Journal of Animal and Feed Sciences, 32, 1, 2023, 59–67 https://doi.org/ 10.22358/jafs/156018/2022
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The potency of *Lactobacillus acidophillus* and *L. lactis* probiotics and *Guazuma ulmifolia* Lam. extract as feed additives with different application times to improve nutrient intake and feed efficiency in *Coturnix coturnix japonica* females

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KEY WORDS: *Coturnix coturnix japonica*, *Guazuma ulmifolia* Lam., health, probiotic

Received: 3 August 2022
Revised: 18 October 2022
Accepted: 24 October 2022

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ABSTRACT. Research on the combination of *Lactobacillus acidophillus* and *L. lactis* probiotics and *Guazuma ulmifolia* Lam. to find an alternative to antibiotic growth promoters has never been conducted, thus the present study aimed to evaluate their effect on nutrient intake and feed efficiency in females of *Coturnix coturnix japonica*. The experiment was conducted for 35 days, including the adaptation period. Supplementation was administered for 7 and 14 days, and the parameters were analysed accordingly. The experiment was set up as a completely randomized factorial design (2 × 3), where the first factor was the period of administration (a0 – one week and a1 – two weeks), and the second factor was feed additive (b0 – without feed additive, b1 – probiotics, b2 – *G. ulmifolia* Lam. extract). The results showed that there was a significant difference ($P < 0.05$) between the treatments in terms of nutrient intake (dry matter, organic matter, ash, crude protein, ether extract, crude fibre, nitrogen free extract, metabolisable energy) and feed efficiency ($P < 0.05$). Based on the results of the study, it can be concluded that the application of *L. acidophillus* and *L. lactis* probiotics and *G. ulmifolia* Lam. extract has the potential to improve nutrient intake and feed efficiency in females of *C. coturnix japonica*, thereby improving safety and health-promoting properties of livestock products for humans and reducing animal production costs.

Introduction

Since 2006, the European Union and several other countries have banned the use of antibiotic growth promoters (AGP) in livestock (Wu et al., 2021). This is the initial step in addressing the problem of antibiotic resistance due to their long-term use. To balance and maintain a healthy digestive tract condition and increase animal growth without the use of antibiotics, an effective, safe and more economical alternatives to antibiotics are needed (Cogliani et al., 2011; Wu et al., 2021). Probiotics are viable and non-pathogenic organisms that improve the host's health by balancing the diversity of intestinal microbes, improving the intestinal barrier function, quality of external and internal eggs, and these microbials particularly include *Lactobacilli* and *Bifidobacterium* (van Belkum et al., 2007; Figueroa et al., 2011; Lokapirnasari et al., 2019; Jayani et al., 2020). Probiotics as feed additives have been reported to improve nutrient digestibility, growth performance, ceacal microflora balance, plasma immunoglobulins, and chicken immunity (Tang et al., 2017; Alaqil et al., 2020). Probiotic species include *Lactobacillus bulgaricus*, *L. plantarum*, *Pediococcus pentosaceus*, *Bifidobacterim bifidum*, *L. acidophilus, L. casei* or *L. lactis* (Khaksefidi and Rahimi, 2005; Yulianto et al., 2021; Lokapirnasari et al., 2022; Agustono et al., 2022). *L. acidophilus* is a non-spore species of lactic acid bacteria and is used as a probiotic to improve the qualitative performance of broilers (Salarmoini and Fooladi, 2011).

In addition to probiotics, phytobiotics are other feed additives that can be used to improve growth performance of livestock. *Guazuma ulmifolia* Lam. is among the phytobiotics that show high potential. Scientific classification of *G. ulmifolia* Lam. derived from the National Inventory of the Natural Heritage Database is as follows: Kingdom: Plantae, Class: Equisetopsida, Order: Malvales, Family: Malvaceae, Genus: *Guazuma* Mill., Species: *Guazuma ulmifolia* Lamarck. (INPN-FR, 2019). The leaves and fruits of *G. ulmifolia* can be used as a source of proteins in animal feeds (Pereira et al., 2019). The main secondary metabolites of *G. ulmifolia* Lam. include bioactive compounds, especially proanthocyanidins, flavonoids and phenolic acids, and among the identified antioxidant compounds are chlorogenic acid, caffeic acid, rutin, quercitrin, quercetin and luteolin. Previous phytochemical studies on secondary metabolites such as proanthocyanidins showed their antimicrobial and antioxidant effects; other activities include antidiarrheal, antiprotozoal and cardioprotective properties (Morais et al., 2017; dos Santos et al., 2018; Assis et al., 2019; Pereira et al., 2019). Phytochemical analyses of *G. ulmifolia* Lam. leaf tissue identified phenolic acids (caffeic acid and chlorogenic acid) and flavonoids (quercetin 3-O-rhamnosyl-glucoside, luteolin and quercetin 3-O-rhamnosyl) as the main compounds. Moreover, chlorogenic acid (25.3 mg/g extract), quercetin (24.6 mg/g extract) (Junior et al., 2016; Morais et al., 2017), octacosanol, taraxeroloac, friedelin-3-áoac, â-sitosterol, Friedelinol-3-acetate (Kumar and Gurunani, 2019), procyanidin dimer B and glycosylated derivatives of catechins, kaempferol and luteolin were also detected (de Souza et al., 2018; dos Santos et al., 2018). Tannin content in *G. ulmifolia* Lam. was reported at the level of 1.2 g/kg (Manríquez-Mendoza et al., 2011), and ent-catechin, epicatechin, ent-gallocatechin, epigallocatechin, epiafzelechin-(4β→8)-epicatechin, epicatechin-(4β→8)-catechin (procyanidin B1), epicatechin-(4β→8)-epicatechin (procyanidin B2), epicatechin-(4β→8)-epigallocatechin, and 4'-O-methyl-epiafzelechin have also been identified (Lopes et al., 2009).

