content of fermented chicken litter, namely water, fat, BETN and TDN content, but did not affect ash content and fiber content. Different fermentation time affected dry matter, protein, fiber fraction digestibility (ADF, NDF, Hemicellulose), but did not affect organic matter digestibility, VFA concentration, NH_3 concentration and total protein production of chicken litter. Based on dry matter, ADF, NDF hemicellulose digestibility and VFA concentration, it is concluded that recommended ripen time for chicken litter fermentation is 6 weeks.

Key Words: Broiler Litter, Chemical Content, Digestibility, Fermentation, *In vitro*

INTRODUCTION

Poultry farming in Indonesia is becoming a widely developed as livestock industry due to the increasing demand for poultry meat by 0.3% annually (BPS, 2019). Poultry farms produce meat and eggs as the main product also produce farm waste in the form of litter. Waste from chicken farms in the form of litter consisting of husks and manure. Litter is a cage base that is generally made of husks that serve to absorb water and excreta produced by chickens during maintenance in the cage (Muharlien et al. 2011). Unprocessed litter will have a negative impact on the environment (Ibrahim & Allaily 2012). The litter usually is processed into organic fertilizer.

Chicken litter is potentially to be used as ruminant feed because it is easy to obtain and has good nutritional content. It contains 25–50% crude protein and a total digested nutrients of 55 – 60% (Rahimi et al. 2018). Manure contained in litter has a nutrient content such as crude protein by 24.9%, extract ether 2.39%, Nitrogen Free Extract 27.96%, Ca 2.31%, P 1.56% and crude fiber 23.6% (Stephenson et al. 1990). Litter has high fiber levels and there are pathogenic microorganisms in it, so it must be processed to be safe, palatabel and have high digestibility when fed to livestock. Litter processing is to eliminate pathogenic microbes, including by: chemical processing, fermentation, ensiling and heating (Bolan et al. 2010). Litter fermentation is expected to increase the

digestibility of the material so as to increase livestock productivity.

In addition to providing a more economical processing solution, litter processing is also important as an alternative way to maintain availability of feed for ruminants because of its potential nutritional content such as protein. The availability and quality of feed in the dry season tends to decrease, compared to the rainy season (Mbatha & Bakare 2018). Litter processing with fermentation can be an alternative source of feed for ruminants. Some ruminant farms have actually implemented the use of litter as animal feed. However, untreated litter can have a negative impact on livestock, due to the presence of pathogenic microorganisms in the litter. Fermented litter can be used as an option.

Fermentation is a processing material with the help of microorganisms, aiming to improve the quality of the litter, so that the complex components in the litter break down into simpler ones. Fermentation can also suppress growth of pathogenic microorganisms in the litter. Litter fermentation can reduce total number of pathogenic bacteria that can interfere with livestock health if used for feed (Najibulloh et al. 2020). The higher lactic acid bacteria population during fermentation can increase protein content of chicken litter. During the process, microorganisms will synthesize protein through the protein encryption process, and enzymes produced by microorganisms will degrade complex compounds into compounds that are easier to be digested. Fermentation can reduce crude fiber content and improve quality in terms of nutritional content and digestibility of materials due to the activity of microorganisms during fermentation (Prastyawan et al. 2012).

Previous research on chicken litter fermentation was carried out using a semi-continuous stirred reactor tank, under thermophilic conditions (55±1°C) with a fermentation duration of up to 8 months (Qiao et al. 2018), which is not applicable in the field and requires and expensive. Therefore, it is necessary to study the fermentation with an applicable and inexpensive method. This study aimed to examine chicken litter fermentation at different duration on chemical content and *in vitro* digestibility [dry matter, organic matter, protein, fiber fractions (ADF, NDF, hemicellulose), concentration of NH3 and VFA].

MATERIALS AND METHODS

The experimental design used in this study was a complete randomized design (CRD) with 4 treatments and 4 replications namely T0 = no fermentation, T1 = fermented for 3 weeks, T2 = fermented for 6 weeks, and T3 = fermented for 9 weeks. The differences among treatment means was compared using Duncan's Multiple Range Test (DMRT) at a 95% confidence level (Utama & Christiyanto 2021).

Fermentation stage

Broiler chicken litter was collected from 16 broiler cages in Cemerlang Unggas Lestari Inc, Semarang City, Central Java. The collected litter was then mixed homogeneously. The liter was weighed as much as 1 kg and then 60 grams of starter mix culture (lactic acid bacteria, cellulolytic, amylolytic and lipolytic) were added. A substrate consisting of 60 grams of mineral mix, 60 grams of salt, 60 ml of molasses mixed with 100 ml of water, were added to the litter. All ingredients are mixed homogeneously, and put into a fermentation plastic container that is tightly tied. The litter was then fermented according to the treatment (T0, T1, T2 and T3). After the fermentation was complete, all samples were dried in a mesh-covered tray to avoid contamination by microorganisms under the sun for 12 hours. The dried samples were ground using a blender until they were powdered and ready for analysis.

Chemical content analysis

Proximate analysis consisting of moisture content, ash content, crude fat, crude protein and crude fiber was carried out using the AOAC method (2005). The content of nitrogen free extract is calculated using the formula according to the method of (Pratiwi et al. 2015), while the Total Digestible Nutrients (TDN) is calculated using equation according (Widodo et al. 2012).

Nitrogen Free Extract:

$$100 - (\text{ash} + \text{crude fat} + \text{crrude fiber} + \text{crude Protein}$$

$$TDN = 70{,}6 + 0{,}259a + 1{,}01b - 0{,}76c + 0{,}0991d$$

where a is percentage of digestible crude protein, b is percentage of crude fat, c is percentage of crude fiber, and d is percentage of NE.

In vitro digestibility

In vitro parameter measurements were carried out by making conditions in accordance to the actual rumen, the experiment was carried out using the Tilley & Terry (1963) method. This technique uses an artificial rumen in the form of a 100 ml fermenter tube, McDougall's solution as a substitute for saliva and fresh cow rumen from the Slaughterhouse as a source of inoculum. The sample was put as much as 0.56 g into a sterilized fermenter tube, then the sample was given 40 ml of McDougall's solution and 10 ml of beef rumen fluid. The fermenter tube is then added with CO_2 gas for 10 – 20 seconds to create anaerobic conditions and closed with a rubber cap. The tubes were then incubated

in a water bath for 3 hours and after incubation the tubes were placed in ice water to stop the fermentation. Each parameter was tested using 32 tubes for *in vitro*.

Meanwhile, the measurement of NH3 and VFA concentrations was carried out using the supernatant. The supernatant used came from the results of the first incubation for 3 hours (before the addition of HCl).

Dry matter and organic matter digestibility

Digestibility of dry matter and organic matter was calculated using the formula:

$$In\ vitro\ \text{Dry Matter digestibility} = \frac{\text{A - (B-C)}}{\text{D}} \text{ x } 100$$

where A is dry matter weight of the sample in gram, B is residual dry matter weight in gram, C is blank dry matter weight in gram, and D is dry matter weight of the sample in gram.

$$In\ vitro\ \text{organic matter digestility} = \frac{\text{E - F - G}}{\text{H}}\ x\ 100$$

where E is organic matter weight of the sample in gram, F is residual organic matter weight in gram, G is blank organic matter weight in gram, and H is dry matter weight of the sample in gram.

Total protein

The sample was mixed with 40 ml of McDougall's solution and 10 ml of rumen fluid in the fermenter tube which was then folowed with CO_2 gas. The incubation was carried out for 48 hours at a temperature of 39^0C. After incubation, 10 ml of the sample was taken and TCA + SSA was added to settle for 4-5 hours and filtered. The filtered residue was analyzed for protein by the Kjeldhal method.

Measurement of *in vitro* total protein used Kjeldahl method. Sample proteins and residual proteins were analyzed by Kjeldahl method according to AOAC, (2005). Total protein can be calculated using the formula (Sumadi et al. 2017):

$$\text{Total Protein} = \frac{\text{(I-J)}\times\text{K}\times 14\times 6{,}25}{L}\ x\ mg/g$$

where I is ml HCl titrant, J is ml HCl blank, K is N HCl, L is Sample weight of sediment (mg/g) of residue.

Protein digestibility

Protein digestibility was calculated accoding to kjeldahl method (1963). Sample proteins and residual proteins were analyzed by kjeldahl method according to AOAC (2005). The formula of protein digestibility calculation is as follows:

$$\text{Protein Digestibility} = \frac{\text{M}\times\text{N - P}}{\text{M}\times\text{N}}\ x\ 100\%$$

where M is % crude protein sample, N is weight of samples, P is % crude protein content of residue x weight of residue.

Concentration of volatile fatty acid (VFA)

The VFA total (Mm) concentrations were determined by steam distillation method (General Laboratory procedure) (Abbaticehlo et al. 1983) and calculating as:

$$\text{Blank titrant volume} - \text{sample titrant volume}) \times \text{N} - \text{HCL} \times \frac{1000}{5})$$

where N-HCL is Normality of HCL, Blank titrant volume is Number of HCL titer for 5 ml NaOH (blank), and sample titrant volume is Number of HCL titer to dissolve the distillate.

Ammonia concentration (NH_3)

Analysis of ammonia concentration (NH_3) using spectrophotometer method (Azizah & Humairoh 2015).

Digestibility of NDF, ADF and hemicellulose

The method used in this study was an experimental method, namely the analysis of the digestibility of ADF, NDF, Lignin and Hemicellulose by *in vitro*. Fiber analysis used Van Soest method, while calculation of values calculated based on Tilley & Terry (1963).

%Digestibility of NDF:

$$\frac{\text{NDF sample (g)-(NDF residue (g)-NDF Blanko (g))}}{\text{NDF sample (g)}}\ x\ 100\%$$

% Digestibility of ADF:

$$\frac{\text{ADF sample (g)-(ADF residue (g)-ADF Blanko (g))}}{\text{ADF sample (g)}}\ x\ 100\%$$

% Hemicellulose Digestibility:

$$\frac{\text{hemicel. sample (g)-(hemicel. residue (g)-hemicel. blanko (g))}}{\text{hemicel. sample (g)}}\ x\ 100\%$$

where hemicel. is hemicellulose.

RESULTS AND DISCUSSION

Analysis of variance showed that T3 was significantly different from T0, T1, T2 ($P<0.05$), and T1 was not significantly different from T2 on the water content of fermented litter. Treatment T3 had a higher water content than treatments T0, T1, and T2. Factors that cause an increase in water content can be caused by microorganisms using dry matter substrates for development and growth during the fermentation process, causing a decrease in dry matter levels and resulting in an increase in fermented water content

(Driehuis et al. 1997). The average water content was 44.09%. Litter has an average moisture content ranging from 16,32 - 19,14% (Marang et al. 2019). The increase occurred in the 63rd day fermentation. The increase in water content can also be caused by the fermentation process. During the process, there is a decrease in dry matter and an increase in water content caused by the first fermentation stage, namely during respiration, glucose is converted into CO_2, H_2O and heat (McDonald 1981).

Analysis of variance showed that difference in fermentation time was not significant (P>0.05) on ash content. Different ripen time had no significant effect in reducing fermented litter ash content. This was because the starter used organic matter as a source of nutrition and at the same time breaks down crude fiber into simple carbohydrates so that organic matter increases. The process will affect the content of organic matter because the compound will degrade complex compounds into simple ones (Setyawati et al. 2014). The average ash content in the litter was 33%, this value was higher than the results reported by Chaudhry et al. (1993) (17.8%). The ash content increased starting from day 0 to day 63. An increase in ash content of fermented litter occurs because during fermentation process there is a decrease in organic matter due to substrate degradation by starter microbes (Collett 2012).

Analysis of variance showed that T0 was significantly different from T1, T2, T3 (P<0.05) on the crude protein content of fermented litter. In fermented litter there is a decrease in value of crude protein. Fermentation is carried out with the aim of increasing the nutritional value of feed ingredients, especially at increasing protein levels (Prastyawan et al. 2012). The protein value of the litter was reported 18.9% (Chaudhry et al. 1993). The decrease in crude protein levels started from T0 treatment to T2 treatment which was caused by the number of mixed microorganisms in the feed being less than optimal. The increase in protein content occurred in treatment T3 on day 63 into 19.27%. Factors that cause an increase in protein content in the activity of microorganisms that hydrolyze proteins in the substrate. Microorganisms will hydrolyze proteins in the substrate with the help of proteolytic enzymes produced by lactic acid bacteria (Hilakore et al. 2013).

