



## Effect of Probiotics and Magnetic Technology in Drinking Water on Production Performance and Egg Quality of Laying Hens

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### ABSTRACT

The ban of antibiotics encourages the use of probiotics as natural feed additives for poultry. However, the effect of probiotics highly depends on the quality of drinking water. The use of Magnetic Technology (MT) could improve water quality, and potentially enhances the efficacy of probiotics. In the present study, the effect of probiotics and MT in drinking water on the production performance and egg quality of laying hens were evaluated using the inclusion of either non-encapsulated probiotic (PRO) and encapsulated probiotic (EPRO) along with drinking water exposure to 2,700 gauss of the magnetic field. A total of 288 57-week-old ISA Brown laying hens were randomly divided into six treatment groups with four replicates of 12 laying hens in each. The treatments consisted of untreated drinking water (control) and drinking water treated with PRO, EPRO, MT, PRO + MT, and EPRO + MT. The results indicated a highly significant improvement in feed conversion ratio, income over feed cost, and egg weight, as well as a significant improvement in egg mass, when EPRO was combined with MT. However, there was no significant effect on the other variables of the production performance and egg quality. It was, therefore, concluded that the use of MT with EPRO improved the egg mass, feed conversion ratio, income over feed cost, and egg weight of the laying hens.

**Keywords:** Drinking water, Encapsulated, Laying hens, Magnetic, Probiotic

### INTRODUCTION

The use of Antibiotic Growth Promoters (AGPs) is a strategy to maintain the production and health of laying hens. However, the use of AGPs has been prohibited due to the chemical residue and antimicrobial resistance issues. The use of probiotics (direct-fed microbial) is currently proposed as an effort to eliminate the use of AGPs. Probiotics are relatively safe because they will adapt and symbiose with the intestinal microflora of laying hens. It will modulate the balance of intestinal microflora, and improve the immune functions, performance production, as well as meat and egg quality (Zhang et al., 2012a; Adhikari et al., 2019; Xiang et al., 2019; Khan et al., 2020).

Although possessing several beneficial effects, probiotics are highly susceptible to environmental changes. Therefore, probiotics need to be prepared by

encapsulation technology to protect the active microbial ingredients against unfavorable environmental conditions (Yao et al., 2020). In the previous studies, it was reported that the use of encapsulated ingredients can lead to the improved production performance, egg quality, and immune response, as well as increased beneficial bacteria, and reduced pathogenic bacteria in the small intestine (Lee et al., 2020; Liu et al., 2020; Natsir et al., 2010). The application of encapsulation technology also potentially increases the durability of probiotics, when they are administered through drinking water.

Many factors may affect the efficacy of probiotics usage, such as the quality of drinking water. The poorer water quality, the lower effects of probiotics. Magnetic Technology (MT) uses a specific level of magnet to increase the quality of drinking water (Ebrahim and Azab, 2017). The application of MT in drinking water could improve the production performance, egg quality, and

reproduction hormones of laying hens (El Sabry et al., 2018; Mitre, 2018; El Sabry et al., 2020).

To date, the exploration of the association between probiotics and MT application in laying hens is still limited. Therefore, the present experiment was designed to evaluate the effect of the supplementation of either non-encapsulated probiotic (PRO) or encapsulated probiotic (EPRO) along with MT-treated drinking water on the production performance and egg quality of laying hens.

## MATERIALS AND METHODS

### Materials

A probiotic that contains *Lactobacillus sp.*, *Bacillus sp.*, *Saccharomyces sp.*, and *Pseudomonas sp* with a total CFU of  $1.8 \times 10^7$  cfu/ml was used in the present research. Encapsulating process was conducted in the Animal Feed Industry Laboratory, Faculty of Animal Science, Universitas Brawijaya, Indonesia. The encapsulation of probiotics consisted of two coating systems. The first coating system was chitosan (Amiri et al., 2021), while the second one was whey protein and Arabic gum (Heidebach et al., 2012; Zhang et al., 2015; Natsir et al., 2017). A magnet bar with the size of 20 cm (width) × 10 cm (length) × 5 cm (height) was used as a source of the magnetic field. The composition of nutrient content of AGPs-free feed used in the present research is presented in Table 1. A total of 288 57-weeks-old ISA Brown laying hens (PT. Japfa Comfeed Indonesia, Tbk., Indonesia) were used in the current study to know the effect after a peak production of laying hens. Each laying hen was placed in a battery cage of 40 cm (width) × 35 cm (length) × 30 cm (height).