Feed efficiency is influenced by its intake and production rate. Low feed intake but high production equals to high feed efficiency, and thus reduced livestock production costs. Nutrients in the feed are used for maintenance and animal production. Feed efficiency can be calculated by comparing the egg production with the amount of feed consumed at a certain time. Research to find an alternative to AGP using a combination of probiotics and *G. ulmifolia* Lam. has never been carried out, thus this study aimed to evaluate their effect as feed additives on nutrient intake (dry matter, organic matter, ash, crude protein, ether extract, crude fibre, metabolisable energy) and feed efficiency in *Coturnix coturnix japonica*.

Material and methods

Ethanol extract of *G. ulmifolia* Lam. leaves

Fresh leaves of *G. ulmifolia* Lam. were collected in Surabaya city. The leaves were dried in an oven at 65 °C and ground using a hammer mill. Extracts were obtained by adding *G. ulmifolia* Lam. leaf powder (1000 g) to 70% alcohol (7000 ml), and 24-h reaction was performed twice to completely remove the compounds. The obtained extracts were filtered and evaporated at 40 °C (modified from Adedapo et al., 2009; Assis et al., 2019).

Study period and location

The study was conducted for 35 days, including the adaptation period. Days 1–14 days were the adaptation period in the cage, days 15–21 were the adaptation period of feeding the additives, and days 22–35 were the feed additive treatment. The treatment was administered for 7 and 14 days. The data collection was performed after 7 and 14 days of the trial. *C. coturnix japonica* were reared in colony cages on the farm in Kediri Regency. Proximate analysis of the feed and variables was conducted at the Laboratory of Animal Nutrition, Division of Animal Husbandry, Department of Veterinary Medicine Science, Faculty of Veterinary Medicine, Universitas Airlangga.

Experimental design

The study material consisted of *L. acidophillus* and *L. lactis* probiotics from the W.P. Lokapirnasari collection (from the Division of Animal Husbandry, Universitas Airlangga, Surabaya, Indonesia) and *G. ulmifolia* Lam. extract in drinking water. The commercial feed contained ash (11.4923%), dry matter (90.8835%), and crude fibre (4.8105%), crude lipid (6.7717%) and crude protein (23.2626%). This study used a factorial complete randomized design, and 72 females of *C. coturnix japonica* (52 days old) divided into 2 × 3 treatments in six replications, with each replication consisting of two *C. coturnix japonica* females. The treatments in this study were as follows: a0b0 –week-1, without feed additive; a0b1 – week-1, 4 ml probiotic/l drinking water; a0b2 – week-1, 4 ml *G. ulmifolia* Lam. extract /l drinking water; a1b0 – week-2, without feed additive; a1b1 – week-2, 4 ml probiotic /l drinking water; a1b2 – week-2, 4 ml *G. ulmifolia* Lam. extract /l drinking water. The method of treatment administration was as follows: probiotics and *G. ulmifolia* Lam. extract was mixed with drinking water, according to the dose and treatment period (7 and 14 days). Drinking water and feed were provided *ad libitum*. Probiotics and *G. ulmifolia* Lam. extract were added to drinking water and stirred evenly. Preparation of probiotics and *G. ulmifolia* Lam. extract was carried out every morning. The tested variables included dry matter intake, organic matter intake, ash intake, crude protein intake, crude fat intake, crude fibre intake, nitrogen-free extract (NFE) intake, metabolisable energy (ME) intake, and feed efficiency. All variables were calculated using the following formula (Yulianto et al., 2020; Suwignyo et al., 2021; Hadi et al., 2021):

Dry matter intake (g) = feed intake (g) × feed dry matter (%),

Organic matter intake (g) = feed intake (g) × feed organic matter (%) × feed dry matter (%),

Ash intake (g) = feed intake (g) × feed ash (%) × feed dry matter (%),

Crude protein intake (g) = feed intake (g) × feed crude protein (%) × feed dry matter (%),

Crude fat intake (g) = feed intake (g) × feed crude fat (%) × feed dry matter (%),

Crude fibre intake (g) = feed intake (g) × feed crude fibre (%) × feed dry matter (%),

NFE intake (g) = feed intake (g) × feed NFE (%) × feed dry matter (%),

ME intake (g) = feed intake (g) × feed ME (kcal/kg) × feed dry matter (%),

Feed efficiency (%) = (egg production / feed intake) × 100.

Statistical analysis

The data collected during this study were statistically analysed in a fully randomized 2 × 3 factorial design. All data were tested for normality of distribution and homogeneity of variance. A 2 × 3 factorial design was used to analyse data of nutrient intake and feed efficiency in a response to 2 levels of administration time periods (a0 – one week and a1 – two weeks) and 3 levels of feed additives (b0 – without feed additives, b1 – probiotics, b2 – *G. ulmifolia* Lam. extract). Differences between means were detected using a 2-way analysis of variance. The differences between means were determined using Duncan's test ($P < 0.05$).

Results

Dry matter intake

The result showed that there was a significant difference ($P < 0.05$) in the 1st and 2nd week of the administration period, as well as a significant difference ($P < 0.05$) regarding the type of feed additive; however, no interaction was observed between the time of administration and the type of feed additive. The average dry matter intake is listed in Table 1.

