World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Herbal-pro supplementation in drinking water to promote the growth and suppress *Coliform* and *Escherichia coli* in the Intestines of ducklings

Ni Wayan Siti *, Desak Putu Mas Ari Candrawati and I Gusti Nyoman Gde Bidura

Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia.

World Journal of Biology Pharmacy and Health Sciences, 2023, 15(02), 164–169

Publication history: Received on 01 July 2023; revised on 14 August 2023; accepted on 17 August 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.15.2.0351

Abstract

Herbal-Pro is a combination of herbal extracts (*Carrot* leaves) and probiotics (*Effective Microorganism-4*). Probiotic microbes have been shown to improve performance and nutrient absorption. Meanwhile, savonins in herbal leaves can suppress pathogenic bacteria. It is interesting to study the effect of Herbal-Pro supplementation in drinking water to improve performance and suppress pathogenic bacteria in the intestines of duckling. A total of 216 male Bali duckling aged 2 weeks with an average homogeneous body weight were randomly divided into 4 treatments and 6 replications in a completely randomized design. The "Herbal-Pro" treatment was administered via drinking water, each at the level of: 0% as a control (A); 2% (B), 4% (C), and 6% (D), respectively. The results showed that the inclusion of Herbal-pro in drinking water were increased significantly ($P<0.05$) body weight gain, feed consumption, and feed efficiency. Herbal-Pro administration at a level of 4-6% via drinking water was significantly different ($P<0.05$) in reducing the number of *Coliform* and *E. coli* bacteria in the duckling intestine. It can be concluded that addition of 2-6% Herbal-Pro on drinking water of duckling from 2-10 weeks of age significantly improves growth and feed efficiency, and vice versa is able to suppress pathogenic bacteria in the intestines of ducklings.

Keywords: Carrot leaves; Duckling; *Eschericia coli*; Growth; Probiotics

1. Introduction

In general, intensive poultry farms often experience losses caused by *Escherichia coli* bacteria which can cause *Colibacillosis* [1]. Therefore, it is necessary to find alternatives to utilize the properties of herbal plants in combination with probiotics to replace antibiotics, because the use of antibiotics in poultry feed has been banned.

In developing countries to treat various diseases, it is very dependent on herbal leaves. Because herbal medicinal plants have fewer side effects on the human body than antibiotics. Several research results show that compounds isolated from herbal leaves have anti-diabetic, anti-inflammatory,anti-hypertensive properties and anti-oxidant, and anti-carcinogenic [2,3,4,5]. However, the results of studies on poultry showed different results. This is suspected by the dose, type of herb, and method of administration [6].

Research on carrot herbal leaves is interesting to study, because of its phytochemical properties and secondary metabolites which have antimicrobial activity that can degrade cell membranes of pathogenic bacteria in the intestine of poultry [7,8,9].

Probiotics can change the digestive microbial ecosystem, and also produce natural antibiotics (bacitracin, hydrogen peroxide, acidolin), thereby affecting the health and performance of the host. Probiotics have a good effect on livestock including improving health and feed efficiency [10]. Supplementation of probiotics in feed significantly increases the height of the villi and the depth of the jejunum crypts, so that the absorption of nutrients can be optimal. In addition,

*Corresponding author: Ni Wayan Siti

probiotic microbes were able to suppress the population of *E.coli* and *Clostridium* perfringens in the jejunum and cecum of pigs [11].

Giving herbal extracts through broiler drinking water can stimulate growth and suppress pathogenic bacteria, and does not affect the performance of the liver, kidneys and blood lipid profile, and has the potential as an antidiabetic drug [1]. Likewise, giving probiotics to poultry improves performance and feed digestibility, but reduces the population of *E.coli* and *Coliform* in the intestines of chickens and pigs [12,13,14,15].

The combination of herbal leaf extracts and probiotics is expected to improve the performance and health of ducklings. This study aims to examine the effect of giving a mixture of carrot leaf extract and probiotics (Herbal-Pro) in drinking water on the growth of pathogenic bacteria in the intestine of ducklings.

2. Material and methods

2.1. Material

The research was conducted at the Research Station, Faculty of Animal Husbandry, Udayana University on Jl. Raya Sesetan, Denpasar. The ducks used were healthy male Bali ducklings aged 2 weeks with homogeneous body weight. The ducklings were obtained from a Balinese duckling breeding business in Kediri District, Tabanan Regency, Bali. The cage used was a battery colony cage made of wire and bamboo slats. The dimensions of each cage plot were: length 220 cm, width 150 cm and height 50 cm. Each plot contained 9 healthy male Bali ducklings aged 2 weeks with homogeneous body weight. Each cage plot was equipped with a feeding trough made of PVC pipe and drinking water from a plastic gallon with a capacity of 5 liters.

