Effect of Fermented *Moringa oleifera* Leaf with *Saccharomyces Sp.* Extract in Drinking Water on Egg Production, Yolk Colour, and Egg Cholesterol Levels in Laying Chicken

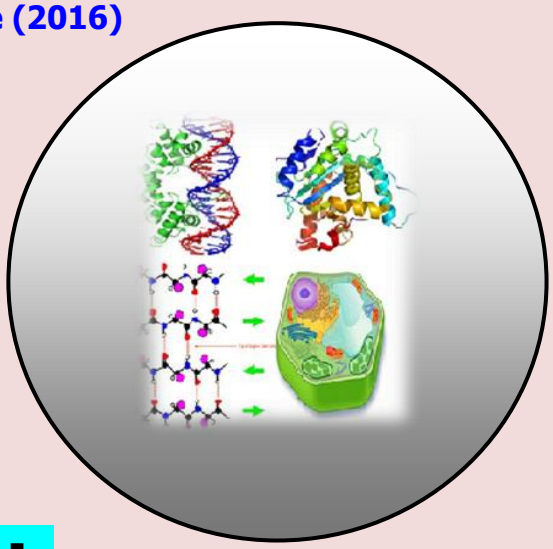
By

NI Wayan Siti, I. G. N. G. Bidura and I. A. P. Utami

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 35 (2) 2018 Pages No. 662-669



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 35, No. 2: 662-669, 2018
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 35/02/9902/2018

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

NI Wayan Siti
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER
Received: 31/08/2018 Revised: 09/09/2018 Accepted: 10/09/2018

Effect of Fermented *Moringa oleifera* Leaf with *Saccharomyces Sp.* Extract in Drinking Water on Egg Production, Yolk Colour, and Egg Cholesterol Levels in Laying Chicken

NI Wayan Siti, I. G. N. G. Bidura and I. A. P. Utami
Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia,
Jl. PB. Soedirman, Denpasar-Bali, Indonesia (80223)

ABSTRACT

The present study was conducted to examine the effect of fermented Moringa leaf with Saccharomyces sp. (FMLS) extract in drinking water on egg production, yolk colour, and egg cholesterol levels in laying chicken. One hundred and twenty 40-wk-old hens were colony caged in an environmentally controlled house to evaluate the levels of FMLS extract in administration on hens. FMLS extract was prepared by macerating FMLS in distilled water (1:1, w/w). Hens were randomly divided into four equal groups: one served as a control and was administered with drinking water only. Theother three groups were administered 2%; 4%; and 6% water extract of FMLS, respectively. Fermented Moringa leaf (FMLS) extract increased significantly (P<0.05) egg production and yolk colour, but not in egg weight (P>0.05). Fermented Moringa leaf extract administration results in lower (P<0.05) yolk cholesterol contents. Fermented Moringa leaf extract in drinking water increased egg production and yolk colour, but decreased yolk cholesterol contents of egg laying hens.

Keywords: Moringa leaf, Fermented, Cholesterol and Yolk Colour.

INTRODUCTION

Public awareness of the importance of animal protein fulfillment leads to high demand for chicken eggs. High market demand for chicken eggs makes this type of chicken much maintained by farmers. High cholesterol content in foodstuffs tends to be the main consideration of consumers in consuming food of animal origin, because it is a source of cholesterol for upper middle class people that can cause degenerative diseases such as coronary heart, characterized by hardening of walls arterial and high fat content (hyperlipidemia) in the blood especially cholesterol (hypercholesterolemia).

Moringa oleifera leaf is a traditional medicinal plant that has high nutrients, as an antibacterial, and contains beta-carotene as an egg yolk active substance. Phytochemical compounds enclosed there in are: flavonoids, saponins, tannins, and some other phenolic compounds that have antimicrobial activity (Bukar *et al*., 2010). Estrogen-like flavonoids can reduce bone loss (osteomalacia), can lower blood cholesterol levels, and increase HDL levels, while saponins are shown to be anticancer, antimicrobial, and lower serum cholesterol levels (Bidura *et al*., 2017). Producing quality egg products with high ration efficiency using the *Moringa leaf* extract (Sugiharto *et al.*, 2017); Bidura *et al*., 2012) will provide knowledge to small-scale farmers to achieve food security, as well as enhance their business competitiveness to increase their income.