Table 1. Dry matter intake (g/quail/day) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	$21.5950^{c} \pm 0.29$	$21.5350^{c} \pm 0.39$	$21.5650^{b} \pm 0.51$
b1	$19.2867^{a} \pm 0.30$	$19.4600^{ab} \pm 0.58$	$19.3733^{a} \pm 0.52$
b2	$19.9083^{b} \pm 0.38$	$19.3567^{a} \pm 0.37$	$19.6325^{b} \pm 0.52$
Average	20.263 ± 1.0494	20.117 ± 1.1170	

data are presented as mean value ± SEM (standard error of the mean); abc – means with different letters in the same column and row are significantly different ($P < 0.05$) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Ash intake

The results of statistical analysis showed that there was no significant difference ($P > 0.05$) between the treatments in ash intake of *C. coturnix japonica* females when feed additives were administered for one and two weeks; however, a significant difference ($P < 0.05$) was recorded for the types of feed additives. There was no interaction between the duration of administration and the type of feed additive. The average ash intake is provided in Table 2.

Table 2. Ash intake (g/quail/day) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	2.4833[c] ± 0.03	2.4750[c] ± 0.05	2.4792[b] ± 0.06
b1	2.2167[a] ± 0.04	2.2367[ab] ± 0.07	2.2267[a] ± 0.06
b2	2.2883[b] ± 0.04	2.2250[a] ± 0.04	2.2567[a] ± 0.07
Average	2.329 ± 0.1209	2.312 ± 0.1289	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (P < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Organic matter intake

The results of statistical analysis showed that there was no significant difference (P > 0.05) between the treatments with respect to organic matter intake of *C. coturnix japonica* females when feed additives were administered for one or two weeks; however, there was a significant difference found (P < 0.05) for the type of feed additives. There was no interaction between the time of administration and the type of feed additive. The average organic matter intake is listed in Table 3.

Table 3. Organic matter intake (g/quail/day) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	17.1433[c] ± 0.23	17.0967[c]± 0.31	17.1200[b] ± 0.2597
b1	15.3117[a] ± 0.23	15.45[ab] ± 0.46	15.3808[a] ± 0.3566
b2	15.8050[b] ± 0.30	15.3667[a]± 0.29	15.5858[a] ± 0.3645
Average	16.0867 ± 0.8326	15.9711 ± 0.887	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (P < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Crude protein intake

The results of statistical analysis showed that there was no significant difference (P > 0.05) between the treatments in crude protein intake of female *C. coturnix japonica* during the supply of feed additives during week 1 and 2. However, a significant difference (P < 0.05) was recorded for the types of feed additives. There was no interaction between the duration of administration and the type of feed additive. The average crude protein intake is listed in Table 4.

Table 4. Crude protein intake (g/quail/day) of *Coturnix coturnix japonica* by during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	5.0233[c] ± 0.07	5.0100[c] ± 0.08	5.0167[b] ± 0.0742
b1	4.4867[a] ± 0.07	4.5267[ab] ± 0.13	4.5067[a] ± 0.1050
b2	4.6317[b] ± 0.09	4.5033[a] ± 0.09	4.5675[a] ± 0.1068
Average	4.7139 ± 0.2439	4.6800 ± 0.2598	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (P < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Crude fat intake

The results of statistical analysis showed that there was no significant difference (P > 0.05) between the treatments in crude fat intake of *C. coturnix japonica* females during the supply of feed additives in the 1st and 2nd week; however, a significant difference was observed (P < 0.05) in the types of feed additives. There was no interaction between administration time and the type of feed additive. The average crude fat intake is listed in Table 5.

Table 5. Crude fat intake (g/quail/day) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	1.4633[c]± 0.02	1.4583[c] ± 0.02	1.4608[b] ± 0.0215
b1	1.3050[a]± 0.02	1.3183[ab] ± 0.04	1.3117[a] ± 0.0304
b2	1.3500[b]± 0.03	1.3117[a] ± 0.03	1.3308[a] ± 0.0323
Average	1.3728 ± 0.0717	1.3628 ± 0.0752	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (P < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Crude fibre intake

The results of statistical analysis showed that there was no significant difference (P > 0.05) between the treatments with respect to crude fibre intake in *C. coturnix japonica* females during the supply of feed additives for one or two weeks; however, there was a significant difference (P < 0.05) in the types of feed additives. There was no interaction between the duration of administration and the type of feed additive. The average crude fibre intake is provided in Table 6.

Table 6. Crude fibre intake (g/quail/day) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	1.0400[c] ± 0.01	1.0367[c] ± 0.01	1.0383[b] ± 0.0158
b1	0.9283[a] ± 0.02	0.9367[a] ± 0.03	0.9325[a] ± 0.0217
b2	0.9600[b] ± 0.02	0.9317[a] ± 0.02	0.9458[a] ± 0.0227
Average	0.9761 ± 0.0507	0.9683 ± 0.0537	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (*P* < 0.05) between treatments (a0 – week-1 a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

NFE intake

The results of statistical analysis showed that there was no significant difference ($P > 0.05$) between the treatments regarding NFE intake in *C. coturnix japonica* females in the supply of feed additives for one or two weeks. However, there was a significant difference ($P < 0.05$) in the types of feed additives. There was no interaction observed between the time of administration and the type of feed additive. The average NFE intake is given in Table 7.

