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Effects of dietary medium-chain fatty acids, probiotics, and organic acid salts on laying hen performance and egg quality

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ABSTRACT. The elimination of antibiotic growth promoters from poultry production has increased demand for alternative feed additives. This study evaluated the impact of several gastrointestinal-stabilising formulations (medium-chain fatty acids, probiotics, and organic acids) on the productivity and egg quality of laying hens. A total of 90 (Lohmann Brown) laying hens were randomly assigned to 5 treatment groups, with 6 replicates of three hens each (18 hens per group). The control group (C) received a basal diet, whereas the experimental groups were additionally supplemented with 0.2% medium-chain fatty acids (A), 0.1% probiotics (L), a 0.1% blend of calcium butyrate, calcium lactate, and bentonite encapsulated in vegetable fats (BE), or 0.05% calcium butyrate encapsulated in vegetable fats (BP). During the 56-day trial, additives exerted no significant effect on performance in hens aged 30–33 weeks. However, in hens aged 34–37 weeks, medium-chain fatty acids significantly increased egg weight and improved feed conversion ratio compared to controls. All additives also markedly increased eggshell thickness in younger hens (30–33 weeks) ($P < 0.05$). Additionally, significantly higher Haugh unit values ($P < 0.05$) were recorded for the L, A, and BE groups compared to the C group. For the 34–37 week period, eggs from the A, BE and BP groups had significantly greater shell thickness ($P < 0.05$) compared to the C group. No significant differences in bird mortality were observed between the groups. These results suggest that medium-chain fatty acids and organic acid salts can effectively support gastrointestinal health and egg quality as antibiotic alternatives in laying hens.

Introduction

The poultry industry has been steadily shifting towards sustainable production practices in response to antimicrobial resistance (AMR) concerns, particularly by seeking alternatives to antibiotic growth promoters (AGPs). The European Union prohibited the use of AGPs in animal feeds in 2006. Nevertheless, antibiotics are still widely used in poultry production for disease control and performance enhancement, particularly through individualised treatments and water or feed supplementation (Singer and Hofacre, 2006; Asghar et al., 2021, 2022; Naımatı et al., 2022). While effective for microbial control and productivity, antibiotic overuse has led to significant AMR emergence (Van Boeckel et al., 2014; Gresse et al., 2017; Akram et al., 2019), detectable egg residues (Wongsuvan et al., 2018), and consumer health risks (Donoghue, 2003). Therefore, there is

an urgent need for effective antibiotic alternatives that improve egg quality, maintain performance, and protect gut health while mitigating AMR (Xiang et al., 2019; Akram et al., 2021).

Medium-chain fatty acids (MCFAs), probiotics, and organic acid salts have emerged as promising AGP alternatives due to their beneficial effects on gut health and productivity. MCFAs (saturated fatty acids with 6–12 carbon atoms) possess well-documented antibacterial and antimicrobial properties (Fusieger et al., 2022). MCFAs, including caproic (C6:0), caprylic (C8:0), capric (C10:0), and lauric acid (C12:0), are naturally present in lipid sources such as cow's milk, coconut oil, and palm oil (Baltić et al., 2017; Çenesiz and Çiftci, 2020). These acids show antibacterial, antifungal, and coccidiostatic (Perdok et al., 2011; Price et al., 2013) properties and may synergistically enhance the efficacy of other feed additives while optimising the performance of laying hens. Prior research has shown that both organic acids and MCFAs can reduce pathogenic bacterial population in the gastrointestinal tract, improve feed efficiency, and egg production metrics, making them a viable substitutes for AGPs in layer diets (Nguyen et al., 2018; Dauksiene et al., 2021). Organic acids such as propionic, lactic, and butyric acid offer a distinct antibacterial approach by lowering the intracellular pH of pathogenic bacteria, interfering with enzyme activity, damaging bacterial cells, and stimulating the development of beneficial microbiota in the gastrointestinal tract (Araujo et al., 2019). Organic acid supplementation also increases the availability of nutrients, while reducing the production of undesirable metabolites by pathogenic microorganisms. However, the effectiveness of these organic acids is limited by their rapid metabolism in the upper digestive system, reducing their antimicrobial activity in the lower intestine (Banupriya et al., 2016; Khan and Iqbal, 2016; Jadhao et al., 2019). Other disadvantages of organic acids include their instability and unpleasant odour that complicate feed handling. To overcome these limitations, organic acids are often encapsulated in lipid-based coatings to enable controlled release in the duodenum, thereby significantly improving their stability and effectiveness (Broom, 2015; Tabata et al., 2018). When incorporated into poultry diets, these protected organic acid formulations have been shown to consistently improve feed conversion ratio (FCR), growth performance, and overall production quality in laying hens (Broom, 2015).