Fermentation of feed ingredients turned out to increase the nutritional value of feed, as reported by Santoso *et al*. (2016); Sugiharto *et al*. (2015); and Sugiharto *et al*. (2017) can significantly increase the nutrient content of feed, and can improve poultry perfomance compared with non fermentation. Beta-carotene can be increased in fermentation with fungus (*Trichoderma harzianum*), because these fungi are carotenogenic (producing beta-carotene) (Hirschberg, 2001; Hsieh and Yang, 2003; Ma *et al*., 2000). The ability of beta-carotene decreasing cholesterol associated with hydroxy methyl glutaril enzyme-CoA (Wang and Keasling, 2002). Fermentation of feed by using microbes that act as probiotics become interesting to study. Because probiotic microbes remain in fermented feed before and after consumed by poultry host. Previous studies have indicated that probiotics have beneficial effects on growth performances of poultry and blood parameters (Dlamini *et al*., 2017), increased body weight gain and improved feed efficiensies (Bidura *et al.,* 2012; Bidura *et al*., 2016; Hasan *et al*., 2016; Siti *et al*., 2014), improve nutrient quality of feed (Bidura *et al*., 2014; Bidura *et al*., 2015; Bidura and Siti, 2017; Candrawati *et al*., 2014), and can decrease egg cholesterol levels (Bidura *et al*., 2016). Besides being used separately, probiotic *Saccharomyces sp* and other herbs (*Moringa oleifera*) may be used simultaneously to improve the quantity and quality of poultry products.

Khamir *S.cereviseae* in the digestive tract of poultry is expected to be able to synergize with poultry digestive microbe, so it will be able to synergize the beneficial effects together with active compounds on *Moringa leaves* to increase the quantity and quality of poultry production. Therefore, this study was conducted to examine the effect of *FMLS* in drinking water on on egg production, yolk colour, and egg cholesterol levels in laying chicken.

MATERIALS AND METHODS

Animals, treatments, and experimental design: This study used 240 Lohmann Brown hens, 40 weeks of age, with a homogeneous body weight of 1758.25 ± 25.82 grams obtained from a commercial poultry farm. All chickens were given commercial feed specific for laying hens containing 2.750 kcal/kg of metabolizable energy (ME); 17% of CP; 3.5% of Ca; and available phosphor of 0.45%. Each cage equipped with food and drinking holder placed outside the cage. Sanitation of the equipment is done everyday by cleaning places for feeding and drinking. For the treatments, hens were placed into four groups each containing 10 hens: (A) hens were only given water as a drink, (B) hens were given 2 cc of FMLS extract in 100 cc of drinking water; (C) hens were given 4 cc of FMLS leaf extract in 100 cc of drinking water; and (D) hens were given 6 cc of FMLS leaf extract in 100 cc of drinking water. Each treatment was repeated 6 times for a total of 240 hens. Food and drinking water were given *ad libitum*. The individual hens were weighted weekly, food consumption and egg production was recorded daily.

Performance, egg quality metrics, and laboratory analysis: Eggs were collected and labeled on a daily basis at 08.00 h and 14.00 h throughout the experimental period. The percent egg production was calculated. Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk colour was determined by using a Roche yolk colour fan (1 to 15). Yolk cholesterol content was analyzed for two consecutive weeks. The yolk samples were randomly collected from two (2) birds per replicate at the end of the study at 10^{th} week and analyzed for the estimation of yolk to determine the total yolk cholesterol content.

Process of making flour *Moringa* leaves and fermentation: Fresh leaves of the *Moringa* leaf were obtained from the local fresh food market. Before being fermented, *Moringa* leaves that have dark green, thinly sliced and dried in room temperature for 1-2 days, then dried in oven at temperature 50°C for 24 hours. Furthermore *Moringa* leaves were ground to fine powder form. Powdered *Moringa* leaves were then analyzed and prepared for fermentation using khamir *Saccharomyces spp*.N-2 (isolated from yeast culture) (Bidura *et al.*, 2012), with inoculum dose of 5% incubated for 4 days with the thickness of 2 cm substrate at pH 5.5 and temperature 30°C and than macerated overnight in distilled water (1:1, w/w) (Parwata *et al*., 2016). The blended extract was then filtered using a cheese cloth. This extract was used for the treatment.

Performance, egg quality metrics, and laboratory analysis: Eggs were collected and labeled on a daily basis at 08.00 h and 14.00 h throughout the experimental period. The percent egg production was calculated, feed conversion ratio (FCR, feed DM intake/total egg weight). Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk colour was determined by using a Roche colour fan (1 to 15). Yolk cholesterol content was analyzed for two consecutive weeks. Cholesterol levels were analyzed following the Liberman-Burchard methods (Lieberman and Burchard, 1980).

Statistical analysis: All data were analyzed with ANOVA to determine the differences among treatments. If differences were found, then further analysis was performed with Duncan's multiple range test.