Table 7. Nitrogen-free extract intake (g/quail/day) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	9.6200[c] ± 0.1279	9.5967[c] ± 0.1702	9.6083[b] ± 0.1440
b1	8.5917[a] ± 0.1337	8.6683[ab] ± 0.2570	8.6300[a] ± 0.1994
b2	8.8667[b] ± 0.1687	8.6233[a] ± 0.1630	8.7450[a] ± 0.2029
Average	9.0261 ± 0.4674	8.9628 ± 0.4988	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (*P* < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

ME intake

The results of statistical analysis showed that there was no significant difference ($P > 0.05$) between the treatments with respect to ME intake in *C. coturnix japonica* females in the supply of feed additives during week 1 and 2; however, a significant difference ($P < 0.05$) in the types of feed additives was recorded. There was no interaction between the time of administration and the type of feed additive. The average ME intake is listed in Table 8.

Table 8. Metabolisable energy intake (cal/g) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	69.2133[c] ± 0.9271	69.0250 ± 1.2379	69.1192[b] ± 1.0473
b1	61.8117[a] ± 0.9621	62.3667[ab] ± 1.8560	62.0892[a] ± 1.4389
b2	63.8067[b] ±1.2251	62.0367[a] ± 1.1721	62.9217[a] ± 1.4700
Average	64.9439 ± 3.3643	64.4761 ± 3.5835	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (*P* < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Feed efficiency

The results of statistical analysis showed that there was no significant difference ($P > 0.05$) between the treatments regarding feed efficiency in *C. coturnix japonica* females in the supply of feed additives for one or two weeks; however, there was a significant difference ($P < 0.05$) in the types of feed additives. No interaction was found between the duration of administration and the type of feed additive. The average feed efficiency is summarised in Table 9.

Table 9. Feed efficiency (%) of *Coturnix coturnix japonica* during administration of probiotics and *Guazuma ulmifolia* Lam. extract in individual periods

Feed additive	Periods		
	a0	a1	Average
b0	32.9283[a] ± 1.7290	34.0367[a] ± 0.4643	33.4825[a] ± 1.3386
b1	40.2717[c] ± 1.8825	41.3167[c] ± 2.5345	40.7942[c] ± 2.1974
b2	37.6650[b] ± 0.9050	40.0300[c] ± 0.9046	38.8475[b] ± 1.5065
Average	36.9550 ± 3.4562	38.4611 ± 3.5845	

data are presented as mean values ± SEM (standard error of the mean); [abc] – means with different letters in the same column and row are significantly different (*P* < 0.05) between treatments (a0 – week-1, a1 – week-2, b0 – without feed additive, b1 – 4 ml probiotic /l drinking water, b2 – 4 ml *G. ulmifolia* Lam. extract /l drinking water)

Discussion

Dry matter intake. The average dry matter intake of *C. coturnix japonica* during probiotics and *G. ulmifolia* Lam. extract supplementation was 19.6325–21.5650 (g/quail/day) and in individual time periods it ranged from 20.117 to 20.263 (g/quail/day) (listed in Table 1). Low dry matter consumption was observed for *G. ulmifolia* Lam. extract administration for two weeks and probiotics for one week, which did not differ significantly from probiotic administration for two weeks. High dry matter intake was found in controls without probiotics or without *G. ulmifolia* Lam. extract supplementation. The results of this study are consistent with other studies in terms that feed intake in chicks fed a diet supplemented with *L. acidophilus* probiotic was significantly lower than in control; moreover, the number of *L. acidophilus* bacteria in the ileum and colon of treated birds was higher than in control, while the abundance of coliform bacteria was lower, as previously described (Salarmoini and Fooladi, 2011). *L. acidophilus* is widely used as a probiotic for humans and animals (Li et al., 2018). These probiotic bacteria can inhibit the invasion of pathogens and is known to modulate the immune response *in vitro* and *in vivo*. *L. acidophilus* is known to produce bacteriocins and acids, thus it can lower intestinal pH. The addition of *L. acidophilus* to poultry feed helps prevent the proliferation of pathogenic bacteria and balance the gut flora through competitive exclusion and antagonism (Lin et al., 2007; Cogliani et al., 2011). In poultry, the addition of probiotics to feed is known to modulate the balance of the intestinal microbiota, increase feed digestibility and nutrient absorption, enhance the immune response, and reduce stress (Patterson and Burkholder, 2003; Revolledo et al., 2006; Prado-Rebolledo et al., 2017; Li et al., 2018).

Ash intake. The average ash intake of *C. coturnix japonica* during the supplementation of probiotics and *G. ulmifolia* Lam. extract was 2.2567–2.4792 (g/quail/day) and in individual periods, it was in the range of 2.312–2.329 (g/quail/day) (listed in Table 2). Low ash consumption was recorded during the administration of *G. ulmifolia Lam*. extract for two weeks and probiotics for one week, and these results were not significantly different from probiotic supplementation for two weeks. High ash consumption was shown in the control treatment without probiotics or *G. ulmifolia* Lam. extract. The administration of probiotics and *G. ulmifolia* Lam. extract resulted in a lower ash intake than in control, which was related to healthy intestinal conditions and better digestion, so that the birds consumed less than the control animals. This was in line with other studies showing that *L. acidophilus* supplementation could improve growth performance and intestinal health (Cogliani et al., 2011). Probiotic supplementation has been shown to balance the microbiota in the gastrointestinal tract, which is essential for the development of the intestine and leads to a more efficient feed intake (Hamasalim, 2016).