Probiotics are live microbial supplements that improve the intestinal microbial balance and exert beneficial effects on the host animal. They are considered good candidates for antibiotic substitutes in terms of supporting gut health, immune system function, and, in certain cases, egg production and quality. The most commonly used microorganisms in probiotic formulations include bacteria from the genera *Bifidobacterium*, *Lactococcus*, *Lactobacillus*, *Bacillus*, *Streptococcus*, and yeasts such as *Candida* spp. (Krysiak et al., 2021). Probiotics not only support the intestinal microbiota but also improve FCR, egg production, and shell quality. Probiotic compositions often include bacterial isolates capable of producing enzymes such as phytases, cellulases, proteases or xylanases. Modern probiotic preparations typically use bacterial spores rather than live cells, ensuring thermal stability during feed processing and enabling incorporation into pelleted feeds without compromising viability (Park et al., 2016). These preparations offer a compelling antibiotic alternative as they improve intestinal health and promote the development of beneficial gastrointestinal microflora (Park et al., 2016; Krysiak et al., 2021). Probiotics have emerged as an effective solution for maintaining healthy intestinal microbiota in laying hens, improving nutrient absorption, and overall production performance. Numerous studies have also indicated that probiotic supplementation can increase egg production, improve FCR (Hargis et al., 2021) and eggshell quality (Jiang et al., 2017).

While the effects of these additives have already been relatively well documented, especially in the context of growing animals such as broiler chickens and pigs, limited research is available regarding the impact of MCFAs and organic acid salts on laying hen performance and egg quality. Considering that all the feed additives analysed in the present study are known to support digestive tract function and stimulate the growth of beneficial microorganisms, we hypothesised that their inclusion in the diet of laying hens would improve nutrient utilisation, leading to better performance and egg quality.

The purpose of this study was to evaluate the effects of dietary inclusion of MCFAs, probiotics, and organic acid salts on key performance indicators, including egg production, egg weight, feed intake, FCR, and egg quality parameters. A comprehensive understanding of how these feed additives influence laying hen performance and egg quality will enable the development of optimised dietary strategies for antibiotic-free poultry production. These findings will provide critical data for identifying effective alternatives to antibiotic growth promoters that maintain both animal health and production efficiency.

Material and methods

Ethical approval

The experiments in this study were conducted in full compliance with Polish Law (2015) and European Union (Directive 2010/63/EU) animal welfare regulations and did not require ethics committee approval. All procedures followed the guidelines for animal experimentation and animal care, and all efforts were made to minimise animal suffering.

Animals, diets and housing

The study was conducted using 90 Lohmann Brown laying hens housed in enclosures from 30 weeks of age. The hens were randomly assigned to five experimental groups, each consisting of six replicate cages containing three hens (18 birds per group). Table 1 provides a comprehensive description of the basal diet administered to all hens.

Table 1. Composition and nutritional value of the basal diet

Diet composition	
Component	Inclusion, %
wheat	37.85
maize	30.00
soybean meal 46% crude protein	20.20
coarsely structured limestone	6.45
finely structured limestone	2.50
Premix*	2.00
soybean oil	1.00
Nutritional value of feed mixture	
nutrient	Level, %DM
dry matter	88.74%
crude ash	12.62
crude fat	3.530
crude protein	16.52
crude fibre	3.430
metabolizable energy	11.38 MJ/kg DM
total Ca	3.920
total P	0.510
available P	0.425
Ca/P ratio	7.686
lysine	0.858
methionine	0.394
methionine+cysteine	0.691
threonine	0.579
tryptophan	0.202
isoleucine	0.647
arginine	0.976
valine	0.757

* 2% premix contained: IU: vitamin A 500 000, vitamin D_3 150 000, vitamin E 1000; mg: vitamin B_1 100, vitamin B_2 250, vitamin B_6 150, vitamin K 125, niacin 1500, folic acid 50, pantothenic acid 500, choline 15 000, Fe 2500, Mn 5000, Zn 3500, Cu 750, I 50, Se 15; g: P 80, Ca 60, Na 80, lysine 50, methionine 80, threonine 5; mcg: vitamin B_{12} 1000, biotin 5000; FTU: phytase 45 000; DM – dry matter