RESULTS

The results study shows that 2-6% FMLS extract in drinking water significantly increased (P<0.05) the average total egg weight and the average number of eggs produced as well as the average hen-day production were noted among the treatments (Table 1). However, no significant differences (P>0.05) in the feed consumption, water consumption, and egg weight per head (g/head). The average value of FCR (feed consumption : total egg weight) over ten weeks of observation in the control group was 2.43/head (Table 1). This was significantly different (P<0.05) from hens in treatment groups B, C, and D an average of 7.41%; 4.94%; and 4.53% more higher than control (Group A), respectively.

Table 1.The effect of FMLS aqueous extract added in drinking water and administered to 40-50 weeks aged of egg laying hens to the egg production and feed efficiencies.

Variables	Groups[1)]				SEM[2)]
	A	B	C	D	
Feed Consumption (g/head/70days)	9290.38a	9307.45a	9348.92a	9295.36a	78.481
Water consumption (l/head/70days)	27.361a	28.074a	28.048a	27.695a	1.052
Total egg weight (g/head/70 days)	3818.34b	4136.64a	4047.15a	4015.11a	52.925
Egg weight (g/head)	67.68a	66.68a	65.02a	64.51a	1.973
The number of eggs (egg/70 days)	56.42b	62.04a	62.24a	62.24a	1.402
Hen-day production (%)	80.06a	88.63b	88.92b	87.85b	1.375
Feed conversion ratio (feed consumption: total egg weight)	2.43a	2.25b	2.31b	2.32b	0.025
Yolk colour (1 to 15)	7.42a	8.61b	8.84b	8.75b	0.327
Yolk cholesterol (mg/dl)	527.31a	489.53b	492.96b	483.05b	5.813

Notes:

1. A: drinking water without FMLS extract as control; (B): drinking water with 2 cc/100 cc FMLS extract; and (C): drinking water with 4 cc/100 cc FMLS extract, and drinking water with 6 cc/100 cc FMLS extract, respectively.
2. SEM: Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different (P<0.05)

In addition, the results show that an additional FMLS extract (2-6 cc/100 cc) in drinking water resulted in a significant (P<0.05) increase in yolk colour of eggs (Table 1). Eggs yolk colour of the group C was 8.84 followed by group D was 8.75; 8.61 (B); and 7.42 (Group A), respectively.

The cholesterol content in cholesterol content in egg yolk in chickens decreased significantly (P<0.05) in the presence of FMLS extract in drinking water. Increased level of FMLS in drinking water was significantly (P<0.05) lowered yolk cholesterol levels. Yolk cholesterol content of the group D was 8.39 followed by group B was 7.16%; and 6.51% (C) were lowered significantly different (P<0.05) than control (A), respectively.

DISCUSSION

The results of the first year study showed that phytochemical compounds contained in *Moringa* leaves are: flavonoids, saponins, tannins, and some other phenolic compounds that have antimicrobial activity. Supplementation of *Moringa oleifera* leaf extract at a concentration of 2-6 cc/100 cc on drinking water were increase egg production and lower cholesterol content in chicken eggs (Siti *et al*., 2017). Supplementation of FMLS extract in drinking water was found to have the same effect with first year research result, that is the increase of total egg weight and number of eggs compared with control, and no effect on feed consumption. Oka *et al*. (2016) reported that the antioxidant properties of the leaves of *Moringa* are very high, also high tannin content, that is equal to 831.92 mg/100 ml. Phytochemical compounds contained in Moringa leaves grown in Bali are alkaloids, flavonoids, phenolics, triterpenoids/ steroids, and tannins (Putra *et al*., 2016). This is due to the active compounds in fermented *Moringa* leaf extract, such as flavonoids, saponins, tannins, and beta-corotene.

The main workings of these active ingredients are by inhibition of pathogenic microbes and endotoxins in the intestines, increasing pancreatic activity, increasing metabolism, and absorption of nutrients in the gastrointestinal tract of chicken (Windisch *et al.,* 2008; Grashorn, 2010). *Moringa* can be a phytogenic compound in feed based on its bioactive compounds, which can improve egg quality and have a positive impact on the health and performance of poultry (Yang *et al*., 2006; Portugaliza and Fernandez, 2011; Zanu *et al.,* 2012; Ola-Fadunsin and Ademola, 2013). The content of β-carotene and quercetin compounds in *Moringa oleifera* leaves, ranging from 2.7-3.10 mg/100 g and 80-150 mg/100 g, respectively (Amaglo, 2010; Saini *et al*., 2014a; Saini *et al*., 2014b). When added to the ration, the bioactive compound along with other phytochemical compounds can improve egg quality and have a positive effect on the health and performance of the chicken.