Organic matter intake. The average organic matter intake of *C. coturnix japonica* during the supplementation of probiotics and *G. ulmifolia Lam*. extract was 15.5858–17.1200 (g/quail/day) and in individual periods, it ranged from 15.9711 to 16.0867 (g/quail/day) (listed in Table 3). The low intake of organic matter was recorded during the administration of *G. ulmifolia* Lam. extract for two weeks and probiotics for one week. The results were not significantly different from probiotic administration for 2 weeks. High organic matter intake was found in the control treatment without the supplementation of probiotics or *G. ulmifolia* Lam. extract. This is due to the fact that the addition of probiotics to poultry is known to increase digestibility and nutrient absorption, modulate intestinal microbiota balance and immune responses, maintain intestinal tract health and reduce stress levels (Patterson and Burkholder, 2003; Revolledo et al., 2006; Prado-Rebolledo et al., 2017; Li et al., 2018).

Crude protein intake. The average crude protein intake of *C. coturnix japonica* during the administration of probiotics and *G. ulmifolia* Lam. extract ranged from 4.5675 to 5.0167 (g/quail/day) and in individual periods varied from 4.6800 to 4.7139 (g/quail/day) (listed in Table 4). Low crude protein intake was recorded during the administration of *G. ulmifolia* Lam. extract for two weeks and probiotics for one week. These results were not significantly different from the variant with probiotic supplementation for two weeks. High crude protein intake was found in the control treatment without probiotics or *G. ulmifolia* Lam. extract. *L. acidophilus* is a Gram-positive bacterium widely used as a probiotic for animals and humans (Li et al., 2018). The results of other studies have shown that *L. acidophilus* is able to inhibit the invasion of pathogenic bacteria and modulate the immune response both *in vivo* and *in vitro* (Lin et al., 2007). The aforementioned studies have demonstrated that *L. acidophilus* has the potential to control pathogens in animals (Cogliani et al., 2011) and administration of probiotic microorganisms can increase protein availability. Symbiotics in turn can increase nutrient uptake as well as nitrogen stability (Falaki et al., 2011).

Crude fat intake. The average crude fat intake of *C. coturnix japonica* during probiotics and *G. ulmifolia* Lam. extract administration was 1.3117–1.4608 (g/quail/day) and in individual periods it was 1.3628–1.3728 (g/quail/day) (listed in Table 5). Low intake of crude fat was observed during the administration of *G. ulmifolia* Lam. extract for two weeks and probiotics for one week. These results were not significantly different from the outcomes of probiotic supplementation for two weeks. High crude fat consumption was found in the control treatment without the addition of probiotics or *G. ulmifolia* Lam. extract. Administration of probiotics was shown to have a modifying effect on the intestinal ecosystem by activating enzymes in the digestive tract and lowering pH (Kabir, 2009; Abd El-Hack et al., 2020). The colonization of probiotics in the intestine depends on several factors, including the availability of fermented substrates (prebiotics), intestinal pH, dose and frequency of probiotic administration, age, genetics, health, nutritional status of the host, and stress factors (Bomba et al., 2002; Rehman et al., 2020). These results are thought to be related to the ability of probiotics to secrete enzymes such as proteases, amylase, and lipase, thereby increasing the level of digestion of feed nutrients, aiding fat, starch and protein digestion, and increasing nutrient availability (Bedford, 2000).

Crude fibre intake. The average crude fibre intake of *C. coturnix japonica* during probiotic and *G. ulmifolia* Lam. extract supplementation was 0.9325–1.0383 (g/quail/day) and in individual periods, it ranged from 0.9683 to 0.9761 (g/quail/day) (listed in Table 6). The intake of crude fibre was low during the administration of *G. ulmifolia* Lam. extract for two weeks and probiotics for one week and two weeks. High crude fibre consumption was recorded in the control treatment without probiotics or *G. ulmifolia* Lam. extract. Low crude fibre intake with *G. ulmifolia* Lam. extract could be related to the presence of phenolic compounds. Phenolic compounds occur in both insoluble and soluble-bound forms. The bound phenolics cannot be absorbed by the small intestine because they are attached to insoluble macromolecules such as hemicellulose, cellulose, pectin or structural proteins (Shahidi and Yeo, 2016). Thus, these compounds enter the large intestine (colon), where a fermentation process by the colon microbiota is carried out that releases immobilised phenols. The released phenolic compounds can lower the pH and modulate the development of fermentation microflora and prevent the growth of pathogenic bacteria, thereby playing a role in improving health (Shahidi and Yeo, 2016; de Rezende et al., 2018).

NFE intake. The average NFE intake of *C. coturnix japonica* during the supplementation of probiotics and *G. ulmifolia* Lam. extract was 8.6300–9.6083 (g/quail/day) and in individual periods it was 8.9628–9.0261 (g/quail/day) (listed in Table 7). Low NFE levels were found in birds administered *G. ulmifolia* Lam. extract for two weeks and probiotics for one and two weeks. High NFE assimilation was found in the control treatment without probiotics or *G. ulmifolia* Lam. extract. Consistently with these findings, some researchers observed that feed intake was decreased after the addition of probiotics and prebiotics to broiler diets (Mokhtari et al., 2010; Falaki et al., 2011; Amerah et al., 2013; Olnood et al., 2015), which was attributed to good intestinal health, better nutrient digestion and absorption due to increased nutrient availability (Bedford, 2000).

ME intake. The average ME intake by *C. coturnix japonica* after probiotic and *G. ulmifolia* Lam. extract administration was 62.0892–69.1192 (cal/g) and in individual periods ranged from 64.4761 to 64.9439 (cal/g) (listed in Table 8). Low ME intake was observed during the administration of *G. ulmifolia* Lam. extract for two weeks and probiotics for one and two weeks. High ME consumption was found in the control treatment without probiotics or *G. ulmifolia* Lam. extract supplementation. ME intake obtained with the addition of probiotics and extracts was lower than in controls. This is believed to be related to efficiency and increased feed nutrient (protein and energy) utilisation and inhibition of gut colonisation by pathogens (Toghyani et al., 2011).