A total of 192 male ducklings (*Anas sp*.) 2 weeks old were randomized into 4 treatment groups, namely: drinking water without the addition of Herbal-Pro as a control (A), drinking water with 2% addition (B); 4% (C); and 6% (D) Herbal-Pro, respectively. Each treatment group with 6 replications with 48 ducklings. All ducks were housed in battery colony cages made of wire and bamboo slats.

The ration given was in the form of a mash consisting of a mixture of several feed ingredients, including: commercial concentrate, rice bran, corn, and a vitamin-mineral mix. Ration was made according to the needs of ducks based on the table of needs according to [16].

2.2. Methods

The mechanism for carrying out the research began with the collection of carrot leaves obtained from post-harvest carrots in the Baturiti area, Tabanan Regency, Bali Province. After being washed clean, the carrot leaves were then blended and filtered with double cloth.The green liquid was then put into a plastic bottle and ready to be given to the ducks.

2.3. The preparation of "Herbal-Pro"

The preparation of "Herbal-pro" begins with mixing carrot leaf juice (herbal) with *Effective Microorganism*-4 (probiotics) immediately before being given to the ducks, namely in a ratio of 9:1 (v/v). *Effective Microorganism*-4 (EM-4) contains: fermenting microorganisms and synthetic microorganisms consisting of: lactic acid bacteria (*Lactobacillus sp*.), photosynthetic bacteria (*Rhodopseudomonas sp*.), *Actinomycetes sp., Streptomyces sp.,* Yeast (yeast), and fungi that can decompose cellulose (PT. Songgolangit Persada, Jakarta). Giving was done simultaneously with feeding.

2.4. Observed variables.

The variables observed were: final body weight (FBW), body weight gain (LWG), feed consumption (FC), and feed efficiency (comparison between FC and LWG). To obtain total *Escherichia coli* used scatter method[1] in EMBA media, namely 5 g of digesta sample was put into an Erlenmeyer containing 0.1% peptone water solution with a volume of 45 ml, resulting in a 10^{-1} dilution. Planting at a dilution level of 10^{-1} to 10^{-7} was to count bacterial colonies that grow using the cup count method (30-300 colonies).

The data obtained were analyzed with one-way analysis of variance, if there was a significant difference ($P<0.05$) between the treatments, then it was continued with Duncan's multiple range test.

3. Results and discussion

3.1. Growth performance

The impact of adding "Herbal-Pro" via drinking water on the performance of male ducklings aged 2-10 weeks is presented in Table 1. Administration of "Herbal-Pro" via drinking water significantly (P<0.05) increased FBW, LWG, feed consumption (FC), and feed efficiency. The average FBW in duckling groups B, C and D were: 11.65%; 10.96%; 12.32% higher (P<0.05) than the duckling group A. Live weight gains for 8 weeks in duckling groups B, C, and D, were: 14.56%; 13.62%; and 15.40% higher than the duckling group control.

Table 1 The effect of adding "Herbal-Pro" via drinking water to male Balinese ducklings aged 2-10 weeks on the performance of ducklings

Variables	Herbal-Pro level in dringking water (cc/100 cc)				SEM
	0	2	4	6	
FBW (g/birds)	1405.21a	1568.92b	1559.18b	1578.32b	41.937
LWGs (g/bird)	1109.23a	1270.77b	1260.35b	1280.04b	40.072
FC (g/bird/56 days)	4414.74a	4739.97b	4650.69b	4748.95b	61.735
Feed conversion ratio	3.98a	3.73b	3.69b	3.71b	0.0305

Note: [a,b] Values with different letters in the same line are significantly different (P<0.05); SEM = standard error of the treatment means

Feed consumption for 8 weeks significantly (P<0.05) increased with the supplementation of "Herbal-Pro" via drinking water. Feed consumption in duckling groups B, C and D were: 7.37%; 5.35%; and 7.57% significantly (P<0.05) higher than the control duckling group (A).

The FCR value is a comparison between FC and LWG in the same unit and time. The lower the FCR value, the better the feed efficiency.FCR in duckling groups B, C, and D were: 6.28%; 7.29%; and 6.78%, higher (P<.0.05) than control grups. More detail is presented in Table 1.