The fermentation process on *Moringa* leaves by probiotic microbes (FMLS) will be able to double function. First, before it is given to livestock, probiotic microbes will be able to help break down complex compounds into simple compounds that are easily digested by digestive enzymes. In addition, the fermentation process will be able to remove antinutrition compounds in feed ingredients, such as tannins. The second advantage of fermentation process by using probiotic microbe is when given to chicken, hence probiotic microbe will be able to assist metabolism process of nutrient in gastrointestinal tract of poultry. The main concept of fermentation of feed with probiotics is to increase the activity of probiotic microbes or provide a suitable condition to increase the number of bacteria involved in probiotics. Basically, fermentation is a chemical transformation of organic matter into simple compounds by active enzymes, ie complex organic catalysts produced by microorganisms, such as bacteria, yeast, or fungi. The use of wet fermented feed with probiotics can improve the performance of chickens (Hasan *et al.,* 2016; Lokaewmanee *et al*., 2012; Sugiharto, 2016).

As reported by Bidura *et al*. (2012), supplementation of probiotics as fermented feeding inoculants is very important because it can increase the rate of growth, weight gain, feed use efficiency, and poultry performance. In addition, fermentation products affect the ecology of bacteria in the gastrointestinal tract and reduce the level of *Enterobacteriaceae* in various parts of the gastrointestinal tract of broiler chickens (Heres *et al*., 2003). The administration of fermented products by probiotics can lead to the reduction of pathogenic bacteria, including *Salmonella* and *Campylobacter* in the livestock digestive tract (Yamamoto *et al*., 2004; 2007). The same thing was reported by Hasal *et al.* (2016) that the use of wet fermented feed products with probiotic microbes can improve chicken performance. Lokman *et al.* (2015) reported that the use of dried fermented products with probiotics had a very significant improvement on chicken performance parameters. It is assumed that fermented feeds generally improve the bacterial ecology of the gastrointestinal tract and the immune response in chicks. Therefore, it becomes an interesting study to improve the efficacy of *Moringa oleifera* leaf herbs through fermentation with probiotic microbes before being used as feed supplements in chickens, as well as to control chicken disease. This is interesting because the efficacy of herbs products fermented by microbial probiotics is double efficacious when compared with the original product (Bidura *et al*., 2014; Santoso *et al*., 2015; Wibawa *et al*., 2016). In addition to the goal of improving health, probiotics *Saccharomyces sp*. has long been used to improve the digestion and growth of poultry performance (Bidura *et al*., 2012; 2014; 2016). Besides being used separately, probiotics *Saccharomyces sp*. and other herbs additives can be used simultaneously to increase weight gain and feed efficiency of poultry. Provision of probiotics is associated with improved chemical, nutritional, and quality of broiler meat (Liu *et al.,* 2012). Supplementation of the FMLS extract in drinking water resulted significantly increased the yolk colour of eggs. Probiotics can improve egg weight, feed efficiency, yolk colour, and egg shell quality (Mallo *et al*., 2010; Mountzouris *et al*., 2010). The colour is an important quality trait of foods since it affects the consumers' perception of quality and intensity of aroma and flavour and their decision on purchase (Loetscher *et al*., 2013). Eggs yolk colour from the FMLS extract extract in drinking water had significantly higher eggs yolk colour compared to the control. Changes observed in yolk colour are largely associated with the ingredients used in diets. Carotenoids play an important role in the development of different colour scores of egg yolk, especially, lutein is active yolk colourant. Some researchers reported that supplementing herbal extract showed potential for increasing in egg yolk colour. It was the same observed by Lokaewmanee *et al.* (2009); Zhao *et al.* (2013) and Bidura *et al.* (2017) on the effects of herbs leaf increase in egg yolk colour. That the increase of the amount of FMLS extract in drinking water resulted in a linear increase in egg yolk colour. Cayan and Erener (2015) reported that this increase in egg yolk colour can be attributed to the carotenoid contents of olive leaf powder. Beta-carotene and vitamin E are natural antioxidants, and antioxidants have an important role in inhibiting and scavenging free radicals. Mabusela *et al.* (2018) reported that *Moringa oleifera* meal can improved yolk colour, maintained external egg quality, and improved the fatty acid profile.