Feed efficiency. The average feed efficiency in *C. coturnix japonica* females administered probiotics and *G. ulmifolia* Lam. extract was 33.4825–40.7942% and in individual periods it ranged from 36.9550 to 38.4611% (listed in Table 9). High feed efficiency was recorded during the administration of *G. ulmifolia* Lam. extract for two weeks and probiotics for one and two weeks. Feed efficiency in the control treatment without probiotics or *G. ulmifolia* Lam. extract was low. Probiotic administrations resulted in a higher feed efficiency than in control; this was related to the viability requirements of probiotics, namely that probiotic isolates must be able to survive during processing and storage.

Administration of *G. ulmifolia* Lam. extract also resulted in higher feed efficiency compared to control. This was related to the presence of proanthocyanidins, which show antioxidant, antimicrobial, anti-inflammatory, vascular and pro-cardiac properties (Shahidi and Yeo, 2016; Tsao, 2010; Shahat and Marzouk, 2013). Proanthocyanidins, known as condensed tannins (catechins and epicatechins), occur in plants and show a protective effect against abiotic and biotic stresses. Supplementing *G. ulmifolia* Lam. extract can improve feed efficiency as these plants produce secondary metabolites that can provide astringency and flavour properties to food, as well as form complexes both with macromolecules (polysaccharides and proteins) and metal ions (Xie and Dixon, 2005; Shahat and Marzouk, 2013). In our study, the results regarding feed efficiency were consistent with the study of Nikpiran et al. (2013), who showed better feed conversion results after the addition of prebiotics to broiler rations compared to controls. This is believed to be related to a more balanced population of the gut biota as substrate availability can lead to improved efficiency of feed digestion and utilisation (Bedford, 2000; Salianeh et al., 2011).

Conclusions

Based on the presented data, it can be concluded that the use of *L. acidophillus* and *L. lactis* probiotics and *G. ulmifolia* Lam. extract has the potential to improve nutrient intake, including dry matter, organic matter, ash, crude protein, ether extract, crude fibre, NFE, ME and feed efficiency in *C. coturnix japonica* females.

Acknowledgment

The Authors are grateful to the Dean of Faculty of Veterinary Medicine, Universitas Airlangga.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Abd El-Hack M.E., El-Saadony M.T., Shafi M.E., Qattan S.Y.A., Batiha G.E., Khafaga A.F., Abdel-Moneim A.M.E., Alagawany M., 2020. Probiotics in poultry feed: a comprehensive review. J. Anim. Physiol. Anim. Nutr. 104, https://doi.org/10.1111/jpn.13454

Adedapo A.A., Mogbojuri O.M., Emikpe B.O., 2009. Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. J. Med. Plant. Res. 3, 586–591

Agustono B., Lokapirnasari W.P., Yunita M.N., Kinanti R.N., Cesa A.E., Windria S., 2022. Efficacy of dietary supplementary probiotics as substitutes for antibiotic growth promoters during the starter period on growth performances, carcass traits, and immune organs of male layer chicken. Vet. World 15, 324–330, https://doi.org/10.14202/vetworld.2022.324-330

Alaqil A.A., Abbas A.O., El-Beltagi H.S., El-Atty H.K.A., Mehaisen G.M., Moustafa E.S., 2020. Dietary supplementation of probiotic *Lactobacillus acidophilus* modulates cholesterol levels, immune response, and productive performance of laying hens. Animals 10, 1588, https://doi.org/10.3390/ani10091588

Amerah A.M., Quiles A., Medel P., Sánchez J., Lehtinen M.J., Gracia M.I., 2013. Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. Anim. Feed Sci. Technol. 180, 55–63, https://doi.org/10.1016/j.anifeedsci.2013.01.002

Assis R.Q., Andrade K.L., Batista L.E.G., de Oliveira Rios A., Dias D.R., Ndiaye E.A., de Souza É.C., 2019. Characterization of mutamba (*Guazuma ulmifolia* LAM.) fruit flour and development of bread. Biocatal. Agric. Biotechnol. 19, 101120, https://doi.org/10.1016/j.bcab.2019.101120

Bedford M., 2000. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. Worlds. Poult. Sci. J. 56, 347–365, https://doi.org/10.1079/WPS20000024

Bomba A., Nemcova R., Gancarcikova S., Herich R., Guba P., Mudronova D., 2002. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acids. Br. J. Nutr. 88, S95–S99, https://doi.org/10.1079/BJN2002634

Cogliani C., Goossens H., Greko C., 2011. Restricting antimicrobial use in food animals: lessons from Europe. Microbe 6, 274–279

de Rezende Mudenuti N.V., de Camargo A.C., Shahidi F., Madeira T.B., Hirooka E.Y., Grossmann M.V.E., 2018. Soluble and insoluble-bound fractions of phenolics and alkaloids and their antioxidant activities in raw and traditional chocolate: a comparative study. J. Funct. Foods 50, 164–171, https://doi.org/10.1016/j.jff.2018.10.003

de Souza J.A.L., da Silva W.A.V., Bezerra I.C.F., Ferreira M.R.A., Soares L.A.L., 2018. Chemical profiles by thin-layer chromatography and high-performance liquid chromatography of plant species from Northeast Brazil. Pharmacogn. Mag. 14, 437–443, https://doi.org/10.4103/pm.pm_225_17

dos Santos J.M., Alfredo T.M., Antunes K.Á. et al., 2018. *Guazuma ulmifolia* Lam. decreases oxidative stress in blood cells and prevents doxorubicin-induced cardiotoxicity. Oxid. Med. Cell. Longev. 2018, 2935051, https://doi.org/10.1155/2018/2935051