### Ethical approval

All animal housing and experiments conducted in this research were approved by the animal care and use committee of Universitas Brawijaya, Indonesia (no. 066-KEP-UB-2020) which was signed by the head of ethics (Aulanni'am, Prof. PhD. drh. DES).

### Methods

A completely randomized design was used in the present study. Laying hens were randomly divided into six treatment groups with four replicates of 12 laying hens in each. The treatments consisted of untreated drinking water (control) and drinking water treated with PRO, EPRO, MT, PRO + MT, and EPRO + MT. According to the previous study, the optimum level of probiotic

supplementation (*Lactobacillus sp* with  $1.4 \times 10^{10}$  cfu/ml) in laying hens was 0.6% (Pradikta et al., 2018). For that reason, both probiotics (PRO and EPRO) were supplied at the level of 0.6% in drinking water. The application of MT was done by exposing the drinking water to 2,700 gauss magnetic fields. The treatments were delivered through the nipple drinking system for six weeks (42 days). The drinking water was provided *ad libitum*, while the feed was supplied once daily by the restricted feeding method with the amount of 120 g/hen/day (Afandi et al., 2020).

**Table 1.** Composition of feed ingredients and analyzed nutrient contents of the feed.

Ingredients	Composition (%)
Corn	52.7
Rice brain	13.95
Soybean meal	24.5
Meat bone meal	4.7
Grit	3.1
Lysine	0.1
Methionine	0.15
Premix <sup>a</sup>	0.2
Salt	0.2
Monocalcium Phosphate	0.4
Total	100
Nutrient content	Value (%) <sup>b</sup>
Dry Matter	90.28
Metabolism Energy (kcal/Kg)	2,959
Crude Protein	19.44
Crude Fiber	2.95
Crude Fat	4.93
Ash	7.99

<sup>a</sup>Premix from PT. MITRAVET (Composition/1kg: vitamin A: 2.000.000 IU, vitamin D3: 400.000 IU, vitamin E: 3.000 mg, vitamin K: 400 mg, vitamin B12: 4 mcg, thiamin HCl/B1: 400 mg, riboflavin HCl/B2: 1.200 mg, pyridoxin HCl/B6: 800 mg, Ca-d-pantothenate: 2.160 mg, niacinamide: 8.000 mg, folic acid: 200 mg, biotin: 4 mg, L-Carnitine : 10.000 mg, copper sulphate: 4.000 mg, cobalt sulphate: 300 mg, ferro sulphate: 10.000 mg, Mn oxide: 20.000 mg, sodium selenite: 150 mg, carrier ad: 1.000 mg). <sup>b</sup>Nutrient contents expressed as % unless otherwise stated.

### Production performance

The production performance traits observed in the current study was Feed Intake (FI), Hen Day Production (HDP), Egg Mass (EM), Feed Conversion Ratio (FCR), and Income Over Feed Cost (IOFC). Feed Intake was determined once a week while HDP was registered daily. The egg mass was calculated by multiplying HDP with the total EW (Andri et al., 2016). The FCR was calculated by FI divided by EM (Sjofjan et al., 2020). Income over feed cost was calculated by subtracting the revenue from egg selling with total feed cost (Sjofjan et al., 2020).

### Egg quality

The observed egg quality variables in the present study included Egg Weight (EW), Shape Index (SI), Shell

Weight (SW), Shell Thickness (ST), Haugh Unit (HU), Albumen Height (AH), Yolk Weight (YW), Yolk Index (YI), and Yolk Color (YC). Egg weight was obtained by weighting the egg with a digital balance. Shape index was calculated by egg width divided by egg length and then multiplied by 100 (Alasahan and Copur, 2016). Shell weight was obtained by weighting the shell with a digital balance. Shell thickness was determined by using a micrometer. Haugh unit was calculated using a formula:  $100 \times \log (AH - 1.7 \times EW^{0.37} + 7.57)$  (Andri et al., 2018). Albumen height was determined by using a tripod micrometer. Yolk weight was obtained by weighting the yolk with a digital balance. Yolk index was calculated by yolk height divided by yolk diameter and then multiplied by 100 (Liu et al., 2021). Yolk color was determined by using DSM yolk color fan with the color score ranging from one to 15.

### Statistical analysis

The data were statistically assessed by the analysis of variance (ANOVA) using the SPSS software (version 26, IBM, USA). The difference among the treatments mean

was analyzed by using Duncan's multiple range test (Duncan, 1955).