Analysis of variance showed that T0 was significantly different from T1, T2 (P<0.05) on crude fat content of fermented litter. There was a decrease in the crude fat content among the treatment. The decrease is influenced by the process due to the reshuffle of cell wall composition and saponification reaction so that water-soluble cell walls become dissolved. Average crude fat content in the litter is 2.2%. Crude fat content in chicken litter is 1.22% (Setyaningrum & Ismail 2019). Litter fermentation process has microbial activity that produces high fatty acids so that the fat content increases (Bakshi & Fontenot 1998). The increased free fat content is utilized by lipolytic microorganisms as an energy source, resulting in a decrease in crude fat content (Suningsih et al. 2019).

Analysis of variance showed that effect of the treatment was not significantly different (P>0.05) on crude fiber content of fermented litter. The average crude fiber was 19.62%. Crude fiber content in fermented litter is 13.24% (Telew et al. 2013). The factor causing no decrease in fiber content is the high crude fiber content of components that make up the litter, especially lignin and cellulose. The litter used is derived from husk material. Microorganisms during the fermentation process are difficult to degrade lignin (Ratnakomala et al. 2006). The high content of lignin and cellulose in the litter decreased the ability of microorganism enzymes to digest crude fiber content of the feed. The value of fiber content is still relatively safe to be given to ruminants. Digestion of crude fiber in ruminants occurs in the rumen with the help of microorganisms (Irawati et al. 2019).

Analysis of variance showed that T0 was significantly different from T1, T2, T3 (P<0.05) and T2 was not significantly different from T3 on the content of nitrogen free extract. Compared to control, NFE increased due to fermentation (T1, T2 and T3). The highest increased of NFE content was in T1. The increased of NFE content due to the increased of bacteria population so that they degrade complex compounds into simple compounds. The decrease in fiber content in feed ingredients will increase the NFE content (Pratiwi et al. 2015).

Analysis of variance showed that different length of fermentation affected TDN content. T0, T3 was significantly contained higher TDN than T1, T2 (P<0.05). Litter of organic chicken has a TDN content of 55-60% (Kwak et al. 2008). The increase in TDN levels occurred due to a decrease in crude fiber in T3 treatment by the cellulose enzyme produced by starter microorganisms, thereby increasing digestibility of feed nutrients (Amrullah 2019). The higher the value the better the quality of the feed, this is due to the more nutrients being digested by the animal's body (Riyanto et al. 2020). Digestibility of dry matter, organic matter, Volatile Fatty Acids (VFA), ammonia (NH_3), crude protein digestibility of the fermented litter is presented in Table 2.

Dry Matter Digestibility

In vitro DM digestibility of fermented chicken litter in sheep rumen was influenced by different fermentation times (P<0.05). Dry matter digestibility of T0 was significantly different from T1 and T2, but not significantly different from T3, the DM digestibility of T2 was the highest. The value of this research results is

Table 1. Water content, ash, crude fat, crude protein, crude fiber, BETN and TDN of fermented chicken litter at different fermentation times

Parameter	Fermentation Duration			
	T0	T1	T2	T3
Water Content (%)	38.39 ± 1.02 [c]	43.34 ± 1.31 [b]	44.30 ± 1.08 [b]	50.34 ± 1.59 [a]
Ash (%)	31.26 ± 0.43	31.38 ± 2.53	32.30 ± 0.79	33.08 ± 1.57
Crude Protein (%)	25.73 ± 1.06 [a]	18.34 ± 1.10 [b]	18.12 ± 0.52 [b]	19.27 ± 0.71 [b]
Crude Fat (%)	2.69± 0.36 [a]	1.85 ± 0.65 [b]	1.79 ± 0.39 [b]	2.50 ± 0.38 [ab]
Crude Fiber (%)	19.52± 1.08	19.90 ± 1.13	20.83 ± 1.58	18.22 ± 1.82
NFE (%)	20.80 ± 1.26 [c]	28.53 ± 1.46 [a]	26.96 ± 0.43 [b]	26.93 ± 1.97 [b]
TDN (%)	48.57 ± 2.15 [a]	44.49 ± 3.66 [b]	42.62 ± 1.73 [b]	45.92 ± 1.90 [a]
ADF Level (%)	26.17±0.40 [c]	30.91±0.76 [a]	28.60±0.16 [b]	31.80±0.93 [a]
NDF Level (%)	40.11±0.54 [a]	37.91±0.44 [b]	36.60±0.35 [c]	34.32±0.57 [d]

NFE = Nitrogen free extract, TDN = Total digestible nutrient, ADF= Acid detergent fiber, NDF= Neutral detergent fiber. Different superscripts on the same line indicate significant differences (P <0.05)

Table 2. Dry matter digestibility, organic matter digestibility, VFA, NH_3, and Crude protein digestibility, total protein production of fermented chicken litter at different fermentation times

Parameter	Fermentation Duration			
	T0	T1	T2	T3
Dry matter digestibility (%)	48.4±0.46 [c]	51.5±0.56 [b]	54.8±0.63 [a]	48.9±0.69 [c]
Organic matter digestibility (%)	71.9±1.79	69.8±1.39	70.1±1.22	68.9±1.28
VFA (mM)	77.5±5.00	80.0±5.77	85.0±11.55	75.0±11.55
NH_3 N (mM)	25.5±2.50	27.3±5.85	24.2±7.29	26.3±2.77
Total Protein (mg/g)	1632±140	1373±145	1458±114	1180±129
Crude protein digestibility (%)	43.6±0.58 [b]	43.7±0.74 [b]	45.2±0.98 [a]	45.8±0.48 [a]

Mean values within a row with different superscripts differ significantly (P≤0.05)

almost the same as that reported by Jokthan et al. (2013) which stated that the dry matter digestibility litter value was 44.31 – 51.33%. The lower dry matter digestibility value in T0 could occur because there was no time for fermentation in that treatment, so the components of the litter at T0 were more complex. The more complex litter components at T0 made the degrading bacteria *in vitro* not optimally degrade the litter, thus giving the lowest dry matter digestibility yield.

The low digestibility value was affected by microbial activity of the rumen fluid, content of feed ingredients used, and type of feed. Priyanto et al. (2017) stated that the factors that influence dry matter digestibility value can come from: form of feed, composition in feed, and microbial activity in the rumen fluid. The low dry matter digestibility value also occurs due to the low ability of rumen microorganisms to digest litter components. Setiyaningsih (2013) stated that the low dry matter digestibility value could be caused by microbial conditions in rumen fluid that could not utilize nutritional content of feed ingredients. Components that are difficult to digest are especially crude fiber composition of the litter that comes from husks. Krogdahl & Dalsgard (1981) stated that the digestibility of feed ingredients is influenced by several factors including form of feed, feed composition and nutritional content of feed but not affected by pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*, that present in chicken litter.

Organic matter digestibility

In vitro digestibility of fermented chicken litter organic matter with sheep rumen was not affected by different ripen times (P>0.05). The digestibility value ranged from 69.8 - 71.9%. The digestibility value in this study was higher than the results reported by Hadjipanayiotou (1982), litter organic matter in this study indicated that either fermented or not fermented chicken litter has the potential to be used as an alternative feed for ruminants. Al-Arif et al. (2017) stated that the fermentation treatment resulted in a

degradation process of fiber fractions such as cellulose, hemicellulose and lignin and had an impact on increasing the digestibility value. The digestibility of organic matter can be influenced by the digestibility of components of organic matter, namely protein, fat and carbohydrates.

The digestibility value can be influenced by nutrition content of the material especially fiber and ash content. The relatively similar organic matter digestibility values were thought to be due to the same fiber content and fermented litter ash content (Table 2). Suharlina et al. (2017) stated that digestibility value of organic matter in feed ingredients is determined by nutrition content present in the material, especially fiber, that will be difficult to digest by rumen microbes. The ash content of fermented chicken litter was not affected by the treatment, so that the digestibility of organic matter was not too influential. This is because organic matter is all the nutrients in the fermented chicken litter except the ash. Al-Arif et al. (2017) stated that digestibility value of organic matter can be used to measure the total amount of nutrients that can be absorbed in the digestive tract of ruminants, estimate protein synthesis of microorganisms and measure the energy produced, where dry matter digestibility is affected by the presence of ash content.

Concentration of volatile fatty acid (VFA)

Volatile Fatty Acid (VFA) *in vitro* of fermented chicken litter was not affected by different fermentation times (P>0.05). The concentration ranged from 75 - 77.5 Mm. Sutardi (1997) stated that good rumen microbial VFA concentrations ranged from 80 – 160 Mm. The VFA measured in this study was the total VFA, namely propionate, acetate and butyrate. The value was still below the good VFA standard for ruminants, indicated that fermented chicken litter can't be used as a single feed, but must be combined with other feed ingredients, so that VFA concentrations can be achieved according to the standard. VFA that is too low will inhibit activity of rumen microorganisms. Singh et al. (2020) stated that in addition to being influenced by the substrate used, activity of microorganisms was also influenced the VFA concentration.

Ripen time did not affect VFA concentration, presumably because crude fiber content in fermented chicken litter was also relatively the same. Widodo & Sutrisno (2012) stated that the fiber fraction in feed will be converted into simple sugars which undergo glycolysis to pyruvic acid and become VFA. The amount of soluble carbohydrates in the fermented chicken litter was assumed to be the same, so the VFA produced was also the same. Wijayanti et al. (2012) stated that factors that affect concentration of VFA include: amount of feed, feed fermentability, amount of soluble carbohydrates, type and pH of the rumen.

Concentration of ammonia (NH_3) in sheep

Concentration of NH_3 in fermented chicken litter *in vitro* was not affected by different ripen times (P>0.05). The NH_3 concentration ranged from 24.2 - 27.3 mM. This value is higher than that reported by Sandi et al. (2016) which states that the levels of NH_3 to support the growth of rumen microbes are in the range of 6-21 mM. Cabeza et al. (2018) stated ammonia is the result of degradation of protein and non-protein nitrogen (NPN) that enters the rumen of ruminants.

Different fermentation time did not affect the production of NH_3 which could be due to relatively the same protein degradation process in the fermented litter. Wijayanti et al. (2012) stated that the factors that affect the concentration of NH_3 are carbohydrates in the ration of the amount of feed, protein degradation, as well as solubility and rumen pH. The higher NH_3 concentration of fermented chicken litter indicated that more protein was hydrolyzed into ammonia. Prayitno et al. (2018) stated that a high NH_3 value is influenced by level of protein solubility in the feed, the higher the protein solubility in the feed, the more easily the protein will be degraded by microbes. NH_3 for rumen microorganisms acts as the main nitrogen source to support the protein synthesis process.

Total protein

Total protein of fermented chicken litter *in vitro* was not affected by different fermentation times (P>0.05). The value was 1180.167 – 1632.49 mg/g. Pal et al. (2016) stated that several factors that affect total protein include NH_3 production, carbon skeletons, and energy sources. Total protein is an indication of microbial protein from the rumen and protein litter of fermented chicken that is not degraded in the rumen of ruminants. Sumadi et al. (2017) stated that total protein plays a role in evaluating the value of protein that escapes degradation of rumen microorganisms, as well as how much concentration of microbial protein is in the post-rumen digestive organs. Priyanto et al. (2017) stated that high total protein can occur due to ideal conditions and the availability of energy sources as quickly as the formation of NH_3, so that when NH_3 is formed, the fermentation product from carbohydrates will function as an energy source and carbon source.

Crude protein digestibility

In vitro crude protein digestibility in this study was influenced by different fermentation times (P<0.05). Compared to control, crude protein digestibility of T2

Table 3. Digestibility of fermented chicken litter fiber fraction at different ripening time

Parameter	Fermentation Duration			
	T0	T1	T2	T3
ADF digestibility (%)	35.3±0.31 [d]	47.9±0.93 [b]	49.4±0.57 [a]	44.5±0.97 [c]
NDF digestibility (%)	45.9±1.00 [d]	59.4±0.51 [b]	64.1±0.85 [a]	54.9±1.10 [c]
Hemicellulose digestibility (%)	20.8±0.96 [b]	17.3±0.66 [a]	21.9±1.03 [b]	15.1±0.57 [a]

Different superscripts on the same line indicate significant differences (P <0.05)

and T3 were higher, but not significantly different from T1. The high values in T2 and T3 could be due to the crude protein and TDN content in the fermented chicken litter which was more optimally utilized compared to other fermented chicken litters. Teti et al. (2018) stated that high crude protein content in the ration will increase the rate of rumen microbial population and the ability to degrade feed increases, besides being influenced by crude protein and TDN levels.

The highest protein content of fermented chicken litter was at T0, but not comparable to the digestibility value of the protein produced, thought to occur due to chemical processes during fermentation and protein was not digested optimally by microorganisms *in vitro*. Ayasan et al. (2018) stated that the increase in protein digestibility was related to the degradation of trypsin inhibitors and the process of nucleic acid loss of secondary and tertiary structures (protein denaturation). T2 and T3 treatments gave the best crude protein digestibility value because the N content was proportional to the TDN content in the material, due to the optimal fermentation time. Ayuningsih et al. (2018) stated that the supply of nitrogen balanced with high TDN will lead to a balance of protein and energy which has an impact on digestibility and higher feed efficiency.