The results of the study found that the administration of Herbal-Pro through drinking water increased the FBW and LWG of ducklings. This increase was caused by Herbal-Pro containing various phytochemical compounds that have antimicrobial activity [17]. Herbal-Pro also contains probiotic *EM-4* which can increase LWG and feed efficiency [18]. Probiotics in the digestive tract of ducklings can increase nutrient digestibility, so growth and feed efficiency can be optimal.As reported by [19] that probiotics through feed can increase production and feed efficiency in duckling. The increase in body weight gain of ducks is inseparable from the increase in feed consumption, so that the need for nutrients to support growth is met. Feed consumption is increased by the presence of Herbal-Pro in drinking water.Contrary to the research of [1] that addition of herbal leaves in feed can't improve feed intake.

Ducklings that received Herbal-Pro had better feed efficiency compared to those without Herbal-Pro. Carrot leaves in Herbal-Pro contain high β-carotene which can be converted into vitamin A. Vitamin A functions in the differentiation of epithelial cells and maintains the digestive organs, so that nutrient absorption can be increased. Increasing the absorption of nutrients, especially protein, will increase protein synthesis and calcium intake, thereby increasing protein synthesis in meat [20]. Nutrient absorption will be optimal by reducing pathogenic bacteria in the duckling intestine,thus increasing duckling performance and feed efficiency. Giving herbal extracts to broilers significantly increases LWG and feed efficiency [21]. Different results were reported by [21], that the use of more than 4% carrot leaf meal in feed can reduce FBW, while FC have no effect.

Probiotic *Effective Microorganism*-4 used in Herbal-Pro can act as a probiotic in the digestive tract of ducklings, thereby increasing enzymatic activity and absorption of food substances. Reported by [10,19] that the use of probiotics in feed can increase feed digestibility , as well as improve the nutritional quality of feed.

3.2. *Coliform* and *Eschericia coli* bacteria

In Table 2, the effect of giving "Herbal-Pro" via drinking water to ducklings from 2-10 weeks of age on the population of *Coliform* and *E.coli* in the duckling intestine is presented. Total *Coliform* both in the duckling groups C and D decreased significantly (P<0.05) lower than the duckling group A.

Table 2 The effect of giving "Herbal-Pro" via drinking water to male Bali ducklings from 2-10 weeks of age on the population of *Eschericia coli* and *Coliform* bacteria in the duckling intestines

Variable	Herbal-Pro" level in dringking water (cc/100 cc)				Normal
	0	2	4	6	
Total *Coliform* (CFU/g)	$1.38 \times 10^6 \pm 0.75 \times 10^6$a	$0.19 \times 10^6 \pm 0.24 \times 10^6$a	$7.29 \times 10^5 \pm 0.13 \times 10^5$b	$6.95 \times 10^5 \pm 0.36 \times 10^5$b	$4.0 \times 10^6 - 9.4 \times 10^6$
Total *E. coli* (CFU/g)	$1.35 \times 10^4 \pm 0.82 \times 10^4$a	$1.24 \times 10^4 \pm 0.18 \times 10^4$a	$9.71 \times 10^3 \pm 0.53 \times 10^3$ b	$8.52 \times 10^3 \pm 0.31 \times 10^3$b	$10^4 - 10^5$

Note: [a,b] Values with different letters in the same line are significantly different (P<0.05); Cfu = cell forming unit

Administration of"Herbal-Pro" via drinking water to ducklings from 2-10 weeks of age at a level of 4-6% (duckling group C and D), can significantly decrease *E.coli* in the intestine. The total *E.coli* in duckling C and D were: 28.97% and 36.89% significantly (P<0.05) lower than the control.

The results of laboratory tests, it turns out that herbal leaf extract has quite strong *E. coli* antibacterial activity [1], so administration via drinking water can reduce the number of pathogenic bacteria in the duckling intestine. *Eschericia coli* bacteria are commensalistic bacteria in poultry and their presence in excreta is very high, so they can be agents of disease transmission [22]. *Eschericia coli* bacteria cause *Colibasilosis* in poultry [23]. The *E. coli* bacteria consists of a single layer of peptide glycan on its cell wall, does not contain teichoic acid, but contains polysaccharides and is more susceptible to mechanical and chemical damage [22]. Phenolic and terphenoid compounds can inhibit the growth of *S. aureus* and *E.coli* bacteria [1,24]. The ability of inhibition against bacteria is highly dependent on the type and concentration of herbal extracts [9].