## RESULTS

The effect of probiotics and MT application on the production performance of laying hens can be seen in Table 2. The use of probiotics and MT had no significant effect ( $p > 0.05$ ) on FI. The hens that received EPRO + MT had a numerically higher HDP than the control group. The use of EPRO along with MT showed a substantial ( $p < 0.05$ ) improvement on EM, and a highly major ( $p < 0.01$ ) enhancement on FCR and IOFC as compared to the control group.

Table 3 shows the effect of probiotic and MT on the egg quality of laying hens. The hens that received MT + EPRO treatment had a higher ( $p < 0.01$ ) EW as compared to those receiving the control treatment. On the other hand, there was no significant effect ( $p > 0.05$ ) of probiotics inclusion along with MT application on the other traits of egg quality (SI, SW, ST, HU, AH, YW, YI, and YC).

**Table 2.** Effect of drinking water treated with the supplementation of either non-encapsulated probiotic or encapsulated probiotic along with magnetic technology on production performance of laying hens

Treatment	FI	HDP	EM*	FCR**	IOFC**
Control	115.51	86.11	52.63 <sup>a</sup>	2.20 <sup>b</sup>	364.58 <sup>a</sup>
PRO	111.64	90.83	57.11 <sup>ab</sup>	1.96 <sup>ab</sup>	462.96 <sup>bc</sup>
EPRO	112.27	90.24	55.88 <sup>ab</sup>	2.01 <sup>ab</sup>	482.08 <sup>c</sup>
MT	111.62	87.60	53.97 <sup>ab</sup>	2.07 <sup>ab</sup>	411.46 <sup>abc</sup>
PRO + MT	111.97	86.30	53.06 <sup>ab</sup>	2.12 <sup>b</sup>	384.22 <sup>ab</sup>
EPRO + MT	111.96	92.87	58.77 <sup>b</sup>	1.91 <sup>a</sup>	488.71 <sup>c</sup>
SEM	0.60	0.92	0.64	0.03	11.55
p-value	0.431	0.191	< 0.05	< 0.01	< 0.01

FI: Feed Intake (g/hen/day), HDP: Hen Day Production (%), EM: Egg Mass (g/hen/day), FCR: Feed Conversion Ratio, IOFC: Income Over Feed Cost (IDR/hen/day), PRO: Non-encapsulated Probiotic, EPRO: Encapsulated Probiotic, MT: Magnetic Technology, SEM: Standard Error of Means. <sup>a-c</sup> Different letter indicates significant differences between the means. \*Superscript shows a significant difference ( $p < 0.05$ ). \*\*Superscript shows a highly significant difference ( $p < 0.01$ ).

**Table 3.** Effect of drinking water treated with the supplementation of either non-encapsulated probiotic or encapsulated probiotic along with magnetic technology on egg quality of laying hens

Treatments	EW**	SI	SW	ST	HU	AH	YW	YI	YC
Control	61.11 <sup>a</sup>	75.96	7.58	0.57	72.49	5.72	16.50	40.96	8.08
PRO	62.86 <sup>bc</sup>	78.36	7.71	0.55	77.73	6.37	17.04	42.39	7.67
EPRO	61.97 <sup>abc</sup>	78.13	7.88	0.58	83.41	7.37	17.17	43.69	7.63
MT	61.59 <sup>ab</sup>	75.66	7.29	0.56	76.46	6.34	16.83	42.93	7.63
PRO + MT	61.51 <sup>ab</sup>	76.85	7.38	0.56	79.53	6.65	16.75	42.31	7.63
EPRO + MT	63.29 <sup>c</sup>	77.26	7.58	0.55	82.15	7.17	17.29	41.95	7.46
SEM	0.21	0.36	0.16	0.01	1.20	0.18	0.11	0.30	0.08
p-value	< 0.01	0.143	0.114	0.664	0.076	0.073	0.389	0.141	0.307

EW: Egg Weight (g), SI: Shape Index, SW: Shell Weight (g), ST: Shell Thickness (mm), HU: Haugh Unit, Albumen Height (mm), YW: Yolk Weight (g), YI: Yolk Index, YC: Yolk Color, PRO: Non-encapsulated Probiotic, EPRO: Encapsulated Probiotic, MT: Magnetic Technology, SEM: Standard Error of Means. <sup>a-c</sup> Different letter indicates significant differences between the means. \*\*Superscript showed a highly significant difference ( $p < 0.01$ ).

## DISCUSSION

### **The effect of probiotics inclusion and magnetic technology application in drinking water on production performance of laying hens**

The application of MT in drinking water showed a numerical reduction in FI. The use of MT improved the quality of drinking water, which may improve the gastrointestinal system, and support the absorption of nutrients and energy. Fulfilling the energy requirements will decrease the feed intake. In line with this finding, the use of magnetic water treatment also improved the growth performance, feed efficiency, productivity, and health of poultry (El-Katcha et al., 2017; El-Sabroun and El-Hanoun, 2019).