Fiber digestibility

The digestibility of ADF, NDF, hemicellulose of fermented chicken litter at different ripening times is presented in Table 3.

Acid detergent fiber (ADF) digestibility

Digestibility of ADF of fermented chicken litter was influenced by different fermentation times (P<0.05). Rahimi et al. (2018) stated that the digestibility value of ADF in various types of broiler litter was 38.11–43.20%. This value indicates that there is an effect of the length of fermentation to increase the digestibility of ADF. Hambakodu et al. (2020) stated that the digestibility value of ADF is a combination of digestibility value which contains cellulose, and lignin. Different fermentation times affect the levels of ADF thus affecting better acceptance of rumen microorganisms or affecting ADF digestibility. Wijaya et al. (2018) stated that digestibility of ADF in sheep was caused by adaptation of rumen microbes to a feed ingredient. The highest value of ADF digestibility was in T2. This shows that ripen time for 6 weeks increases the digestibility of ADF in broiler litter. The increase was caused by the breaking of the lignin-cellulose bonds by microbes during fermentation. Putri (2020) stated that fermentation causes lignin cellulose bonds consisting of lignin, cellulose and also hemicellulose to be broken so that they are easily digested by rumen microbes.

Neutral Detergent Fiber (NDF) Digestibility

NDF digestibility of fermented chicken litter was influenced by different fermentation times (P<0.05). All treatments were significantly different from each other. NDF is the main component of fiber in fermented chicken litter. The presence of NDF in chicken litter comes from the husk which is part of the litter which is high in fiber content. Zhao et al. (2019) stated that NDF is a constituent of cell walls consisting of hemicellulose, lignin, cellulose, and other small components such as silicates and proteins. Turangan et al. (2018) stated that NDF digestibility levels were influenced by crude fiber content such as lignin, silica, energy sources, protein, minerals and vitamins. In fermentation process there are enzymes that work in the process of breaking lignin bonds from fermented chicken manure. Yanti et al. (2021) stated that the penetration of rumen microorganism enzymeswould be easier to degrade NDF due to the presence of lignase enzymes that break the bonds of lignohemicellulose and lignocellulose.

Hemicellulose digestibility

Hemicellulose digestibility of fermented chicken litter was influenced by different length of fermentation (P<0.05). Treatment T0 was significantly higher than T1 and T3, but not significantly different from T2; T1 was significantly different from treatment T2 and T3; T2 and T3 were significantly different. The digestibility

of the material is influenced by crude fiber content, because crude fiber content will result in a low degradation value. Angelidaki & Ahring (2000) stated that crude fiber in the form of cellulose and hemicellulose often binds to lignin, and will be difficult to be broken down by digestive enzymes, which causes lower digestibility if a feed ingredient contains high fiber.

Higher hemicellulose digestibility results at T2 can occur because in this treatment digestibility of NDF is also high, so it has an impact on high hemicellulose digestibility as well. Yanti et al. (2021) stated that digestibility of hemicellulose is generally higher than digestibility of cellulose and digestibility value is influenced by levels of NDF and ADF. The hemicellulose constituent fractions are generally more easily digested by rumen microorganisms. Zhao et al. (2019) stated that hemicellulose has an amorphous structure and low polymerization rate, so it will be easier to digest than other cell wall components. The use of cellulose and hemicellulose as a source of carbohydrates will be easily fermented by rumen bacteria into VFA which is a source of energy for the growth of sheep.

CONCLUSION

Fermentation increased water and NFE content, decreased CP, Crude Fat, NDF and TDN, but did not affect ash and CF content. Fermentation increased dry matter digestibility, protein digestibility, fiber fraction digestibility (ADF, NDF, Hemicellulose). The recommended treatment is fermenting chicken litter for 6 weeks.

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REFERENCES

Abbaticehlo P, Bailee V, Cera O. 1983. Determination of volatile fatty acids by gas chromatography after a rapid steam distillation of wastes. Environ Technol Lett. 4:179–182. DOI:10.1080/09593338309384191.

Al-Arif MA, Suwanti LT, Estoepangestie AS, Lamid M. 2017. The nutrients contents, dry matter digestibility, organic matter digestibility, total digestible nutrient, and NH_3 rumen production of three kinds of cattle feeding models. KnE Life Sci. 3:338. DOI:10.18502/kls.v3i6.1142.

Amrullah M, Tampoebolon BIM, Prasetyono BW. 2019 Kajian pengaruh proses fermentasi sekam padi amoniasi menggunakan aspergillus niger terhadap serat kasar, protein kasar, dan total digestible nutrients. JPPP. 16:25–31.

Angelidaki I, Ahring BK. 2000. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Sci Technol. 41(3):189–194. DOI: 10.2166/wst.2000.0071.

Ayasan T, Ülger I, Kaliber M, Ergül S, Inci H, Mart D, Türkeri M. 2018. Comparison of *in vitro* gas production, nutritive value, metabolizable energy and organic matter digestibility of some chickpea varieties. Iran J Appl Anim Sci. 8:31–136.

Ayuningsih B, Hernaman I, Ramdani D, Siswoyo S. 2018. Pengaruh imbangan protein dan energi terhadap efisiensi penggunaan ransum pada domba Garut betina. J Ilm Peternak Terpadu. 6:97. DOI:10.23960/jipt.v6i1.p97-100.

Azizah M, Azizah M. 2015. Analisis kadar amonia (NH3) dalam air sungai Cileungsi. Nusa Sylva. 15:47–54.

Bakshi MPS, Fontenot JP. 1998. Processing and nutritive evaluation of broiler litter as livestock feed. Anim Feed Sci Technol. 74:337–345. DOI:10.1016/S0377-8401(98)00181-3.

Bolan NS, Szogi AA, Chuasavathi T, Seshadri B, Rothrock MJ, Panneerselvam P. 2010. Uses and management of poultry litter. Worlds Poult Sci J. 66:673–698. DOI: 10.1017/S0043933910000656.

[BPS] Badan Pusat Statistik. 2019. Broiler Population by Province. Jakarta (Indones): Bada Pusat Statistik.

Cabeza I, Waterhouse T, Sohi S, Rooke JA. 2018. Effect of biochar produced from different biomass sources and at different process temperatures on methane production and ammonia concentrations *in vitro*. Anim Feed Sci Technol. 237:1–7. DOI:10.1016/j.anifeedsci.2018.01.003.

Chaudhry SM, Naseer Z, Chaudhry DM. 1993. Fermentation characteristics and nutritive value of broiler litter ensiled with corn forage. Food Chem. 48:51–55. DOI: 10.1016/0308-8146(93)90220-A.

Collett SR. 2012. Nutrition and wet litter problems in poultry. Anim Feed Sci Technol. 173:65–75. DOI:10.1016/j.anifeedsci.2011.12.013.

Driehuis F, Van Wikselaar PG, Van Vuuren AM, Spoelstra SF. 1997. Effect of a bacterial inoculant on rate of fermentation and chemical composition of high dry matter grass silages. J Agric Sci. 128:323–329. DOI: 10.1017/S0021859696004157.

Hadjipanayiotou M. 1982. Laboratory evaluation of ensiled poultry litter. Anim Prod. 35:157–161. DOI: 10.1017/S0003356100000921.

Hambakodu M, Kaka A, Ina YT. 2020. Kajian In Vitro Kecernaan Fraksi Serat Hijauan Tropis pada Media Cairan Rumen Kambing. JITPT. 7:29. DOI:10.33772/jitro.v7i1.8907.

Ibrahim S, Allaily A. 2012. Pengaruh berbagai bahan litter terhadap konsentrasi ammonia udara ambient kandang dan performan ayam broiler. J Agripet. 12:47–51. DOI: 10.17969/agripet.v12i1.288.

Irawati E, Purnamasari E, Arsyad F. 2019. Kualitas fisik dan nutrisi eceng gondok (*Eichornia crassipes*) dengan lama fermentasi yang berbeda. J Peternak. 16:18. DOI:10. 24014/jupet.v16i1.3679.

Jokthan GE, Muhammad SA, Osuhur CU. 2013. Effect of Cottonseed cake replacement with broiler litter on performance of Yankasa Rams fed maize husk basal diets. 2013:147–155.

Krogdahl A, Dalsgard B. 1981. Estimation of nitrogen digestibility in poultry: content and distribution of major urinary nitrogen compounds in excreta. Poult Sci. 60:2480–2485. DOI:10.3382/ps.0602480.

Kwak WS, Jung SH, Kim YI. 2008. Broiler litter supplementation improves storage and feed-nutritional value of sawdust-based spent mushroom substrate. Bioresour Technol. 99:2947–2955. DOI: 10.1016/j. biortech.2007.06.021.

Marang EAF, Mahfudz LD, Sarjana TA, Setyaningrum S. 2019. Kualitas dan kadar amonia litter akibat penambahan sinbiotik dalam ransum ayam broiler. J Peternak Indones. 21:303. DOI:10.25077/jpi.21.3.303-310.2019.

Maritje Aleonor Hilakore, Suryahadi, Komang Wiryawan DM. 2013. Peningkatan Kadar Protein Putak melalui Fermentasi oleh Kapang *Trichoderma reesei*. 14:250–254.

Mbatha KR, Bakare AG. 2018. Browse silage as potential feed for captive wild ungulates in southern Africa: A review. Anim Nutr. 4:1–10. DOI:10.1016/j.aninu.2017. 12.003.

McDonald P. 1981. The Biochemistry of Silage. London: John Wiley and Sons Ltd.

Muharlien, Achmanu, R.Rachmawati. 2011. Meningkatkan Produksi Ayam Pedaging Melalui Pengaturan Proporsi Sekam, Pasir Dan Kapur Sebagai Litter. J Ternak Trop. 12:38–45.

Najibulloh M, Ulupi N, Salundik S. 2020. Pengaruh daur ulang litter terhadap kualitas litter dan udara dalam pemeliharaan broiler. Livest Anim Res. 18:107. DOI: 10.20961/lar.v18i2.42932.

Nur Eka Styawati, Muhtarudin L. 2014. Pengaruh lama fermentasi trametes sp. Terhadap kadar bahan kering, kadar abu, dan kadar serat kasar daun nenas varietas smooth cayenne. J Ilm Peternak Terpadu. 2:19–24.

Pal RS, Singh KS, Tripathi MK. 2016. Nutrient intake, digestibility, milk production and composition of lactating cows fed oat hay and concentrate containing varying levels of poultry litter. Indian J Anim Res. 50:194–198. DOI:10.18805/ijar.9540.

Prastyawan RMP, Tampoebolon BIM, Surono. 2012. Peningkatan kualitas tongkol jagung melalui teknologi amoniasi fermentasi (amofer) terhadap kecernaan bahan kering dan bahan organik serta protein total secara in vitro. Anim Agric J. 1:611–621.

Pratiwi I, Fathul F, Muhtarudin. 2015. The effect of different additioning starter to making silage on crude fiber content, crude fat, water content, and material extract without nitrogen silage. J Ilm Perten Terpadu. 3:116–120.

Prayitno RS, Wahyono F, Pangestu E. 2018. Pengaruh suplementasi sumber protein hijauan leguminosa terhadap produksi amonia dan protein total ruminal secara in vitro. J Peternak Indones. 20:116. DOI: 10.25077/jpi.20.2.116-123.2018.

Priyanto A, Endraswati A, Rizkiyanshah, Febriyani NC, Nopiansyah T, Nuswantara LK. 2017. Pengaruh pemberian minyak jagung dan suplementasi urea pada ransum terhadap profil cairan rumen (KcBK, KcBO, pH, N-NH 3 dan Total Mikroba Rumen). J Ilmu Ternak. 17:1–9.

Putri PW. 2020. Kandungan neutral detergent fibre (NDF), acid detergent fibre (ADF), hemiselulisa, selulosa dan lignin onggok yang difermentasi trichoderma reesei dengan suplementasi N, S, P. Bull Appl Anim Res. 2:33–37. DOI:10.36423/baar.v2i1.227.

Rahimi MR, Alijoo YA, Pirmohammadi R, Alimirzaei M. 2018. Effects of feeding with broiler litter in pellet-form diet on qizil fattening lambs' performance, nutrient digestibility, blood metabolites and husbandry economics. Vet Res Forum. 9:245–251. DOI:10.30 466/vrf.2018.32081.

Singh R, Park S, Koo JS, Balasubramanian B. 2020. Influence of various concentrations of aflatoxin B 1 on in vitro rumen fermentation of a buffalo diet. Korean J Agric Sci. 47:131–138. DOI:10.7744/kjoas.20200005.