The existence of *Effective Microorganism*-4 in Herbal-Pro can act as a probiotic in the digestive tract of ducklings. Lactic acid bacteria in EM-4 can create an acidic environment in the intestine, thus suppressing the growth of pathogenic bacteria [25]. Besides that, competition between pathogenic bacteria and probiotic microorganisms causes pathogenic bacteria to not live in the digestive tract [10]. According to [26], probiotic microbes are one of several methods to reduce Salmonella infection in poultry. Probiotics can eliminate Salmonella colonization, enhance intestinal immunity, and strengthen the intestinal barrier in chicken intestines [27,28]. Chang et al.[29] reported that feed supplementation with multi-strain probiotics can improve gut microbiota and induce different cytokine expression patterns in *Salmonella* infection. According to [30], *Salmonella* infection can reduce growth performance and cause dysbacteriosis.

4. Conclusion

It was concluded that the administration of 2-6% Herbal-Pro via drinking water could increase growth and feed efficiency of ducklings. Conversely, it can suppress *Coliform* and *E. coli* bacteria in the intestines of ducklings.

Compliance with ethical standards

Acknowledgments

The authors thank the Head of the Research Station of the Faculty of Animal Husbandry, for the stable facilities and staff analysts of the Animal Microbiology Laboratory, Faculty of Animal Husbandry, Udayana University, for their assistance in sample analysis.

Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

The experimental animals in this study were approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia.

References

[1] Sudatri NW. Identification of active compounds of Turmeric rhizome (*Curcuma domestic* Val.) and Tamarind fruit (*Tamarindus indica* L.) and their implementation to improve health performance and broiler production. Dissertation, Doctoral Program, Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia. 2021.

[2] Ashok V, Gomashe PAG, Megha PJ, and Neeta AD. Antimicrobial activity of Indian medicinal plants: *Moringa oleifera* and *Saracaindica*. Int. J. Curr. Microbiol. App. Sci. 2014; 3(6): 161-169.

[3] Dalukdeniya DACK, DeSilva KLSR and Rathnayaka RMUSK. Antimicrobial activity of different extracts of leaves bark and roots of *Moringa oleifera* (Lam). Int. J. Curr. Microbiol. App. Sci., 2016; 5(7): 687-691.

[4] Elangovan M, Dhanarajan MS, Rajalakshmi A, Jayachitra A, Mathi P, Bhogireddy N. Analysis of phytochemicals, antibacterial and antioxidant activities of *Moringa oleifera* Lam. leaf extract-an *in vitro* study. Int. J. Drug Dev. & Res., 2014; 6(4): 173-180.

[5] Godinez-Oviedo A, Guemes-Vera N, and Acevedo-Sandoval OA. Nutritional and Phytochemical composition of *Moringa oleifera* Lam and its potential use as nutraceutical plant: A Review. Pakistan Journal of Nutrition, 2016; 15(4): 397-405

[6] Chukwuebuka E. *Moringa oleifera* "The Mother's Best Friend". International Journal of Nutrition and Food Sciences, 2015; 4(6): 624-630

[7] Hammershoj M, Kidmose U, Steenfeldt S. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. Journal of the Science of Food and Agriculture. 2010; 90: 1163-1171.

[8] Enemuor S, Chukwuemeka, Omale, James, Joseph, and Matthew E. Antibacterial activity: a comparison of ripe and unripe fruit extract of *Cissus multistriata* (*vitaceae*) plant. J. Bacteriol. Res., 2011; 3: 83-87.

[9] YunizaA and Yuherman. Content of phytochemical compound and antibacterial activity of *Cinnamon* leaf (*Cinnamomum burmanii*) and Noni fruit and leaf (*Morinda citrifolia* L) mixture extract to replace antibiotics. Pakistan J. Nutr., 2015; 14(8): 492-497. DOI: 10.3923/pjn.2015.492.497

[10] Zurmiati ME, Mahata MH, Abbas and Wizna. The aplication of probiotic on duck. Jurnal Peternakan Indonesia. 2014; 16(2): 134-144

[11] Hanczakowska E, Świątkiewicz M, Natonek-Wiśniewska M, and Okoń K. Effect of glutamine and/or probiotic (*Enterococcus faecium*) feed supplementation on piglet performance, intestines structure, and antibacterial activity. Czech J. Anim. Sci. 2017; 62(8): 313-322

[12] Bidura IGNG. Supplementation of *Saccharomyces spp.* probiotics intof feed laying hens to increase feed digestibility and reduce ammonia gas content in excreta. Majalah Ilmiah Peternakan, 2020; 23(2): 84-90

[13] Chen KL, Kho WL, You SH, Yeh RH, Tang SW, and Hsieh CW. Effects of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* mixed fermented feed on the enhanced growth performance of broilers. Poult. Sci., 2009; 88: 309-315.