Decreasing of cholesterol levels in egg yolks in chickens supplemented *Moringa* fermented leaf extract. This is caused by bioactive compound flavonoids (quercetin) and carotenoids (β-Carotene) positively affected and reduced the levels of creatinine, glucose and cholesterol levels in the serum, which showed improved liver performance, whereas lowered creatinine levels indicated better kidney functionality in FMLS supplemented groups (Melesse *et al*., 2013; Elkloub *et al*., 2015). Lowered cholesterol levels in the yolk show the hypocholesterolemic effect of *Moringa*, which might be attributed to β-sitosterol-rich plant material, which has same structure as cholesterol and lowers uptake from the intestine (Ghasi *et al.,* 2000). These antioxidants (β-Carotene and quercetin) and phytosterols (β-Sitosterol) affect the functionality of liver, kidneys and heart, resulting in improved metabolism, as indicated in biochemical parameters and antibody titers (Ghasi *et al.,* 2000). *Katuk* (*Sauropus*) leaf can lower cholesterol levels because of the content of beta-carotene (Wardiny, 2006). Beta-carotene can be increased in fermentation with fungus *Trichoderma harzianum*, because these fungi are carotenogenic (producing beta-carotene) (Ma *et al*., 2000; Hirschberg, 2001; Hsieh and Yang, 2003). The ability of beta-carotene decreasing cholesterol associated with hydroxy methyl glutaril enzyme-CoA (HMG) (Wang and Keasling, 2002). This enzyme plays a role in the formation of mevalonic in the biosynthesis of cholesterol. Cholesterol synthesis and synthesis of beta-carotene are together through mevalonic and derived from acetyl CoA. If increasing consumption beta-carotene greater than saturated fatty acid, it makes biosynthesis process by enzyme HMGCoA directed at beta-carotene, so that saturated fatty acids are not converted into cholesterol (McGilvery and Goldstein, 1996; Nuraini, 2006).

The decrease in egg cholesterol levels is also due to the presence of probiotic microbes (*Saccharomyces spp.*) used as inoculants in *Moringa* leaf fermentation. Probiotics can improve egg weight, feed efficiency, yolk colour, egg shell quality, pathogen inhibition (Mallo *et al*., 2010; Mountzouris *et al.,* 2010) and have the ability to lower serum cholesterol levels (Kusumawati *et al*., 2003). The lowest cholesterol level was obtained by feeding the chickens with diets containing 14% fermented *katuk* leaf (Syahruddin *et al.,* 2013). Santoso *et al.* (2015) reported that *Saccharomyces cerevisiae* fermented *Sauropus androgynus* leaves inclusion resulted in the best broiler meat quality as indicated by lower fat and cholesterol. Ekayuni *et al*. (2017) reported that supplementation of *Moringa oleifera* extract of 50 ml/liter of drinking water can reduce abdominal fat and cholesterol levels in meat in broiler chickens.

CONCLUSION

We conclude that an additional 2-6 cc/100 cc of FMLS extract in drinking water were increased egg production and yolk colour, but may decrease yolk cholesterol in laying hens up to fourty weeks of age.

ACKNOWLEDGEMENTS

The authors would like to thank to staff of laboratory attendants at the Nutrition Laboratory, Udayana University for their assistance in chemical analysis of the samples. We also would like to thank the Head of Research and Public Service Department and Rector of Udayana University for their support research funding.

REFERENCES

Amaglo, N.K., Bennett, R.N., Curto, R.B., Rosa, E.A., Turco, V.L., Giuffrida, A., Curto, A.L., Crea, F. and Timpo, G.M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L, grown in Ghana. Food. Chem. 15, 1047-1054.

Bidura, I.G.N.G., and N.W. Siti. (2017). Selection and Implementation of Probiotics *Saccharomyces spp.*Kb-05 and *Saccharomyces spp.*Kb-08 Isolated from Buffalo Ruments to Increase the Nutritional Value of Rice Bran. J. Biol. Chem. Research. Vol. 34 (2): 866-877.

Bidura, I.G.N.G., I.P. Suyadnya, I.G. Mahardika, I.B.Gaga Partama, I.G.L. Oka dan IG.A.I. Aryani (2012). The implementation of *Saccharomyces spp.n-2* isolate culture (isolation from traditional yeast culture) for improving feed quality and performance of male Bali duckling. Agricultural Science Research Journal. ISSN-L: 2026-6073. September: Vol. 2 (9): 486-492.