The control group (C) received only the basal diet without any supplements. The diet of the A group was supplemented with 0.2% MCFAs in the form of a lactate ester compound containing lactic acid, C12 lauric acid, and C14 myristic acid. The L group received the basal diet with the addition of a 0.1% probiotic mixture containing bacterial and yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081, and *Saccharomyces cerevisiae* KKP 2059p), the diet of the BE (bentonite encapsulated) group contained additionally 0.1% encapsulated calcium butyrate (calcium butyrate content 24.6–28.3%) combined with calcium lactate and bentonite as carriers, while the BP group received the basal diet with the addition of 0.05% calcium butyrate encapsulated in refined vegetable lipids (calcium butyrate content 80–87%).

The hens were provided with 130 g of feed daily and unrestricted access to water. The feed mixtures used in the experiment were isonitrogenous and isocaloric, with balanced protein and energy levels. The 56-day feeding trial was conducted at the Research and Didactic Station in Swojczyce, affiliated with the Wrocław University of Environmental and Life Sciences. The hens were housed in cages with controlled environmental conditions maintained at 18 °C, 65% relative humidity, and a 14:10 h light:dark cycle (Konkol et al., 2020) GIII, and GIV contained one, 2, and 3 additional feeders in the cages, respectively. The assessment of bird welfare was based on production, physiological and behavioral parameters, as well as on the basis of external appearance. The experiment lasted 12 wk. The obtained results suggest that enriching laying hens' cages with additional feeders improved the welfare of the hens. Enrichment of cages significantly reduced the number of feather pecking and aggressive behaviors in the GII and GIV groups ($P < 0.01$. Each 3 750 cm² cage was equipped with welfare-enrichment features including a perch, nest, scratcher, feeding trough (20 cm per hen), and two water nipples. The vaccination programme applied during the rearing period was as follows:

- day 1 – vaccination against Marek's disease, Newcastle disease and infectious bronchitis;
- days 10–14 – vaccination against Gumboro disease;
- days 19–21 – vaccination against Gumboro disease;
- week 4 – vaccination against Newcastle disease and infectious bronchitis;

- week 8 – vaccination against Newcastle disease and infectious bronchitis;
- weeks 12–13 – vaccination against encephalomyelitis infection;
- week 16 – vaccination against Newcastle disease, infectious bronchitis, egg drop syndrome and big head syndrome (pneumovirus infection).

Performance measurements

At the start of the experiment, the hens had an average body weight of 1920 g, which increased to approximately 1960 g by the end of the trial. To assess the effects of dietary treatments, egg production, feed intake, and egg weight were recorded. Egg production was determined daily by collecting and counting all eggs laid by each group. Feed intake and egg weight were measured twice weekly. Feed intake was calculated by weighing feed leftovers from each cage and dividing by the number of hens per cage. The feed conversion ratio (FCR), an indicator of feed efficiency relative to egg production, was calculated by dividing feed intake by the total mass of eggs laid.

Egg quality assessments

During the experimental period, six eggs were randomly selected from each replicate cage on days 28 and 56 to be used for quality assessment. Each egg was separated into its components, i.e. the albumen, yolk, and shell, which were individually weighed to obtain component-specific data. Yolk colour and egg freshness, expressed as Haugh units (HU), were assessed using an EggAnalyzer (ORKA Food Technology, West Bountiful, Utah, USA). Shell strength was determined with an Egg Force Reader (ORKA Food Technology, West Bountiful, Utah, USA), and eggshell thickness was measured with a micrometre at three locations: the small end, large end, and equator of each egg. The final eggshell thickness value was calculated as the arithmetic mean of these three measurements to provide a comprehensive evaluation of shell quality across experimental groups.

Statistical analysis

All parameters measured were analysed by calculating means and standard errors of the mean (SEM). The ShapiroWilk test was used to assess the normality of data distribution. For normally distributed data, a one-way analysis of variance (ANOVA) was performed, followed by post hoc group comparisons using the Tukey test. Non-normally distributed data were analysed using the KruskalWallis test. Statistical significance was set at $P < 0.05$. All analyses were conducted using Statistica software (version 13.1).

Results

Performance of laying hens

For hens aged 30–33 weeks (Table 2), no significant differences ($P > 0.05$) were found between the groups for feed intake, FCR, egg weight and egg production.