[14] Ezema C, and Eze DC. Probiotic Effect of Yeast (*Saccharomyces cerevisiae*) on hen-day egg performance, serum and egg cholesterol levels in laying chicken. Pakistan Journal of Nutrition, 2015; 14(1): 44-46

[15] Tufarelli V, Crovace AM, Rossi G, and Laudadio V. Effect of a dietary probiotic blend on performance, blood characteristics, meat quality and faecal microbial shedding in growing-finishing pigs. South African Journal of Animal Science, 2017; 47(6): 875-882

[16] NRC. Nutrient requirements of poultry. 9th ed. Washington, DC, USA: National Academy Press; 1994.

[17] Bukar A, Uba TI, and Oyeyi. Antimicrobical Profile of Moringa oleifera extracts against some food-borne microorganism. Bayero Journal of Pure and Applied Sciences, 2010; 3 (1): 43-48.

[18] Sutan YFGD, Suryatni NPF and Henuk YL. Suplementation of probiotics in drinking water on the performance of finisher broiler. Jurnal Nukleus Peternakan, 2014; 1(1): 44-49

[19] Prabowo LD, Mahfudz, and Atmomarsono U. Calcium and protein meat mass due to the use of waste product of Carrot powder in ration. Jurnal Sain Peternakan Indonesia, 2019; 14(2): 201-207. DOI: https://doi.org/10.31186/jspi.id.14.2.201-207

[20] Muzaki MR, Mahfudz LD, and Muryani R. The effect of waste carrot product (*Daucus carrota* L) powder in the diet on broiler chickens performance. Jurnal Ilmu Ternak. 2017; 17(1): 14-20

[21] Bidura IGNG, Suyadnya IP, Mahardika IG, Partama IBG, Oka IGL, and Aryani IGAI. 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, 2012; 2(9): 486-492

[22] Roth N, Hofacre C, Zitz U, Greg Mathis F, Moder K, Doupovec B, Berghouse R, and Domig KJ. Prevalence of antibiotic-resistant *E. coli* in broilers challenged with a multi-resistant *E. coli* strain and received ampicillin, an organic acid-based feed additive or a synbiotic preparation. Poultry Science, 2019; 98(6): 2598-2607.

[23] Delannoy S, Schouler C, Souillard R, LénaYousfi, Devendec L, Lucas C, Kempf I. Diversity of *Escherichia coli* strains isolated from day-old broiler chicks, their environment and Colibacillosis lesions in 80 flocks in France. Veterinary Microbiology. 2021; 252 https://doi.org/https://doi.org/10.1016/j.vetmic.2020.108923

[24] Pambayun R, Gardjito M, Sudarmadji S, and Kuswanto KR. The content of phenol and antibacterial nature of various species of Gambier product (*Uncaria gambir* Roxb.). Majalah Farmasi Indonesia, 2017; 18: 141-146.

[25] Purwati E, Syukur S, and Hidayat Z. *Lactobacillus*, isolate from *Biovicophitomega* as a probiotic. Lembaga Ilmu Pengetahuan Indonesia, Jakarta, 2005

[26] Wang Y, Zhou J, Wang G, Cai S, Zeng X and Qiao S. Advances in low-protein diets for swine. Journal of Animal Science and Biotechnology, 2018; 9: 60-60. https://doi.org/ 10.1186/s40104-018-0276-7.

[27] Chen CY, Tsen HY, Lin CL, Yu Band Chen CS. Oral administration of a combination of select lactic acid bacteria strains to reduce the Salmonella invasion and inflammation of broiler chicks. Poultry Science, 2012; 91: 2139-2147. https://doi.org/10.3382/ps.2012-02237

[28] Yu LL, Zhou KK, and Parry J. Antioxidant properties of cold pressed black caraway, carrot, cranberry, and hemp seeds oils. Food Chem., 2005;91: 723-729

[29] Chang CH, Teng PY, Lee TT and Yu B. The effects of the supplementation of multi-strain probiotics on intestinal microbiota, metabolites and inflammation of young SPF chickens challenged with *Salmonella enterica* subsp. enterica. Animal Science Journal, 2019;90: 737-746. https://doi.org/10.1111/asj.13205

Kogut MH and Arsenault RJ. A role for the non-canonical Wnt-beta-catenin and TGF-beta signaling pathways in the induction of tolerance during the establishment of a *Salmonella enterica* serovar enteritidis persistent cecal infection in chickens. Frontiers in Veterinary Science, 2015; 2: 33. https://doi.org/10.3389/fvets.2015.00033