Bidura, I.G.N.G., I.B.G. Partama, D.K.H. Putra and U. Santoso (2016). Implementation on diet of probiotic Saccharomyces spp.SB-6 isolated from colon of Bali cattle on egg production and egg cholesterol concentration of Lohmann brown laying hens. Int. J. Curr. Microbiol. App. Sci. Volume 32 (2): 683-699

Bidura, I.G.N.G., D.P.M.A. Candrawati and D.A. Warmadewi (2015). Selection of Khamir *Saccharomyces spp.* Isolated from Colon of Native Chickens as a Probiotics Properties and has CMC-ase Activity. J. Biol. Chem. Research. Vol. 32, (2): 683-699.

Bidura, I.G.N.G., I.B.G. Partama, B.R.T., Putri and N. L. Watiniasih (2017). Effect of Water Extract of Two Leaves (*Allium sativum* and *Sauropus androgynus*) on Egg Production and Yolk Cholesterol Levels in Egg Laying Hens. *Pakistan Journal of Nutrition* Vol. 16 (7): 482-487.

Bidura, I.G.N.G., N. W. Siti and I.A. Putri Utami (2014). Isolation of cellulolytic bacteria from rumen liquid of buffalo both as a probiotics properties and has CMC-ase activity to improve nutrient quality of soybean distillery by-product as feed. International Journal of Pure & Applied Bioscience Vol 2 (5): 10-18.

Bukar, A., T. I. Uba and Oyeyi (2010). Antimicrobical Profile of Moringa oleifera Lam. Ekstracts Against Some Food-Borne Microorganism. Bayero Journal of Pure and Applied Sciences, 3(1): 43-48.

Candrawati, D.P.M.A., D.A. Warmadewi and I.G.N.G. Bidura (2014). Isolation of *Saccharomyces spp* from Manure of Beef Bali cattle as a probiotics properties and has CMC-ase activity to improve nutrient quality of rice bran. Journal of Biological and Chemical Research. January Vol. 31 (1): 39-52.

Cayan, H. and G. Erener (2015). Effect of Olive Leaf (*Olea europaea*) Powder on Laying Hens Performance, Egg Quality and Egg Yolk Cholesterol Levels. *Asian Australas. J. Anim. Sci. 28:538-543*

Dlamini, Z.C., R.L.S. Langa, O. A. Aiyegoro and A.I. Okoh (2017). Effects of probiotics on growth performance, blood parameters, and antibody stimulation in piglets. South African Journal of Animal Science, 47 (6): 81-87

Elkloub, K., Moustafa, M.E.L., Riry, F.H., Mousa, M.A.M. and Hanan, A.H. (2015). Effect of using *Moringa oleifera* leaf meal on performance of Japanese quail. Egypt. Poult. Sci. J. 35, 1095-1108.

Ekayuni, A.A., I.G.N.G. Bidura, and I.B. Gaga Partama (2017). The Effect of Water Extract of Two Leaves (*Moringa oleivera and Sauropus androgynus*) on Growth Performance and Meat Cholesterol Levels in Broilers. J. Biol. Chem. Research. Vol. 34, No. 1: 118-128

Ghasi, S., Nwobodo, E. and Ofili, J.O. (2000). Hypo-cholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. J. Ethno. Pharmacol. 69, 21–25.

Grashorn, M.A. (2010). Use of phytobiotics in broiler nutrition: an alternative to infeed antibiotics. J. Anim. Feed. Sci. 19, 338-347.

Hasan S.A. Jawad, I.H. Lokman, Saad A. Naji, A.B.Z. Zuki and A.B. Kassim (2016). Effects of dietary supplementation of wet fermented feed with probiotic on the production performance of Akar Putra chicken. Asian J. Poult. Sci., 10: 72-77.

Heres, L., B. Engel, F. van Knapen, M.C. de Jong, J.A. Wagenaar and H.A. Urlings (2003). Fermented liquid feed reducessusceptibility of broilers for Salmonella enteritidis. Poult. Sci., 82: 603-611.

Hirschberg, J., (2001). Carotenoid biosynthesis in flowering plants. Curr. Opin. Plant Biol., 4: 210-218.

Hsieh, C. and F.C. Yang (2003). Reusing soy residue for the solid-state fermentation of *Ganoderma lucidum*. Bioresour. Technol., 80: 21-25.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megías, M.D. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science, 83, 169-174.

Kusumawati, N., L.J. Bettysri, S. Siswa, D. Ratih and Hariadi (2003). Selection of lactic acid bacteria of indigenous as strain probiotic with ability to lower cholesterol. J. Microbiol. Indonesian, 8: 39-43.

Liu, X., Yan, H., Lv, L., Xu, Q., Yin, C., Zhang, K., Wang, P. and Hu, J. (2012). Growth performance and meat quality of broiler chickens supplemented with *Bacillus licheniformis* in drinking water. Asian-Aust. J. Anim. Sci. 25, 682-689.