Ratnakomala S, Ridwan R, Kartina G, Widyastuti Y. 2006. The effect of *Lactobacillus plantarum* 1A-2 and 1BL-2 inoculant on the quality of napier grass silage. Biodiversitas J Biol Divers. 7:131–134. DOI:10.130 57/biodiv/d070208.

Riyanto J, Widyawati SD, Sudibya S. 2020. Pengaruh perbedaan rasio menir kedelai proteksi dan tanpa proteksi terhadap konsumsi, kecernaan dan nilai nutrien pakan Domba Ekor Gemuk. Livest Anim Res. 18:240. DOI:10.20961/lar.v18i3.45995.

Sandi S, Ali AIM, Akbar AA. 2016. Uji *in-vitro* wafer ransum komplit dengan bahan perekat yang berbeda. J Peternak Sriwij. 4:7–16. DOI:10.33230/jps.4.2.2015.2802.

Setiyaningsih KDMC dan S. 2013. Kecernaan bahan Kering dan bahan organik secara in vitro hijauan Desmodium cinereum pada berbagai dosis pupuk organik cair dan jarak tanam. J Chem Inf Model. 53:1689–1699.

Setyaningrum S, Ismail D. 2019. Efektivitas pupuk kandang dari kotoran sapi, domba dan ayam terhadap kadar lemak kasar, protein kasar dan serat kasar rumput gajah pada defoliasi kedua. J Anim Sci Agron. 4:18–28.

Stephenson AH, McCaskey TA, Ruffin BG. 1990. A survey of broiler litter composition and potential value as a nutrient resource. Biol Wastes. 34:1–9. DOI: 10.1016/0269-7483(90)90139-J.

Suharlina S, Abdullah L, Astuti D, Nahrowi N, Jayanegara A. 2017. Karakteristik fermentasi rumen terhadap beberapa jenis tanaman leguminosa. Pastura. 5:71. DOI: 10.24843/pastura.2016.v05.i02.p02.

Sumadi S, Subrata A, Sutrisno S. 2017. Produksi Protein total dan kecernaan protein daun kelor secara in vitro. J Sain Peternak Indones. 12:419–423. DOI: 10.31186/jspi. id.12.4.419-423.

Suningsih N, Ibrahim W, Liandris O, Yulianti R. 2019. Kualitas fisik dan nutrisi jerami padi fermentasi pada berbagai penambahan starter. J Sain Peternak Indones. 14:191–200. DOI:10.31186/jspi.id.14.2.191-200.

Sutardi T. 1997. Peluang dan tantangan pengembangan ilmu-ilmu nutrisi ternak. Bogor (Indones): IPB University.

Telew C, Kereh VG, Untu IM, Rembet B. 2017. Pengayaan nilai nutritif sekam padi berbasis bioteknologi “effective microorganisms” (EM4) sebagai bahan pakan organik. Zootec. 32:1–8. DOI:10.35792/zot.32.5.2013.983.

Teti N, Hernaman I, Ayuningsih B, Ramdani D, Siswoyo 2018. Pengaruh imbangan protein dan energi terhadap kecernaan nutrien ransum domba garut betina. J Ilmu Teknol Peternak. 2:97–101.

Tilley JMA, Terry RA. 1963. A Two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci. 18:104–111. DOI:10.1111/j.1365-2494.1963.tb00335.x.

Turangan, G. G., B. Tulung, Y. R. L. Tulung MRW. 2018. Kecernaan NDF dan ADF yang mendapat suplementasi urea molasses multinutrient block (UMMB) dari beberapa jenis limbah pertanian dan rumput lapang pada sapi Peranakan Ongole (PO). Zootec. 38:320–328.

Utama CS, Christiyanto M. 2021. The feasibility of fermented litter as a feed ingredient for ruminant livestock. J Adv Vet Anim Res. 8:312–322. DOI:10.5455/javar.2021 .h517.

Wei Q, ShaoJie B, LinPeng X, Ren, ZhengRan R, Renjie D. 2018. Effect of feed concentration on long-term thermophilic methane fermentation of chicken manure. China Environ Sci. 38:2593–2601.

Widodo, F W, Sutrisno. 2012. Kecernaan Bahan Kering, Kecernaan Bahan Organik, Produksi Vfa Dan Nh3 Pakan Komplit Dengan Level Jerami Padi Berbeda Secara in Vitro. Anim Agric J. 1(1):215–230.

Wijaya AS, Dhalika T, Nurachma S. 2018. Pengaruh pemberian silase campuran Indigofera sp. dan rumput gajah pada berbagai rasio terhadap kecernaan serat kasar dan BETN pada domba garut jantan. J Ilmu Ternak Univ Padjadjaran. 18:51. DOI:10.24198 /jit.v18i1.16499.

Wijayanti E, Wahyono F, Surono. 2012. Kecernaan nutrien dan fermentabilitas pakan komplit dengan level ampas tebu yang berbeda secara *in vitro*. Anim Agric J. 1:167–179.

Yanti G, Jamarun N, Elihasridas, Astuti T. 2021. Quality improvement of sugarcane top as animal feed with biodelignification by phanerochaete chrysosporium fungi on *in-vitro* digestibility of NDF, ADF, Cellulose and hemicellulose. J Phys Conf Ser. 1940. DOI: 10. 1088/1742-6596/1940/1/012063.

Zhao J, Dong Z, Li J, Chen L, Bai Y, Jia Y, Shao T. 2019. Effects of sugar sources and doses on fermentation dynamics, carbohydrates changes, *in vitro* digestibility and gas production of rice straw silage. Ital J Anim Sci. 18:1345–1355. DOI:10.1080/1828051X.2019.1659106.

Protective Effects of Gallic Acid and Curcumin on Serum Levels of Hepatic Transaminases, Blood Plasma Parameters and Pituitary-testicular Hormones in Rats Treated Nickel Nanoparticles

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ABSTRAK

Khezri Motlagh R, Vahdati A, Hosseini SE, Edalatmanesh MA. 2021. Efek protektif asam galat (GA) dan Kurkumin (Cur) pada transaminase hati, parameter plasma darah dan kadar hormon hipofisis-testis pada tikus yang diberi NiNPs. JITV 27(1):45-56. DOI:http://dx.doi.org/10.14334/jitv.v27i1.2971

Nanopartikel nikel (NiNPs) memiliki efek toksik pada sel-sel tubuh karena memproduksi radikal bebas. Tujuan dari penelitian ini adalah untuk mengetahui efek protektif asam galat (GA) dan Kurkumin (Cur) pada transaminase hati, parameter plasma darah dan kadar hormon hipofisis-testis pada tikus yang diberi NiNPs. Tujuh puluh ekor tikus wistar jantan dewasa dibagi dalam 7 kelompok yang terdiri dari 10 ekor yaitu kontrol, Ni50 mg/kg, Ni50+GA150 mg/kg, Ni50+GA300 mg/kg, Ni50+Cur150 mg/kg, Ni50+Cur300 mg/kg dan Ni50+ GA300+CUR300 mg/kg. NiNPs, GA dan Cur diberikan secara oral dengan *gavage* oral selama 28 hari. Pada penelitian tahap terakhir, sampel darah diambil langsung dari jantung dan kadar serum transaminase hati (alanine aminotransferase (ALT) dan aspartate aminotransferase (AST)), parameter plasma darah (Glukosa, protein total (TP), bilirubin (Bil), albumin (Alb), kreatinin (Cr), Blood urea nitrogen (BUN), trigliserida, kolesterol, HDL, LDL dan alkaline phosphatase (ALP)) dan hormon pituitari-testis (FSH, LH, testosteron dan dihidrotestosteron) dinilai. Pemberian NiNPs meningkatkan kadar glukosa serum, ALT, ALP, AST, Bil, BUN, Cr, trigliserida, kolesterol dan LDL dibandingkan dengan kelompok kontrol ($p<0,05$) dan sebaliknya menurunkan kadar serum FSH, LH, testosteron, dihidrotestosteron, Alb, TP dan HDL ($p<0,05$). Namun, pemberian bersama GA dan Cur pada dosis 300 ml/kg pada tikus yang diobati dengan NiNPs meningkatkan semua parameter plasma darah dibandingkan dengan kelompok kontrol ($p>0,05$). Temuan penelitian ini menunjukkan bahwa pemberian bersama GA dan Cur pada dosis 300 mg/kg dapat mengurangi dan meningkatkan efek merusak NiNPs pada parameter plasma darah, transaminase hati dan hormon pituitari-testis pada tikus dewasa.

Kata Kunci: Nickel Nanoparticles, Curcumin, Gallic Acid, Testosterone, Hepatic Transaminases

ABSTRACT

Khezri Motlagh R, Vahdati A, Hosseini SE, Edalatmanesh MA. 2021. Protective Effects of Gallic acid and Curcumin on Serum Levels of Hepatic Transaminases, Blood Plasma Parameters and Pituitary-testicular Hormones in Rats Treated with Nickel Nanoparticles. JITV 27(1):45-56. DOI:http://dx.doi.org/10.14334/jitv.v27i1.2971

Nickel nanoparticles (NiNPs) have toxic effects on body cells due to the production of free radicals. The purpose of this research was to investigate the protective effects of Gallic acid (GA) and Curcumin (Cur) on hepatic transaminases, blood plasma parameters and pituitary-testicular hormones levels in NiNPs-treated rats. Seventy adult male Wistar rats were divided in 7 groups of 10 including control, Ni50 mg/kg, Ni50+GA150 mg/kg, Ni50+GA300 mg/kg, Ni50+Cur150 mg/kg, Ni50+Cur300 mg/kg and Ni50+GA300+CUR300 mg/kg. NiNPs, GA and Cur were administered orally by oral gavage for 28 days. At the last phase of the study, the samples of blood were taken directly from heart and serum levels of hepatic transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), blood plasma parameters (Glucose, total protein (TP), bilirubin (Bil), albumin (Alb), creatinine (Cr), Blood urea nitrogen (BUN), triglyceride, cholesterol, HDL, LDL and alkaline phosphatase (ALP)) and pituitary-testicular hormones (FSH, LH, testosterone and dihydrotestosterone) were assessed. NiNPs administration increased serum levels of glucose, ALT, ALP, AST, Bil, BUN, Cr, triglyceride, cholesterol and LDL compared to the control group ($p<0.05$) and in contrast, it decreased serum levels of FSH, LH, testosterone, dihydrotestosterone, Alb, TP and HDL ($p<0.05$). However, co-administration of GA and Cur at doses of 300 ml/kg in NiNPs -treated rats improved all blood plasma parameters compared to the control group ($p>0.05$). The findings of this study suggest that co-administration of GA and Cur at a dose of 300 mg/kg can reduce and improve the damaging effects of NiNPs on blood plasma parameters, hepatic transaminases and pituitary-testicular hormones in adult rats.

Key Words: Curcumin, Gallic Acid, Hepatic Transaminases, Nickel Nanoparticles, Testosterone

INTRODUCTION

The application of nanoparticles has increased significantly in recent years in domestic and industrial processes. These particles show special physical and chemical behavior due to their high surface-to-volume ratio and small size, therefore, they can penetrate small molecules (such as water, oxygen, and carbon dioxide) and cause rupture in their structure. Metal nanoparticles have wide applications in medicine and industry as catalysts, pigments and sensors (Abudayyak et al. 2020). Nickel nanoparticles (NiNPs) are among those with the highest frequency of use in metal nanoparticles group, therefore, the probability of exposure to NiNPs has greatly increased.

Some studies suggest that NiNPs can induce apoptosis, oxidative stress, and DNA damage (Hu et al. 2020). The accumulation of metal ions in cells increases the production of reactive oxygen species (ROS). If ROS is not neutralized by the body's antioxidant system, it can lead to oxidative stress. Oxidative stress caused by excessive ROS can lead to lipid peroxidation, DNA and protein degradation. Exposure to compounds and nickel ions in different environments can occur through skin contact, gastrointestinal tract, and inhalation of airborne particles (Marzban et al. 2020). Studies have indicated that exposure to nickel or its compounds has the potential to cause a variety of histopathological effects such as skin inflammation and also swelling, redness, eczema and itching on the skin and may also include allergic and teratogenic reactions. The International Agency for Research on Cancer has classified nickel compounds as carcinogens (Kong et al. 2014). In addition, NiNPs can induce liver, pulmonary, renal and reproductive toxicity (Abudayyak et al. 2020; Kong et al. 2014). Nickel ions can induce a wide range of adverse effects on reproduction and growth, such as influencing infertility or fertility in males and females, abortion, and congenital anomalies and defects. Hormonal disorders may play an important role in the reproductive toxicology of NiNPs at neuroendocrine and gonadal levels along the hypothalamic-pituitary-gonadal axis (Forgacs et al. 2012; Kong et al. 2014).