Falaki M., Shargh M.S., Dastar B., Zerehdaran S., 2011. Effects of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. J. Anim. Vet. Adv. 10, 378-384, https://doi.org/10.3923/javaa.2011.378.384

Figueroa-González I., Quijano G., Ramirez G., Cruz-Guerrero A., 2011. Probiotics and prebiotics-perspectives and challenges. J. Sci. Food Agric. 91, 1341–1348, https://doi.org/10.1002/jsfa.4367

Hadi R.F., Sudiyono, Wati A.K., 2021. The effect of vegetable oils (palm oil, canola, coconut and soybean oil) on the ration of slaughter weight, carcass weight, carcass percentage, and abdominal fat percentage of male ducks. IOP Conf. Ser.: Earth Environ. Sci. 828, 012014, https://doi.org/10.1088/1755-1315/828/1/012014

Hamasalim H.J., 2016. Synbiotic as feed additives relating to animal health and performance. Adv. Microbiol. 6, 288-302, https://doi.org/10.4236/aim.2016.64028

INPN-FR (Inventaire national du patrimoine naturel), 2019. *Guazuma ulmifolia* Lam. https://inpn.mnhn.fr/espece/cd_nom/447624/tab/taxo

Jayani T., Sanjeev B., Marimuthu S., Uthandi S., 2020. Bacterial Cellulose Nano Fiber (BCNF) as carrier support for the immobilization of probiotic, *Lactobacillus acidophilus* 016. Carbohydr. Polym. 250, 116965, https://doi.org/10.1016/j.carbpol.2020.116965

Junior J.T.C., de Morais S.M., Gomez C.V., Molas C.C., Rolon M., Boligon A.A., Athayde M.L., de Morais Oliveira C.D., Tintino S.R., Coutinho H.D.M., 2016. Phenolic composition and antiparasitic activity of plants from the Brazilian Northeast "Cerrado". Saudi J. Biol. Sci. 23, 434–440, https://doi.org/10.1016/j.sjbs.2015.10.009

Kabir S.L., 2009. The dynamics of probiotics in enhancing poultry meat production and quality. Int. J. Poult. Sci. 3, 361–364

Khaksefidi A., Rahimi S., 2005. Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. Asian-Australas. J. Anim. Sci. 18, 1153–1156, https://doi.org/10.5713/ajas.2005.1153

Kumar N.S., Gurunani S.G., 2019. Guazuma ulmifolia Lam.: a review for future view. J. Med. Plants Stud. 7, 205-210

Li Z., Wang W., Liu D., Guo Y., 2018. Effects of *Lactobacillus acidophilus* on the growth performance and intestinal health of broilers challenged with *Clostridium perfringens*. J. Anim. Sci. 9, 25, https://doi.org/10.1186/s40104-018-0243-3

Lin W.H., Yu B., Lin C.K., Hwang W.Z., Tsen H.Y., 2007. Immune effect of heat-killed multistrain of *Lactobacillus acidophilus* against *Salmonella typhimurium* invasion to mice. J. Appl. Microbiol. 102, 22–31, https://doi.org/10.1111/j.1365-2672.2006.03073.x

Lokapirnasari W.P., Agustono B., Al Arif M.A., Maslachah L., Chandra E.H., Yulianto A.B., 2022. Effect of probiotic and *Moringa oleifera* extract on performance, carcass yield, and mortality of Peking duck. Vet. World 15, 694–700, https://doi.org/10.14202/vetworld.2022.694-700

Lokapirnasari W.P., Al Arif M.A., Soeharsono S., Fathinah A., Najwan R., Wardhani H.C.P., Noorrahman N.F., Huda K., Ulfah N., Yulianto A.B., 2019. Improves in external and internal egg quality of Japanese quail (*Coturnix coturnix japonica*) by giving lactic acid bacteria as an alternative antibiotic growth promoter. Iran. J. Microbiol. 11, 406–411, https://doi.org/10.18502/ijm.v11i5.1959

Lopes G.C., Rocha J.C., de Almeida G.C.D., de Mello J.C., 2009. Condensed tannins from the bark of *Guazuma ulmifolia* Lam. (Sterculiaceae). J. Braz. Chem. Soc. 20, 1103–1109, https://doi.org/10.1590/S0103-50532009000600016

Manríquez-Mendoza L.Y., López-Ortíz S., Pérez-Hernández P., Ortega-Jiménez E., López-Tecpoyotl Z.G., Villarruel-Fuentes M., 2011. Agronomic and forage characteristics of *Guazuma ulmifolia* Lam. Trop. Subtrop. Agroecosystems 14, 453–463

Mokhtari R., Yazdani A.R., Rezaei M., Ghorbani B., 2010. The effects of different growth promoters on performance and carcass characteristics of broiler chickens. J. Anim. Vet. Adv. 9, 2633–2639, https://doi.org/10.3923/javaa.2010.2633.2639

Morais S.M., Calixto-Júnior J.T., Ribeiro L.M. et al., 2017. Phenolic composition and antioxidant, anticholinesterase and antibiotic-modulating antifungal activities of *Guazuma ulmifolia* Lam. (Malvaceae) ethanol extract. S. Afr. J. Bot. 110, 251–257, https://doi.org/10.1016/j.sajb.2016.08.003