Lieberman, A. and R. Burchard (1980). Enzimatic method to determined cholesterol. Engl. J. Med., 271: 915-924.

Loetscher, Y., M. Kreuzer, and R. E. Messikommer (2013). Utility of nettle (*Urtica dioica*) in layer diets as a natural yellow colorant for egg yolk. Anim. Feed Sci. Technol. 186:158-168.

Lokaewmanee, K., S. Mompanuon, P. Khumpeerawat and K. Yamauchi (2009). Effects of dietary mulberry leaves (*Morus alba L*.) on egg yolk color. J. Poult. Sci. 46:112-115.

Lokaewmanee, K., Yamauchi, K. and Thongwittaya, N. (2012). Effects of fermented plant product on growth performance, some blood variables, carcase characteristics, and intestinal histology in broilers. Br. Poult. Sci. 53, 215-223.

Lokman, I.H., S.H. Jawad, A.B.Z. Zuki and A.B. Kassim (2015). Effect of dry probiotic supplemented fermented feed on production performance of akar putra chicken. Int. J. Poult. Sci., 14: 420-426.

Ma, J., Y. Li, Q. Ye, J. Li, Y. Hua, D. Ju, D. Zhang, R.Cooper and M. Chang (2000). Constituent of red yeast rice, a traditional chinese food and medicine. J. Agric. Food Chem. 48: 5220-5225.

Mabusela, S.P., T. T. Nkukwana, M. Mokoma and V. Muchenje (2018). Layer performance, fatty acid profile and the quality of eggs from hens supplemented with *Moringa oleifera* whole seed meal. South African Journal of Animal Science, 48 (2): 234-243.

McGilvery, R.W. and G.W. Goldstein (1996). Biochemistry: a functional approach. Sumarno dsbk, t.m.(penterjemah). Penerbit airlangga university press,Surabaya.

Mallo, J.J., M.I. Gracia, P. Honrubia and G. Sedano (2010). Use of a Bacillus amyloliquefaciens probiotic in broiler farms. Poult. Sci., 89(E-Suppl. 1): 815-815.

Melesse, A., Getye, Y., Berihun, K. & Banerjee, S. (2013). Effect of feeding graded levels of *Moringa stenopetala* leaf meal on growth performance, carcass traits and some serum biochemical parameters of Koekoek chickens. Livest. Sci. 157(2), 498-505.

Mountzouris, K. C., P. Tsitrsikos, I. Palamidi, A. Arvaniti, M. Mohnl. G. Schatzmayr and K. Fegeros (2010). Effect of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult. Sci. 89:58-67.

Nuraini (2006). Potential karotenogenik mold for producing $-carotene food sources and their effects on the performance of broilers and laying. Dissertation. Graduate programs, university Andalas. Padang.

Oka, A.A., K.A. Wiyana, I.M. Sugitha dan I.N.S. Miwada (2016). Identifikasi Sifat Fungsional dari Daun Jati, Kelor dan Kayu Manis dan Potensinya sebagai Sumber Antioksidan pada *Edible Film (Identification of Functional Properties of Teak Leaves, Moringa and Cinnamon and its Potential as a Source of Antioxidants in Edible Film).* Jurnal Sain Peternakan Indonesia Vol. 11 No 1: 1-7.

Ola-Fadunsin, S.D. and Ademola, I.O. (2013). Direct effects of *Moringa oleifera* Lam (Moringaceae) acetone leaf extract on broiler chickens naturally infected with Eimeria species. Trop. Anim. Health. Prod. 45, 1423-1428.

Portugaliza, H.P. and Fernandez, T.J. (2011). Growth performance of Cobb broilers given varying concentrations of Malunggay (*Moringa oleifera* lam.) aqueous leaf extract. J. Anim. Feed. Res. 2, 465-469.

Putra, I.W.D.P., Anak Agung Gde Oka Dharmayudha and Luh Made Sudimartini (2016). Identifikasi Senyawa Kimia Ekstrak Etanol Daun Kelor (*Moringa oleifera L*) di Bali (*Identification of Chemical Compounds Ethanol Extract Leaf Moringa (Moringa Oleifera L) In Bali*). Indonesia Medicus Veterinus 5(5): 464-473

Parwata, A., P. Manuaba, S. Yasa and I.G.N.G. Bidura. 2016. Characteristics and Antioxidant activities of Gaharu (Gyrinops versteegii) leaves, J.Biol.chem. Research 33(1): 294-301

Saini, R.K., Shetty, N.P., Prakash, M. and Giridhar, P. (2014a). Effect of dehydration methods on retention of carotenoids, tocopherols, ascorbic acid and antioxidant activity in *Moringa oleifera* leaves and preparation of a RTE product. J. Food. Sci. Technol. 51, 2176-2182.