Today, researchers have focused on dietary supplements and natural extracts of various plants to control the toxicity of drugs and chemicals. Studies suggest that phenolic compounds have the ability to neutralize free radicals due to their antioxidant activity. The antioxidant activity of phenolic agents is mainly due to their redox properties, which allows them to act as hydrogen donors and regenerative intermediaries (Moradi et al. 2021). Gallic acid (GA), 3,4,5-trihydroxybenzoic acid, is a polyphenolic compound found in fruits, vegetables and herbal medicines. Biological and pharmacological activities of GA include antioxidant, antimicrobial, anti-cancer, anti-inflammatory and inhibitory activities of metabolic disorders (Yang et al. 2020). Animal studies suggest that oral administration of GA in diabetic rats may be effective in reducing hyperglycemia due to its antioxidant properties. Gallic acid can also significantly reduce oxidative stress by strengthening the body's natural antioxidant system against ROS. Although GA is believed to have low side effects and no serious side effects have been reported, doses greater than 1 mg/kg may have teratogenic effects to the fetus (Hsieh et al. 2015). Therefore, choosing the appropriate dose of GA can be a challenge in studies (Rahimifard et al. 2020; Variya et al. 2019). Gallic acid destroys free radicals by giving them hydrogen. In diclofenac-treated rats, the administration of GA has been shown to reduce diclofenac-induced renal toxicity by modulating oxidative stress and inhibiting inflammation (Moradi et al. 2021). Also, the administration of GA can have protective effects on liver and testicular tissue (Kahkeshani et al. 2019).

Curcumin (Cur, C21H20O6) is a polyphenolic and lipophilic compound. Curcumin as the main active curcuminoid pigment is obtained from turmeric rhizomes and does not dissolve in acid or neutral water but is soluble in acetone, methanol and ethanol. Curcumin has anti-inflammatory, antioxidant and anti-mutagenic activities due to its unique chemical structure as well as the presence of hydroxyl and methoxy groups in its structure (Jakubczyk et al. 2020). In addition, Curcumin can inhibit the proliferation of inflammatory cells due to its interaction with various molecular targets and acts as a chemical inhibitor. These properties are associated with the regulation of proinflammatory cytokines, Nitric oxide synthase enzymes (iNOS), Cyclooxygenase-2 (COX-2), Lipoxygenase, and malondialdehyde reduction. It has been suggested that Curcumin can exert its anti-inflammatory, anti-tumor, and antioxidant effects by modulating various cellular signaling pathways. The anti-inflammatory mechanism of Cur is associated with nuclear factor kappa β (NF-κB) inhibition, which leads to inhibiting the expression of proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor α (TNF-α) (Coelho et al. 2020; Jakubczyk et al. 2020). Also, research shows that Cur as an effective antioxidant may reduce the effects of oxidative stress due to its ability to interact with various molecular mechanisms. This property of Cur is related to its ability to chelate heavy metals or regulate the activity of many enzymes (Alizadeh & Kheirouri 2019). The US Food and Drug Administration (FDA) has approved Cur as a safe compound based on animal and human studies. Specific mutagenicity and genotoxicity following Cur use has not been reported, even at high doses. However, due to its anti-proliferative activity, Cur may reduce cell life in

normal cells (Soleimani et al. 2018; Alizadeh & Kheirouri 2019). The positive effects of Cur on lowering serum levels of glucose, triglycerides, cholesterol and LDL and increasing serum HDL levels have been represented in some studies (Marton et al. 2021). It has been suggested that Cur may increase sperm chromatin quality and prevent the disruption of pituitary-testicular hormones (Shahedi et al. 2021; Onwuemene et al. 2019).

Due to this fact that few studies have been performed on the effect of NiNPs on hepatotoxicity and pituitary-testicular hormones and also, information on the protective effects of applying GA and Cur alone and in combination following the administration of NiNPs in male rats is insufficient, therefore, this study was designed to evaluate the administration of GA and Cur alone and in combination on changes in hepatic transaminases including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), some blood plasma parameters including glucose, total protein (TP), bilirubin (Bil), albumin (Alb), creatinine (Cr), blood urea nitrogen (BUN), triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and alkaline phosphatase (ALP) as well as pituitary-testicular axis hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and dihydrotestosterone in adult rats treated with NiNPs to provide grounds for growth and resistance against these nanoparticles in the body.

MATERIALS AND METHODS

Animals

Seventy adult, male Wistar rats (weighing 230±20 g and 8 weeks old) were purchased from the animal house of Shiraz University of Medical Sciences and kept in the same place for 2 weeks before starting the study to adapt to the environment. During this study, the animals were kept in suitable conditions with a controlled temperature of 23±2 °C, 12 hours of light/darkness and 50-60% moisture. The animals were placed in special transparent macrolone cages with dimensions of 20×30×55 cm^3 with a retractable and movable steel roof. Five rats were kept in each cage. The animal food was in the form of compact food or pellets purchased from Daniran Livestock and Poultry Company in Shiraz. Access to adequate water and food was free during the study. Therefore, the storage conditions were considered same for all animals. The ethical and experimental protocol of this study of working with laboratory animals was approved by the Medical Ethics Review Committee of Shiraz University of Medical Sciences (Ethical code number: A1732-20) and it was observed until the last phase of the study.

The study protocol

In this experimental study, 70 adult, male Wistar rats were grouped in 7 groups of 10 completely randomly. The animals in the control group (group 1) did not receive any treatment during the study period. In the Ni50 group (group 2), animals received 50 mg/kg NiNPs (Pishgam Iranian Group, Iran) by oral gavage for 28 days. Animals in the Ni50+GA150 group (group 3) received 50 mg/kg NiNPs and 150 mg/kg GA (Merck, Germany) by oral gavage for 28 days. In the Ni50+GA300 group (group 4), the animals received 50 mg/kg NiNPs and 300 mg/kg GA by oral gavage for 28 days. Animals in the Ni50+Cur150 group (group 5) received 50 mg/kg NiNPs and 150 mg/kg Cur (Merck, Germany) by oral gavage for 28 days. In the Ni50+Cur300 group (group 6), animals received 50 mg/kg NiNPs and 300 mg/kg Cur by oral gavage for 28 days. Animals in the Ni50+GA300+Cur300 group (group 7) received 50 mg/kg of NiNPs, 300 mg/kg of GA, and 300 mg/kg of Cur by oral gavage for 28 days, respectively. Doses of NiNPs, GA and Cur were administered daily at 8 am, 12 pm and 4 pm, respectively, in all study groups. The selection of NiNPs, GA and Cur doses was determined and prescribed based on the previous studies (Marzban et al. 2020; Rong et al. 2018; Wang et al. 2015). At the last phase of the study, the samples of blood were taken from all animals and serum levels of hepatic transaminases (ALT and AST), blood plasma parameters (Glucose, TP, Bil, Alb, Cr, BUN, triglyceride, cholesterol, HDL, LDL and ALP) and pituitary-testicular axis hormones (FSH, LH, testosterone and dihydrotestosterone) were measured.

Blood parameters analysis

At the last phase of the study, all the animals were anesthetized with ether (Merck, Germany) after 11 hours of fasting, in one day (Wang et al. 2010). All animals were placed in anesthesia Jar with ether-impregnated cotton and were anesthetized. The reason for using ether was that ether anesthesia is mild and has little effect on blood flow velocity. On the other hand, it is less dangerous to breathe than chloroform. After anesthetizing the animals, blood samples were drawn from the left ventricle of the animals without opening the chest using 5 ml syringes. The samples of blood were placed in an incubator at 37 ° C to complete the agglutination process. Blood samples were then centrifuged at 3500 rpm (MSE, England) for 10 minutes to separate the serum. The obtained serum was stored in the freezer at -20 °C until the assessment of serum levels of hepatic transaminases, blood plasma parameters and pituitary-testicular axis hormones.

The glucose oxidase method was used to measure the plasma glucose levels (El-Borady et al. 2020) and

according to the instructions of the glucose measuring kit (Pars Azmoun, Iran). Serum level of total Bil was measured by photometric method using Diazo 2 and 4 Dichloroaniline (DCA) according to the manufacturer instructions (Pars Azmoun, Iran). Serum Cr level was measured using JAFFE method according to the instructions of Cr assay kit (Pezhuhan Teb, Iran). Serum BUN level was measured by DCA method according to the manufacturer's instructions (Pars Azmoun, Iran). Serum levels of triglyceride (Pars Azmon, Iran), cholesterol (Man Company, Iran), HDL (biochemistry, Iran) and LDL (Pars Azmon) were measured using the enzymatic method and according to the manufacturer's kits. Serum levels of FSH (BT Lab, China), LH (BT Lab, China), testosterone (IBL, Germany) and dihydrotestosterone (IBL, Germany) were measured using ELISA kits according to the manufacturer's instructions.

Serum levels of AST, ALT, ALP, Alb and TP were measured using the RA-1000 auto-analyzer (Technicon, USA) and the manufacturer's instructions were followed (Pars Azmoun Company, Iran). Serum ALT and AST levels were measured by IFCC (International Federation of Clinical Chemistry) method without adding Pyridoxal-50phosphate. Serum ALP level was measured using the PGKC method (Deutsche Gesellschaft Fur Klinische Chemie). Also, based on Biuret method, serum TP level was measured using Photometric method and serum Alb level was measured by BCG method (Bromocresol-Green) (Farashbandi et al. 2021).

Statistical analysis

The data resulting from the measurement of serum levels of liver transaminases, plasma blood parameters and pituitary-testicular hormones were analyzed by SPSS software version 20 (SPSS Inc, Chicago, IL, USA) and were analyzed using one-way ANOVA method followed by Tukey's *post-hoc* test to compare the mean data. The obtained values were reported as Mean±SEM (standard error of the mean) and a significant level of P <0.05 was considered. The Figures were drawn by GhraPhpad software version 5 (FigurePad Prism, Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

The results of this study indicated that glucose levels (Figure 1) in Ni50 group have increased significantly compared to the control group ($p<0.05$). NiNPs administration has been shown to be associated with high fasting blood sugar and insulin levels. The evidence suggests that exposure to nickel has a greater effect on glucose level and hyperglycemia induction than other divalent metals by increasing glycogenolysis and altering hepatic gluconeogenesis. Treating rats with nickel increases liver lipid peroxides and decreases the activity of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPx) as well as hepatic glutathione (GSH) concentrations.

Nickel has been reported to reduce glucose regulation by increasing ROS, thereby impairing insulin function (Liu et al. 2015). In Ni50+GA150, Ni50+GA300 and Ni50+Cur150 groups, glucose levels showed a significant increase compared to the control group ($p<0.05$), in contrast, compared to Ni50 group, glucose level decreased significantly ($p<0.05$). Also, in Ni50+Cur300 and Ni50+GA300+Cur300 groups, a significant decrease in glucose levels was observed compared to Ni50 group ($p<0.05$), while in comparison with the control group, no significant difference was observed ($p>0.05$). Natural products and their derived compounds have a long history of clinical use as well as better patient tolerance and acceptance. Curcumin and GA have been shown to inhibit oxidative stress and inflammation (Dludla et al. 2018; Hashemzaei et al. 2020). In the present study, co-administration of GA and Cur at a dose of 300 mg/kg improved glucose levels compared with the control group. Synergistic effects between different compounds may lead to increased pharmacological effects (Pujimulyani et al. 2020).

Co-administration of GA and Cur in rats treated with NiNPs seems to enhance each other's effects compared to administrating them separately. The positive effects of Cur have been reported to increase the sensitivity of cells to insulin. Curcumin leads to increased insulin secretion due to prevention of apoptosis and oxidative stress along with increased activity of antioxidant enzymes. On the other hand, studies show that Cur is associated with increased glucose uptake and utilization by skeletal muscle cells and adipocytes, and the inhibition of gluconeogenesis (Panda et al. 2021). Gallic acid has been shown to improve insulin sensitivity since it regulates the expression of hepatic insulin signaling proteins such as insulin receptor, insulin receptor substrate 1 and phosphatidylinositol-3 kinase as demonstrated in mice fed with a high-fat diet. Gallic acid also reduces the expression of proteins associated with hepatic gluconeogenesis such as fructose 1,6-bisphosphatase and regulates the expression of hepatic glycogen synthase (Variya et al. 2020; Huang et al. 2016). The results of these studies are consistent with the results in improving glucose levels.