The use of EPRO + MT numerically increased HDP and significantly improved EM of laying hens. This result indicated that the encapsulation technology successfully enhances the efficacy of probiotic administration. In the present study, the probiotic was encapsulated using chitosan, whey protein, and Arabic gum. It was reported that the use of chitosan could protect the probiotic during transporting inside the gastrointestinal system (Călinoiu et al., 2019). The use of whey protein as an encapsulant also increased egg production of laying hens (Pineda-Quiroga et al., 2017). In another study, liquid whey inclusion in drinking water also improved hens' performance by modifying gut pH and microflora (Bouassi et al., 2021). Moreover, the positive effect of EPRO is also supported with MT application in drinking water. Magnetic technology could improve water quality (Ebrahim and Azab, 2017), which then could provide a favorable environment for probiotic administration. It was, therefore, speculated that the encapsulation technology along with MT application in drinking water could efficiently deliver the probiotics into the intestinal environment. After that, the probiotics could improve the balance of intestinal microflora, preventing the growth of pathogenic microbes, and supporting the digestive system (De Vrese and Schrezenmeir, 2008), which ultimately could improve HDP and EM of laying hens.

The hens in EPRO + MT group significantly had better FCR and IOFC as compared to those in the control group. Feed conversion ratio is the result of feed intake divided by egg mass of laying hens. The hens that received EPRO + MT treatment had the best result on FCR (1.91). This result was mainly driven by the higher EM in EPRO + MT treatment. The results in the current study were in harmony with the findings of El-Katcha et al. (2017) who

reported that using the magnetic water treatment improved the feed efficiency. Hosseini and Meimandipour (2018) also reported that the use of chitosan as an encapsulant could improve FCR as compared to the control treatment. A better FCR also indicated that the use of feed was efficient to produce an egg. This result was then followed by a better IOFC. Income over feed cost is an income obtained based on the revenue from egg production of layer hens compared to the feed cost. The hens in EPRO + MT group showed the highest result on IOFC (488.71 IDR/hen/day) as compared to other treatments.

### **The effect of probiotics inclusion and magnetic technology application in drinking water on egg quality of laying hens**

The combination of EPRO and MT showed the best results on EW. This result was similar to the previous study which found that probiotic supplementation increased EW compared to the control group (Mazanko et al., 2018; Alaqil et al., 2020). These results indicated that using probiotics in drinking water with encapsulation and MT was more effective to improve the EW of laying hens. The use of MT tended to decrease SI, compared to the treatments without MT. The shape index was classified into three categories namely sharp (< 72), standard (72-76), and round (> 76) (Duman et al., 2016). The result of using probiotics and MT administration showed no significant effect on SW and ST. However, MT application generally tended to decrease SW and ST. Each eggshell contained up to three grams of calcium (Roberts, 2004). The magnetic field inhibited calcium carbonate formation in water (Jiang et al., 2015), which consequently reduced the calcium concentration in water (Gabrielli et al., 2001), and ultimately decreased SW and ST.

The treatments using probiotics had numerically better results on HU and AH than the treatment without them. Probiotics increased the population of lactic acid bacteria, and optimized nutrient absorption (Peralta-Sánchez et al., 2019). This circumstance stimulated amino acid production that balanced ovomucin and lecithin for improving egg quality, mainly HU (Sjofjan et al., 2020).

The treatments had no significant effect on YW, YI, and YC. These results were in agreement with Baghban-Kanani et al. (2019) who found that probiotics did not affect EW and YW. In contrast, Zhang et al. (2012b) found that YW in probiotic-based treatments was significantly decreased, compared with the control group. Mazanko et al. (2018) stated that using probiotic supplements increased YI in laying hens. In another study,

Zhang et al. (2012b) reported that probiotics had no significant effect on YC.

## CONCLUSION

It could be concluded that the application of encapsulation technology on probiotics and the magnetic technology on drinking water had the best result on the improvement of egg mass, feed conversion ratio, income over feed cost, and egg weight of laying hens.

## DECLARATIONS

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### Competing interests

Authors declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence the present work, there is no professional or other.

### Authors' contributions

Professor MHN did the methodology, reviewed, and edited the manuscript. Dr. OS analyzed the data. Dr. AM analyzed magnetized drinking water. FM did the experiments, collected the samples, and wrote the original draft. All authors did the validation, investigation, and approved the final manuscript.

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