Saini, R.K., Shetty, N.P. and Giridhar, P. (2014b). Carotenoid content in vegetative and reproductive parts of commercially grown *Moringa oleifera* Lam. cultivars from India by LC–APCI–MS. European. Food. Res. Tech. 238, 971-978.

Santoso, U., Yosi Fenita, Kususiyah and I Gusti Nyoman Gde. Bidura (2015). Effect of Fermented *Sauropus androgynus* Leaves on Meat Composition, Amino Acid and Fatty Acid Compositions in Broiler Chickens. Pakistan Journal of Nutrition 14 (11): 799-807.

Siti, N.W., DPMA. Candrawati, DA. Warmadewi and IGNG. Bidura (2014). Isolation of *Saccharomyces spp* from Manure of Beef Bali cattle as a probiotics properties and has CMC-ase activity to improve nutrient quality of rice bran. Journal of Biological and Chemical Research Vol. 31 (1): 39-52.

Siti, N.W., I.G.N.G. Bidura, and I.A. Putri Utamai (2017). The Effect of Water Extract of Leaves *Moringa oleifera on Egg Production and Yolk Cholesterol Levels* in Egg Laying Hens. J. Biol. Chem. Research. Vol. 34, No. 2: 657-665.

Sugiharto, S., Yudiarti, T. and Isroli, I. (2015). Functional properties of filamentous fungi isolated from the Indonesian fermented dried cassava, with particular application on poultry. Mycobiology 43, 415-422.

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti and F.D. Putra (2017). Effects of feeding cassava pulp fermented with *Acremonium charticola* on growth performance, nutrient digestibility and meat quality of broiler chicks. South African Journal of Animal Science, 47 (2): 130-138.

Sugiharto, S. (2016). Role of nutraceuticals in gut health and growth performance of poultry. J. Saudi Soc. Agric. Sci. 15, 99-111.

Syahruddin, E., R. Herawaty and R.W.S. Ningrat (2013). Effect of Fermented *Katuk* Leaf (*Sauropus androgynus* L. Merr.) in Diets On Cholesterol Content of Broiler Chicken Carcass. Pakistan Journal of Nutrition 12 (11): 1013-1018.

Wang, G. And J.D. Keasling (2002). Amplification of HMG CoA reduktase production enhances carotenoid accumulation in neurospora crassa. Metabol. Eng.

Wardiny, T.M. (2006). The content of vitamin A, C, and Chicken egg cholesterol in the ration fed noni. Thesis Graduate School. Bogor Agricultural Intitute, Bogor

Wibawa, A.A.P.P., I.A.P. Utami and IGNG. Bidura (2016). The effect of Garlic (*Allium sativum*) extract on performance, abdominal fat and serum cholesterol of broiler. Journal of Biological and Chemical Research Vol. 33 (1): 214-225

Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86, 140-148.

Yamamoto, M., F. Saleh, M. Tahir, A. Ohtuska and K. Hayashi (2007). The effect of Koji-feed (fermented distillery by-product) onthe growth performance and nutrient metabolizability inbroiler. J. Poult. Sci., 44: 291-296.

Yang, R.Y., Chang, L.C., Hsu, J.C., Weng, B.B.C., Palada, M.C., Chadha, M.L. and Levasseur V. (2006). Nutritional and functional properties of Moringa leaves, from germ plasm to plant to food to health. Moringa and other highly nutritious plant resources: Strategies, standards and markets for a better impact on nutrition in Africa. Accra Ghana. November 16-18.

Zanu, H.K., Asiedu, P., Tampouri, M., Abada, M. and Asante, I. (2012). Possibilities of using Moringa (*Moringa oleifera*) leaf meal as a partial substitution for fish meal in broiler chicken diets. J. Anim. Feed. Res. 2, 70-75.

Zhao, L., X. Zhang, F. Cao, D. Sun, T. Wang, and G. Wang (2013). Effect of dietary supplementation with fermented ginkgo-leaves on performance, egg quality, lipid metabolism and egg-yolk fatty acids composition in laying hens. Live. Sci. 155:77-85

Corresponding author: Dr. NI Wayan Siti, Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia
Email: bidura_unud@yahoo.com

Indexed, Abstracted and Cited in Indexed Copernicus International and 20 other databases of National and International repute