In this study, triglyceride level (Figure 2A) in Ni50 group was significantly higher than that of the control group ($p<0.05$). The levels in Ni50+GA150, Ni50+GA300 and Ni50+Cur150 groups were also higher than that in the control group ($p<0.05$), but lower

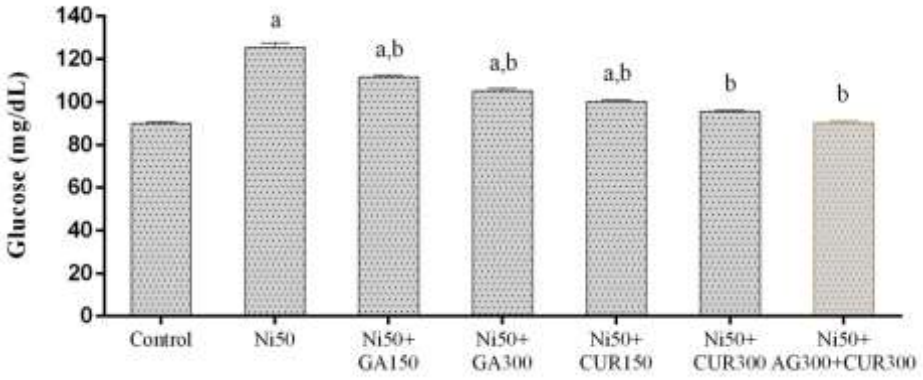

Figure 1. Comparison of mean and SEM of the serum glucose levels in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

than that in the Ni50 group ($p<0.05$). Also, in Ni50+Cur300 and Ni50+GA300+Cur300 groups, a significant lower level was observed as compared to Ni50 group ($p<0.05$), but not to the control group ($p>0.05$). The effects of the treatment on either cholesterol or LDL were similar to those on triglyceride, except that in Ni50+Cur300 group, the levels were significantly higher than that in control and Ni50+GA300+Cur300 groups ($p<0.05$) (Figure 2B and 2C). HDL level (Figure 2D) in the Ni50 and Ni50+GA150 groups showed a significant decrease compared to the control and Ni50+GA300+Cur300 groups ($p<0.05$). In Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 groups, HDL level showed a significant decrease compared to the control and Ni50+GA300+Cur300 groups ($p<0.05$). In contrast, HDL level showed a significant decrease compared to the Ni50 group ($p<0.05$).

Previous studies show that the administration of NiNPs in rats for 28 days increases triglyceride and LDL and decreases HDL compared to the control group, which is consistent with the results of this study (Ali et al. 2021). Increased triglyceride levels appear to be associated with increased levels of LDL, which is responsible for transporting cholesterol to the blood, while HLD, which is responsible for transporting cholesterol to the liver, is significantly reduced (Abdel-Ghafarf et al. 2018). It has been shown that, regular consumption of Cur for 12 weeks in patients with metabolic syndrome lowered triglyceride, LDL and total cholesterol levels, but not HDL levels (Yang et al. 2014). Also, Cur reduces serum lipid levels in rats by affecting fatty acid metabolism (Xia et al. 2020). Curcumin can inhibit expression of LDL receptor gene by activating Peroxisome proliferator- activated receptor gamma (PPAR-γ). In addition, it appears that Cur can affect the synthesis and catabolism of triglyceride-rich lipoproteins and cholesterol metabolism pathways. Thus, Cur appears to play an important role in reducing plasma triglyceride and cholesterol concentrations by reducing the expression of lipogenic genes (Jalali et al. 2020). It has been reported that the administration of Gallic acid at a dose of 100 mg/kg alone for 4 weeks in rats has no effect on triglyceride, HDL and cholesterol levels, however it does reduce LDL levels. Also, the co-administration of GA and Cur in this study had no effect on triglyceride, HDL and cholesterol levels but reduced LDL levels (Abarikwu et al. 2016). The lack of significant reductions in triglyceride, HDL and cholesterol levels contradicts the results of our study. Using various experimental models, it has been shown that the administration of GA for 4 weeks improves lipid profile, antioxidant status and insulin resistance in rats with a high-fat diet (Dludla et al. 2018).

The NiNps caused severe liver damage as shown by the profound increase of ALT, AST, ALP and Bil, and decrease of Alb and TP ($p<0.05$) (Figure 3A, 3B, 3C, 3D, 3E and 3F, respectively). The protective effect of Cur and GA was obvious as all the hepatoxic markers were significantly lower of higher in Ni50+GA150, Ni50+GA300 and Ni50+Cur150 groups than in the Ni50 group. Evaluating the hepatotoxicity of NiNPs in rats with different doses of nanoparticles increased liver functional enzymes (ALT, AST and ALP) and decreased SOD, GPx and Cat (Abdulqadir & Aziz 2019). In rats, NiNPs caused significant increases of Malondialdehyde levels in liver and kidney tissues and decrease of Glutathion level and SOD activity, indicating oxidative stress conditions. These changes were attributed to the overproduction of ROS caused by NiNPs, which leads to altered antioxidant activity at the level of gene and protein expression. The decreases in TP and Alb levels as observed in this study could be associated with the inhibition of protein expression. In addition, overproduction of ROS can damage cell membranes which in turn resulted in the increase of hepatic transaminases (Ali et al. 2021).

Curcumin is believed to be unique because it has both β-diketone and phenolic hydroxyl groups in one

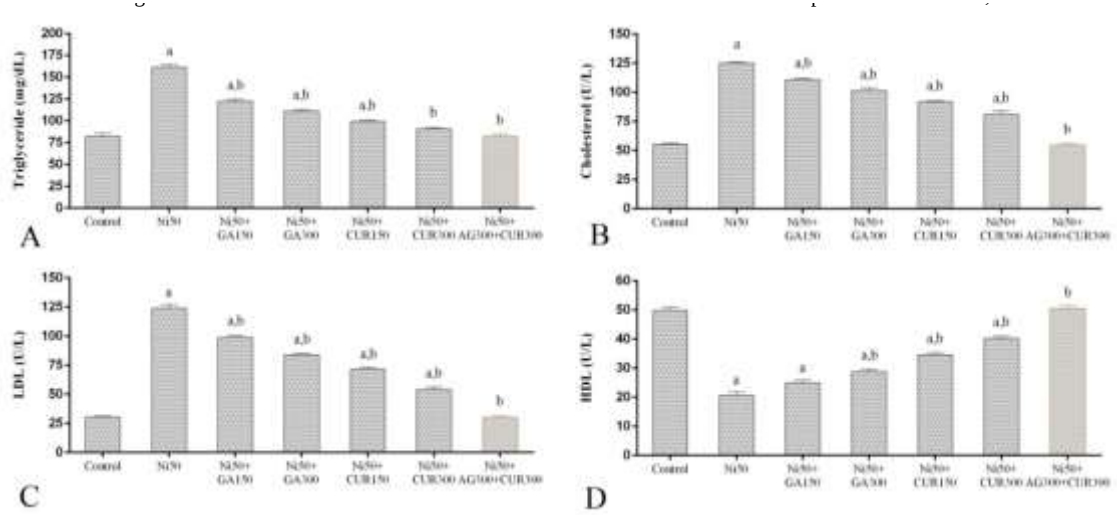

Figure 2. Comparison of mean and SEM of the serum triglyceride (A), cholesterol (B), LDL (C) and HDL (D) levels in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

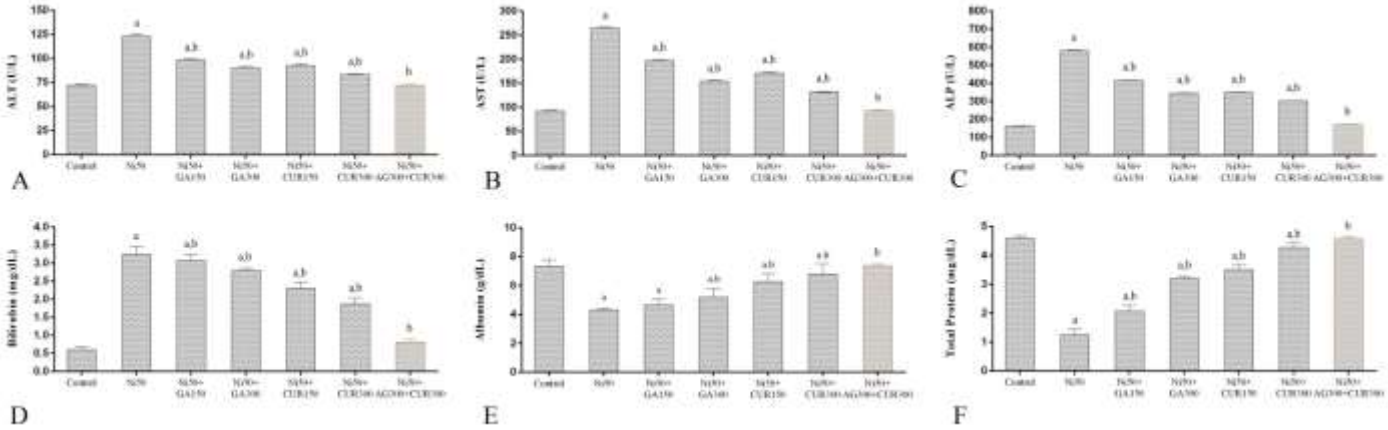

Figure 3. Comparison of mean and SEM of the serum levels of ALT (A), AST (B), ALP (C) and Bil (D), Alb (E) and TP (F) in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

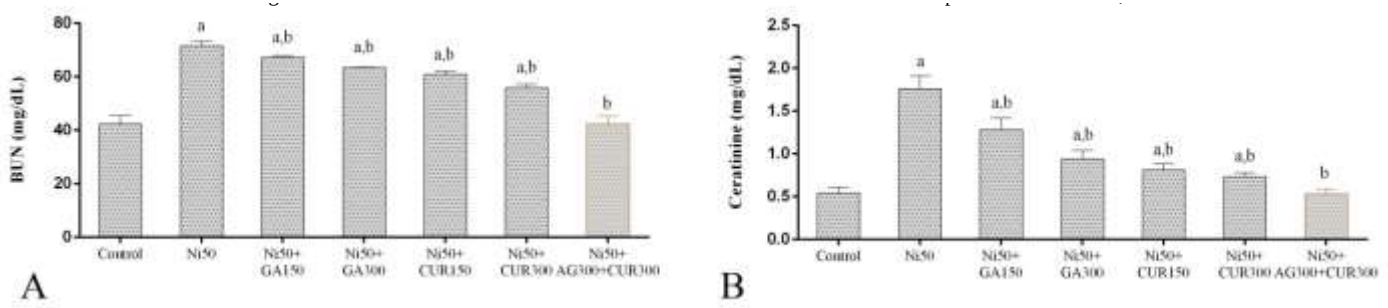

Figure 4. Comparison of mean and SEM of the serum levels of BUN (A) and Cr (B) in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

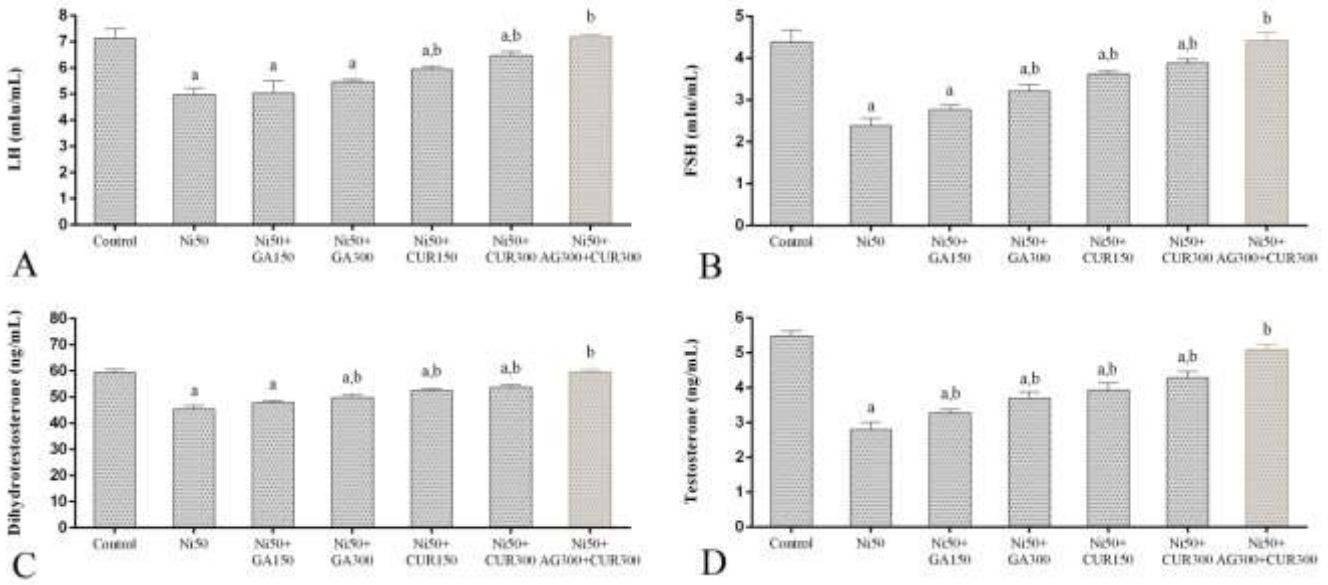

Figure 5. Comparison of mean and SEM of the serum levels of LH (A), FSH (B), testosterone (C) and dihydrotestosterone (D) in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

molecule. This antioxidant molecule by trapping and stabilizing free radicals, especially lipid peroxyl radicals, can prevent the spread of oxidation and thus prevent tissue damage (Sökmen & Khan 2016). Studies showed that Cur has antioxidant and anti-inflammatory properties by suppressing NF-κβ and reducing oxidative stress and inflammation (Pulido-Moran et al. 2016). In addition, it can enhance the activity of antioxidant enzymes such as SOD and GPx by scavenging free radicals. This result suggests that Cur can be effective in protecting the liver by reducing oxidative stress (Jalali et al. 2020). Gallic acid has been reported to be effective in improving hepatic parameters induced by transient ischemia. Serum levels of ALT, AST, ALP and Bil showed a significant decrease with the administration of 50 and 100 mg/kg GA compared to the transient ischemia group (Akbari et al. 2019).