Nikpiran H., Taghavi M., Khodadadi A., Athari S.S., 2013. Influence of probiotic and prebiotic on broiler chickens' performance and immune status. J. Nov. Appl. Sci. 2, 256–259

Olnood C.G., Beski S.S.M., Choct M., Iji P.A., 2015. Novel probiotics: their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim. Nutr. 1, 184–191, https://doi.org/10.1016/j.aninu.2015.07.003

Patterson J.A., Burkholder K.M., 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82, 627–631, https://doi.org/10.1093/ps/82.4.627

Pereira G.A., Araujo N.M.P., Arruda H.S., de Paulo Farias D., Molina G., Pastore G.M., 2019. Phytochemicals and biological activities of mutamba (*Guazuma ulmifolia* Lam.): a review. Food Res. Int. 126, 108713, https://doi.org/10.1016/j.foodres.2019.108713

Prado-Rebolledo O.F., Delgado-Machuca J.D.J., Macedo-Barragan R.J., Garcia-Márquez L.J., Morales-Barrera J.E., Latorre J.D., Hernandez-Velasco X., Tellez G., 2017. Evaluation of a selected lactic acid bacteria-based probiotic on *Salmonella enterica* serovar Enteritidis colonization and intestinal permeability in broiler chickens. Avian Pathol. 46, 90–94, https://doi.org/10.1080/03079457.2016.1222808

Rehman A., Arif M., Sajjad N. et al., 2020. Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. Poult. Sci. 99, 6946–6953, https://doi.org/10.1016/j.psj.2020.09.043

Revolledo L., Ferreira A.J.P., Mead G.C., 2006. Prospects in *Salmonella* control: competitive exclusion, probiotics, and enhancement of avian intestinal immunity. J. Appl. Poult. Res. 15, 341–351, https://doi.org/10.1093/japr/15.2.341

Salarmoini M., Fooladi M.H., 2011. Efficacy of *Lactobacillus acidophilus* as probiotic to improve broiler chicks' performance. J. Agr. Sci. Tech. 13, 165–172

Salianeh N., Shirzad M.R., Seifi S., 2011. Performance and antibody response of broiler chickens fed diets containing probiotic and prebiotic. J. Appl. Anim. Res. 39, 65–67, https://doi.org/10.1080/09712119.2011.565222

Shahat A.A., Marzouk M.S., 2013. Tannins and related compounds from medicinal plants of Africa. In: V. Kuete (Editor). Medicinal plant research in Africa. Elsevier, Amsterdam (Netherlands), pp. 479–555, https://doi.org/10.1016/B978-0-12-405927-6.00013-8

Shahidi F., Yeo J., 2016. Insoluble-bound phenolics in food. Molecules 21, 1216, https://doi.org/10.3390/molecules21091216

Suwignyo B., Suryanto E., Samur S.I.N., Hanim C., 2021. The effect of hay alfalfa (*Medicago sativa* L.) supplementation in different basal feed on the feed intake (FI), body weight, and feed conversion ratio of hybrid ducks. IOP Conf. Ser.: Earth Environ. Sci. 686, 012039, https://doi.org/10.1088/1755-1315/686/1/012039

Tang S.G.H., Sieo C.C., Ramasamy K., Saad W.Z., Wong H.K., Ho Y.W., 2017. Performance, biochemical and haematological responses, and relative organ weights of laying hens fed diets supplemented with prebiotic, probiotic and synbiotic. BMC Vet. Res. 13, 248, https://doi.org/10.1186/s12917-017-1160-y

Toghyani M., Toghyani M., Tabeidian S.A., 2011. Effect of probiotic and prebiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. In: International Conference on Food Engineering and Biotechnology 9. IACSIT Press, (Singapore), pp. 82–86

Tsao R., 2010. Chemistry and biochemistry of dietary polyphenols. Nutrients 2, 1231-1246, https://doi.org/10.3390/nu2121231

van Belkum A., Nieuwenhuis E.E., 2007. Life in commercial probiotics. FEMS Immunol. Med. Microbiol. 50, 281–283, https://doi.org/10.1111/j.1574-695X.2007.00290.x

Wu Z., Yang K., Zhang A., Chang W., Zheng A., Chen Z., Cai H., Liu G., 2021. Effects of *Lactobacillus acidophilus* on the growth performance, immune response, and intestinal barrier function of broiler chickens challenged with *Escherichia coli* O157. Poult. Sci. 100, 101323, https://doi.org/10.1016/j.psj.2021.101323

Xie D.Y., Dixon R.A., 2005. Proanthocyanidin biosynthesis - still more questions than answers? Phytochemistry 66, 2127–2144, https://doi.org/10.1016/j.phytochem.2005.01.008

Yulianto A.B., Lokapirnasari W.P., Najwan R., Wardhani H.C.P., Rahman N.F.N., Huda K., Ulfah N., 2020. Influence of *Lactobacillus casei* WB 315 and crude fish oil (CFO) on growth performance, EPA, DHA, HDL, LDL, cholesterol of meat broiler chickens. Iran. J. Microbiol. 12, 148–155, https://doi.org/10.18502/ijm.v12i2.2620

Yulianto A.B., Suwanti L.T., Widiyatno T.V., Suwarno S., Yunus M., Tyasningsih W., Hidanah S., Sjofjan O., Lokapirnasari W.P., 2021. Probiotic Pediococcus pentosaceus ABY 118 to modulation of ChIFN-γ and ChIL-10 in broilers infected by Eimeria tenella oocyst. Vet. Med. Int. 2021, 1473208, https://doi.org/10.1155/2021/147320