Previous studies on the effects of GA on liver have also shown that it protects liver tissue from damages caused by transient ischemia, Paracetamol and CCl4 by inhibiting ROS and exerting antioxidant activity (Wang et al. 2014; Bayramoglu et al. 2015) The research shows that GA can be an inhibitor of lipid peroxidation. The levels of malondialdehyde and lactate dehydrogenase have significantly increased in cells exposed to NiSO4, while treatment of these cells with Gallic acid has reduced the levels of malondialdehyde and lactate dehydrogenase. The studies have confirmed that GA, produces a free radical system consisting of hydroxyl radical and xanthine oxidase through the Fenton reaction, which leads to the removal of the free radical anion superoxide and it also has an inhibitory effect on the oxidation of cytochrome P450 3A (CYP3A) microsomal human liver to reduce tissue accumulation of ROS. In inducing apoptosis, GA, predominantly has a prooxidative effect and significantly protects cells against NiSO4-induced oxidative stress (An et al. 2016).

Blood urea nitrogen and creatinine are important kidney indicators. Their changes indicate the extent of kidney damage. In the present study, the administration of NINPs increased serum levels of BUN and Cr (Figure 4A and 4B, respectively) in Ni50 group compared to control group ($p<0.05$). Serum levels of BUN and Cr showed a significant increase in Ni50+GA150, Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 ($p <0.05$), in contrast, they showed a significant decrease compared to the Ni50 group ($p<0.05$). Also, in Ni50+GA300+Cur300 group, a significant increase in BUN and Cr levels was observed compared to Ni50 group ($p<0.05$), while in comparison with the control group, no significant difference was observed ($p>0.05$).

Nickel nanoparticles cause the accumulation of inflammatory cells and tissue damage in the kidney, and the inflammatory response in the kidney indicates damage to the filtration capacity of the kidney, and an increase in Cr level is the best confirmation for the kidney damage (Hendi 2011). Creatinine and urea are byproducts of cellular metabolism that are mainly excreted by the kidneys. Renal impairment increases plasma Cr and urea. Therefore, the increase in both products in the Nickel-treated group may be due to the nephrotoxic effects of Nickel on renal cells. Nickel is a nephrotoxin (Yin et al. 2021) and NiNPs increase serum Cr and BUN levels significantly, indicating impaired renal function (Ali et al. 2021). Other studies have reported significant changes in serum BUN and Cr levels after exposure to NiNPs (Ali 2019). This increase may be due to the accumulation of Nickel in the kidney tissue, which may impair the filtration of urea and Cr and increase them in the blood (Dumala et al. 2018).

The administration of Cur in ischemia-induced dysfunction and oxidative stress induced in kidney tissue of rats significantly reduced Cr and BUN. It has been shown that the use of Cur leads to a relative improvement in renal function as well as a reduction in oxidative stress and leukocyte infiltration due to ischemia. Some researchers have shown that Cur can directly inhibit chemokines. In addition, it has been suggested that Cur induces the expression of co-oxygenase-1 in renal epithelial cells, which is also a protective mechanism against oxidative stress. Curcumin has also been shown to inhibit TNF-α by stimulating co-oxygenase-1, thereby preventing leukocyte infiltration (Najafi et al. 2015). The results show that pretreatment with GA as an antioxidant reduces serum malondialdehyde levels and increases GSH levels and GPX activity in the kidney. In addition, GA pretreatment reduces urea and Cr levels (Ahmadvand et al. 2019). ROS or reactive nitrogen species (RNS) production is the major cause of kidney damage due to treatment with NiNPs. ROS is involved in the pathophysiology of NiNPs by inducing apoptosis, increasing lipid peroxidation, and activating cellular stress signaling pathways (Genchi et al. 2020). Antioxidants such as SOD, CAT, GPX and GSH are responsible for protecting cells against free radicals and oxidative stress. Gallic acid as a polyhydroxyphenolic compound is able to eliminate ROS. Oxidative stress caused by NiNPs leads to damage to macromolecules due to lipid peroxidation, DNA oxidation, protein oxidation, enzyme inactivation and dysfunction of various membranes. Glycolic acid reduces these destructive events by increasing the enzymatic activity of antioxidants such as GSH and GPX (Ahmadvand et al. 2019).

Serum levels of pituitary-testicular hormones play an important role in the process of spermatogenesis and fertility. Any change in the levels of these hormones can cause fertility problems. In this study, LH hormone levels (Figure 5A) in the groups of Ni50, Ni50+GA150 and Ni50+GA300 showed a significant decrease

compared to the control group ($p<0.05$). In Ni50+Cur150 and Ni50+Cur300 groups, serum LH levels showed a significant decrease compared to the control group ($p<0.05$), in contrast, compared with the Ni50 group showed a significant increase ($p<0.05$). Also, in Ni50+GA300+Cur300 group, an increase in serum LH level was observed in comparison with Ni50 group ($p<0.05$) while in comparison with the control group, no significant difference was observed ($p>0.05$). Serum levels of FSH and dihydrotestosterone (Figure 5B and 5C, respectively) in Ni50 and Ni50+GA150 groups showed a significant decrease compared to the control group ($p<0.05$). In Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 groups, serum levels of FSH and dihydrotestosterone showed a significant decrease ($p <0.05$) compared to the control group, but in contrast, showed a significant increase compared to the Ni50 group ($p<0.05$). Also, in Ni50+GA300+Cur300 group, an increase in serum levels of FSH and dihydrotestosterone was observed in comparison with the Ni50 group ($p<0.05$) while in the control group, no significant difference was observed ($p>0.05$). Serum testosterone level (Figure 5D) in Ni50 group showed a significant decrease compared to the control group ($p<0.05$). Serum testosterone levels in Ni50+GA150, Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 showed a significant decrease compared to the control group ($p<0.05$), in contrast, they showed a significant increase compared to the Ni50 group ($p<0.05$). Also, in Ni50+GA300+Cur300 group, an increase in serum testosterone levels was observed compared to the Ni50 group ($p<0.05$), while no significant difference was observed compared to the control group ($p>0.05$). Decreased circulating LH, FSH, testosterone and dihydrotestosterone after treatment with NiNPs may be related to two mechanisms. Firstly, nanoparticles are able to affect the pituitary-testicular axis by altering sex hormone secretion and cellular activity (Forgacs et al. 2012). Secondly, NINPs may alters gene expression in the testis. In a study on the effect of NiNPs density on Zebrafish reproduction, it was found that the higher the density, the more severe the damage the caused on fertility (Ispas et al. 2009). These findings were in agreement with the present study. Nickel nanoparticles have been shown to produce oxidative stress that induces plasma membrane peroxidation, DNA fragmentation, mitochondrial membrane change and sperm morphology (Gallo et al. 2016).

The positive effects of Cur on improving the levels of pituitary-testicular hormones appear to be due to its protective effects on Leydig cells. Curcumin probably exerts its protective effects due to its anti-apoptotic, antioxidant and anti-genotoxic properties. In addition, Cur inhibits cortisol secretion by suppressing adrenocorticotropic hormone and increasing mRNAs encoding steroid-controlling proteins (Mohamadpour et al. 2017). Oxidative stress is known to be a potent mediator of apoptosis. In this process, mitochondria are recognized as an important factor. Mitochondrial dysfunction due to oxidative stress can lead to the release of cytochrome *c* and activation of caspase, followed by cell death (Ilbey et al. 2009). The studies have shown that in the male mammalian reproductive system, LH stimulates Leydig cells to produce testosterone, which is important for initiating and maintaining spermatogenesis through the Sertoli cellular androgen receptor. In addition, FSH plays a crucial role in normal spermatogenesis in pubertal rats (Oyagbemi et al. 2016). The LH, FSH and testosterone are known as hormonal biomarkers of androgenic hormone (Adedara & Farombi 2013). In this study, the increase in FSH and LH concentrations was associated with a significant increase in testosterone and dihydrotestosterone levels in the GA-treated groups alone and in combination with Cur, indicating the androgenic potential of GA and Cur. These observations may indicate that GA has an effective stimulatory effect on the pituitary-testicular axis of adult male rats.

CONCLUSION

Treatment of rats with NiNPs at a dose of 50 mg/kg for 28 days caused liver damage as indicated by the changes in serum concentration of ALT, AST, glucose, total protein, bilirubin, albumin, Cr, BUN, triglyceride, HDL, LDL and ALP, and pituitary-testicular axis hormones (FSH, LH, testosterone and dihydrotestosterone). Curcumin at a dose of 150 or 300, or GA at a dose of 300 mg/kg, co-administered with the NiNPs, was effectively inhibited the toxicity of the nickle. The protective effect of Cur and GA is even greater when the two substances are administered simultaneously.

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REFERENCES

Abarikwu SO, Durojaiye M, Alabi A, Asonye B, Akiri O. 2016. Curcumin protects against gallic acid-induced oxidative stress, suppression of glutathione antioxidant defenses, hepatic and renal damage in rats. Ren Fail. 38:321–329. DOI: 10.3109/0886022X.2015.1127743.

Abdel-Ghafarf O, Ali A, Soliman S. 2018. Protective effect of naringenin against isoniazid-induced adverse reactions

in rats. Int J Pharmacol. 14:667–680. DOI: 10.3923/ijp.2018.667.680.

Abdulqadir SZ, Aziz FM. 2019. Hepatotoxicity of nickel nanoparticles in rats. Indian J Anim Res.(of). DOI: 10.18805/ijar.B-1100.

Abudayyak M, GuzelL E, Özhan G. 2020. Cytotoxic, genotoxic, and apoptotic effects of nickel oxide nanoparticles in intestinal epithelial cells. Turkish J Pharm Sci. 17:446–451. DOI: 10.4274/tjps.galenos. 2019.76376

Adedara IA, Farombi EO. 2013. Chemoprotective effects of kolaviron on ethylene glycol monoethyl ether-induced pituitary-thyroid axis toxicity in male rats. Andrologia. 45:111–119. DOI: 10.1111/j.1439-0272.2012.01321.x.

Ahmadvand H, Yalameha B, Adibhesami G, Nasri M, Naderi N, Babaeenezhad E, Nouryazdan N. 2019. The protective role of gallic acid pretreatment on renal ischemia-reperfusion injury in rats. Reports Biochem Mol Biol. 8:42–48.

Akbari G, Savari F, Mard SA, Rezaie A, Moradi M. 2019. Gallic acid protects the liver in rats against injuries induced by transient ischemia-reperfusion through regulating microRNAs expressions. Iran J Basic Med Sci. 22:439–444. DOI: 10.22038/ijbms.2018.31589. 7605

Ali AA-M. 2019. Evaluation of some biological, biochemical, and hematological aspects in male albino rats after acute exposure to the nano-structured oxides of nickel and cobalt. Environ Sci Pollut Res. 26:17407–17417. DOI: 10.1007/s11356-019-05093-2.

Ali AA-M, Mansour AB, Attia SA. 2021. The potential protective role of apigenin against oxidative damage induced by nickel oxide nanoparticles in liver and kidney of male Wistar rat, *Rattus norvegicus*. Environ Sci Pollut Res. 28:27577–27592. DOI: 10.1007/s11356-021-12632-3.

Alizadeh M, Kheirouri S. 2019. Curcumin reduces malondialdehyde and improves antioxidants in humans with diseased conditions: a comprehensive meta-analysis of randomized controlled trials. BioMedicine. 9:23. DOI: 10.1051/bmdcn/2019090423.

An X, Zhou A, Yang Y, Wang Y, Xin R, Tian C, Wu Y. 2016. Protective effects of gallic acid against NiSO4-induced toxicity through down-regulation of the Ras/ERK signaling pathway in Beas-2B cells. Med Sci Monit. 22:3446–3454. DOI: 10.12659/MSM.900460.

Bayramoglu G, Kurt H, Bayramoglu A, Gunes HV, Degirmenci İ, Colak S. 2015. Preventive role of gallic acid on hepatic ischemia and reperfusion injury in rats. Cytotechnology. 67:845–849. DOI: 10.1007/s10616-014-9724-1.

Coelho MR, Romi MD, Ferreira DMTP, Zaltman C, Soares-Mota M. 2020. The use of curcumin as a complementary therapy in ulCrive colitis: a systematic review of randomized controlled clinical trials. Nutrients. 12:2296. DOI: 10.3390/nu12082296.

Dludla P, Nkambule B, Jack B, Mkandla Z, Mutize T, Silvestri S, Orlando P, Tiano L, Louw J, Mazibuko-Mbeje S. 2018. Inflammation and oxidative stress in an obese state and the protective effects of gallic acid. Nutrients. 11:23. DOI: 10.3390/nu11010023.

Dumala N, Mangalampalli B, Kalyan Kamal SS, Grover P. 2018. Biochemical alterations induced by nickel oxide nanoparticles in female Wistar albino rats after acute oral exposure. Biomarkers. 23:33–43. DOI: 10.1080/1354750X.2017.1360943.

El-Borady OM, Othman MS, Atallah HH, Abdel Moneim AE. 2020. Hypoglycemic potential of selenium nanoparticles capped with polyvinyl-pyrrolidone in streptozotocin-induced experimental diabetes in rats. Heliyon. 6:e04045. DOI: 10.1016/j.heliyon.2020.e04 045.

Farashbandi AL, Shariati M, Mokhtari M. 2021. Comparing the Protective Effects of Curcumin and Ursodeoxycholic Acid after Ethanol-Induced Hepatotoxicity in Rat Liver. Ethiop J Health Sci. 31:673–682. DOI: 10.4314/ejhs.v31i3.25.

Forgacs Z, Massányi P, Lukac N, Somosy Z. 2012. Reproductive toxicology of nickel – Review. J Environ Sci Heal Part A. 47:1249–1260. DOI: 10.1080/10934 529.2012.672114.

Gallo A, Boni R, Buttino I, Tosti E. 2016. Spermiotoxicity of nickel nanoparticles in the marine invertebrate Ciona intestinalis (ascidians). Nanotoxicology. 10:1096–1104. DOI: 10.1080/17435390.2016.1177743.

Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. 2020. Nickel: human health and environmental toxicology. Int J Environ Res Public Health. 17:679. DOI: 10.3390/ijerph17030679.

Hashemzaei M, Tabrizian K, Alizadeh Z, Pasandideh S, Rezaee R, Mamoulakis C, Tsatsakis A, Skaperda Z, Kouretas D, Shahraki J. 2020. Resveratrol, curcumin and gallic acid attenuate glyoxal-induced damage to rat renal cells. Toxicol Reports. 7:1571–1577. DOI: 10.1016/j.toxrep.2020.11.008.

Hendi A. 2011. Silver nanoparticles mediate differential responses in some of liver and kidney functions during skin wound healing. J King Saud Univ - Sci. 23:47–52. DOI: 10.1016/j.jksus.2010.06.006.

Hsieh CL, Lin C-H, Chen KC, Peng C-C, Peng RY. 2015. The teratogenicity and the action mechanism of gallic acid relating with brain and cervical muscles.Ho Y-S, editor. PLoS One. 10:e0119516. DOI: 10.1371/journal. pone.0119516.

Hu W, Yu Z, Gao X, Wu Y, Tang M, Kong L. 2020. Study on the damage of sperm induced by nickel nanoparticle exposure. Environ Geochem Health. 42:1715–1724. DOI: 10.1007/s10653-019-00364-w.

Huang D-W, Chang W-C, Wu JS-B, Shih R-W, Shen S-C. 2016. Gallic acid ameliorates hyperglycemia and improves hepatic carbohydrate metabolism in rats fed a high-fructose diet. Nutr Res. 36:150–160. DOI: 10.1016/j.nutres.2015.10.001.

Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A. 2009. Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. Hum Reprod. 24:1717–1725. DOI: 10.1093/humrep/dep058.

Ispas C, Andreescu D, Patel A, Goia D V, Andreescu S, Wallace KN. 2009. Toxicity and developmental defects of different sizes and shape nickel nanoparticles in Zebrafish. Environ Sci Technol. 43:6349–6356. DOI: 10.1021/es9010543.

Jakubczyk K, Drużga A, Katarzyna J, Skonieczna-Żydecka K. 2020. Antioxidant potential of curcumin—a Meta-analysis of randomized clinical trials. Antioxidants. 9:1092. DOI: 10.3390/antiox9111092.

Jalali M, Mahmoodi M, Mosallanezhad Z, Jalali R, Imanieh MH, Moosavian SP. 2020. The effects of curcumin supplementation on liver function, metabolic profile and body composition in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. Complement Ther Med. 48:102283. DOI: 10.1016/j.ctim.2019.102283.

Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, Momtaz S, Abbasabadi Z, Rahimi R, Farzaei MH, Bishayee A. 2019. Pharmacological effects of gallic acid in health and disease: A mechanistic review. Iran J Basic Med Sci. 22:225–237.

Kong L, Tang M, Zhang T, Wang D, Hu K, Lu W, Wei C, Liang G, Pu Y. 2014. Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. Int J Mol Sci. 15:21253–21269. DOI: 10.3390/ijms151121253.

Liu G, Sun L, Pan A, Zhu M, Li Z, Wang Z, Liu X, Ye X, Li H, Zheng H, et al. 2015. Nickel exposure is associated with the prevalence of type 2 diabetes in Chinese adults. Int J Epidemiol. 44:240–248. DOI: 10.1093/ije/dyu200.

Marton LT, Pescinini-e-Salzedas LM, Camargo MEC, Barbalho SM, Haber JF dos S, Sinatora RV, Detregiachi CRP, Girio RJS, Buchaim DV, Cincotto dos Santos Bueno P. 2021. The effects of curcumin on diabetes mellitus: A systematic review. Front Endocrinol (Lausanne). 12:669448. DOI: 10.3389/fendo.2021.669448/full.

Marzban A, Seyedalipour B, Mianabady M, Taravati A, Hoseini SM. 2020. Biochemical, Toxicological, and Histopathological outcome in rat brain following treatment with NiO and NiO nanoparticles. Biol Trace Elem Res. 196:528–536. DOI: 10.1007/s12011-019-01941-x

Mohamadpour M, Noorafshan A, Karbalay-Doust S, Talaei-Khozani T, Aliabadi E. 2017. Protective effects of curcumin co-treatment in rats with establishing chronic variable stress on testis and reproductive hormones. Int J Reprod Biomed. 15:447–452. DOI: 10.29252/ijrm.15.7.447.

Moradi A, Abolfathi M, Javadian M, Heidarian E, Roshanmehr H, Khaledi M, Nouri A. 2021. Gallic acid exerts nephroprotective, anti-oxidative stress, and anti-inflammatory effects against diclofenac-induced renal injury in male rats. Arch Med Res. 52:380–388. DOI: 10.1016/j.arcmed.2020.12.005.

Najafi H, Changizi Ashtiyani S, Sayedzadeh SA, Mohamadi Yarijani Z, Fakhri S. 2015. Therapeutic effects of curcumin on the functional disturbances and oxidative stress induced by renal ischemia/reperfusion in rats. Avicenna J phytomedicine. 5:576–86.

Onwuemene NJ, Imafidon CE, Ayoka AO. 2019. Curcuma longa normalized cimetidine-induced pituitary-testicular dysfunction: Relevance in nutraceutical therapy. Anim Model Exp Med. 2:191–200. DOI: 10.1002/ame2.12081.

Oyagbemi AA, Omobowale TO, Saba AB, Adedara IA, Olowu ER, Akinrinde AS, Dada RO. 2016. Gallic acid protects against cyclophosphamide-induced toxicity in testis and epididymis of rats. Andrologia. 48:393–401. DOI: 10.1111/and.12459.

Panda V, Deshmukh A, Singh S, Shah T, Hingorani L. 2021. An ayurvedic formulation of Emblica officinalis and Curcuma longa alleviates insulin resistance in diabetic rats: Involvement of curcuminoids and polyphenolics. J Ayurveda Integr Med. 12:506–513. DOI: 10.1016/j.jaim.2021.05.005.

Pujimulyani D, Yulianto WA, Setyowati A, Arumwardana S, Sari Widya Kusuma H, Adhani Sholihah I, Rizal R, Widowati W, Maruf A. 2020. Hypoglycemic activity of Curcuma mangga Val. extract via modulation of GLUT4 and PPAR-γ mRNA expression in 3T3-L1 adipocytes. J Exp Pharmacol. 12:363–369. DOI: 10.2147/JEP.S267912.

Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C, Ramirez-Tortosa Mc. 2016. Curcumin and health. Molecules. 21:264. DOI: 10.3390/molecules21030264.

Rahimifard M, Baeeri M, Bahadar H, Moini-Nodeh S, Khalid M, Haghi-Aminjan H, Mohammadian H, Abdollahi M. 2020. Therapeutic effects of gallic acid in regulating senescence and diabetes; an in vitro study. Molecules. 25:5875. DOI: 10.3390/molecules25245875.

Rong Y, Cao B, Liu B, Li W, Chen Y, Chen H, Liu Y, Liu T. 2018. A novel gallic acid derivative attenuates BLM-induced pulmonary fibrosis in mice. Int Immunopharmacol. 64:183–191. DOI: 10.1016/j.intimp.2018.08.024.

Shahedi A, Talebi AR, Mirjalili A, Pourentezari M. 2021. Protective effects of curcumin on chromatin quality, sperm parameters, and apoptosis following testicular torsion-detorsion in mice. Clin Exp Reprod Med. 48(1):27–33. DOI: 10.5653/cerm.2020.03853.

Sökmen M, Khan M. 2016. The antioxidant activity of some curcuminoids and chalcones. Inflammopharmacology. 24:81–86. DOI: 10.1007/s10787-016-0264-5.

Soleimani V, Sahebkar A, Hosseinzadeh H. 2018. Turmeric (*Curcuma longa*) and its major constituent (curcumin) as nontoxic and safe substances: Review. Phyther Res. 32:985–995. DOI: 10.1002/ptr.6054.

Variya BC, Bakrania AK, Madan P, Patel SS. 2019. Acute and 28-days repeated dose sub-acute toxicity study of gallic acid in albino mice. Regul Toxicol Pharmacol. 101:71–78. DOI: 10.1016/j.yrtph.2018.11.010.

Variya BC, Bakrania AK, Patel SS. 2020. Antidiabetic potential of gallic acid from Emblica officinalis: Improved glucose transporters and insulin sensitivity through PPAR-γ and Akt signaling. Phytomedicine. 73:152906. DOI: 10.1016/j.phymed.2019.15290.

Wang B, Cui Z, Zhong Z, Sun Y, Sun Q, Yang G, Bian L. 2015. Curcumin attenuates brain edema in mice with intracerebral hemorrhage through inhibition of AQP4 and AQP9 expression. Acta Pharmacol Sin. 36:939–948. DOI: 10.1038/aps.2015.47.

Wang J, Tang L, White J, Fang J. 2014. Inhibitory effect of gallic acid on CCl4-mediated liver fibrosis in mice. Cell Biochem Biophys. 69:21–26. DOI: 10.1007/s12013-013-9761-y.

Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. 2010. Estimation of the normal range of blood glucose in rats. Wei Sheng Yan Jiu. 39:133–1377.

Xia Z, Chen W, Shi L, Jiang X, Li K, Wang Y, Liu Y. 2020. The underlying mechanisms of curcumin inhibition of hyperglycemia and hyperlipidemia in rats fed a high-fat diet combined with STZ treatment. Molecules. 25:271. DOI: 10.3390/molecules25020271.

Yang K, Zhang L, Liao P, Xiao Z, Zhang F, Sindaye D, Xin Z, Tan C, Deng J, Yin Y, Deng B. 2020. Impact of Gallic Acid on Gut Health: Focus on the Gut Microbiome, Immune Response, and Mechanisms of Action. Front Immunol. 11:580208. DOI: 10.3389/fimmu.2020.580208/full.

Yang Y, Su Y, Yang H, Lee Y, Chou JI, Ueng K. 2014. Lipid-lowering effects of curcumin in patients with metabolic syndrome: A randomized, double-blind, placebo-controlled trial. Phyther Res. 28:1770–1777. DOI: 10.1002/ptr.5197.

Yin H, Zuo Z, Yang Z, Guo H, Fang J, Cui H, Ouyang P, Chen X, Chen J, Geng Y, et al. 2021. Nickel induces autophagy via PI3K/AKT/mTOR and AMPK pathways in mouse kidney. Ecotoxicol Environ Saf . 223:112583. DOI: 10.1016/j.ecoenv.2021.112583.

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