In hens aged 34–37 weeks (Table 3), the MCFA-supplemented group (A) showed significantly greater egg weight ($P < 0.05$) and lower FCR ($P < 0.05$) compared to the control group, indicating improved feed efficiency. No other significant differences in performance parameters were observed during this period. All values are presented as mean ± SEM.

Table 2. Performance of 30–33-week-old laying hens under different dietary treatments

Parameter	C	L	A	BE	BP	SEM	*P*-value
Egg production,%	96.23	96.23	96.41	95.16	96.68	0.64	0.965
Egg weight, g	62.15	63.07	61.86	62.69	62.70	0.23	0.514
Feed intake, g	112.6	122.5	122.4	118.42	117.22	1.61	0.266
FCR, g of feed/g of eggs	1.800	1.940	1.970	1.880	1.860	0.02	0.271

C – control group; L – group fed a basal diet with a mixture of probiotic bacterial yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium–divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) at 0.1%; A – group fed a basal diet with the addition of medium-chain fatty acids in the form of lactate (an ester combination of lactic acid with C12 lauric acid and C14 myristic acid) at 0.2%; BE – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 24.6–28.3% + calcium lactate and bentonite as carriers) at 0.1%; BP – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 80–87%), at 0.05%; FCR – feed conversion ratio; SEM – standard error of the mean; $P > 0.05$

Eggs quality parameters

For eggs from 30–33-week-old hens (Table 4), all supplemented groups (L, A, BE, BP) showed significantly greater shell thickness compared to the control group ($P < 0.05$), with the BP group demonstrating the thickest shells among treat-

Table 3. Performance of 34–37-week-old laying hens under different dietary treatments

Parameter	C	L	A	BE	BP	SEM	*P*-value
Egg production, %	97.91	98.35	98.26	98.61	98.66	0.13	0.775
Egg weight, g/day	62.46[a]	63.59[ab]	64.66[b]	63.90[ab]	63.22[ab]	0.24	0.023
Feed intake, g/day	127.8	127.0	124.78	126.5	125.6	0.47	0.172
FCR, g of feed/g of eggs	2.040[a]	2.000[ab]	1.930[b]	1.980[ab]	1.990[ab]	0.01	0.002

C – control group; L – group fed a basal diet with a mixture of probiotic bacterial yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium–divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) at 0.1%; A – group fed a basal diet with the addition of medium-chain fatty acids in the form of lactate (an ester combination of lactic acid with C12 lauric acid and C14 myristic acid) at 0.2%; BE – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 24.6–28.3% + calcium lactate and bentonite as carriers) at 0.1%; BP – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 80–87%), at 0.05%; FCR – feed conversion ratio; SEM – standard error of the mean; [ab] means with different superscripts within the row are significantly different at $P > 0.05$

Table 4. Effect of dietary treatments on egg quality parameters in 30–33-week-old laying hens

Parameter	C	L	A	BE	BP	SEM	*P*-value
Breaking strength, N	5.404	5.428	5.438	5.434	5.663	0.04	0.602
Thickness, mm	0.371[a]	0.406[b]	0.397[bc]	0.405[b]	0.413[bd]	0.01	0.001
Yolk colour	4.230	4.260	4.130	3.930	4.130	0.05	0.336
Haugh units	76.22[a]	82.46[b]	81.68[b]	81.46[b]	79.14[ab]	0.52	0.001

C – control group; L – group fed a basal diet with a mixture of probiotic bacterial yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium–divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) at 0.1%; A – group fed a basal diet with the addition of medium-chain fatty acids in the form of lactate (an ester combination of lactic acid with C12 lauric acid and C14 myristic acid) at 0.2%; BE – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 24.6–28.3% + calcium lactate and bentonite as carriers) at 0.1%; BP – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 80–87%), at 0.05%; SEM – standard error of the mean; [ab] means with different superscripts within the row are significantly different at $P > 0.05$

ments. The L, A, and BE groups exhibited significantly higher Haugh units than controls ($P < 0.05$), indicating better freshness, while egg freshness in the BP group was comparable to controls. No significant differences were observed in breaking strength or yolk colour between any of the groups. All values are presented as mean ± SEM.

For eggs from 34–37-week-old hens (Table 5), the A, BE, and BP groups showed significantly greater shell thickness compared to the control group ($P < 0.05$), demonstrating a long-lasting improvement in shell quality. No statistically significant differences were observed between the groups for breaking strength, Haugh units (freshness), or yolk colour, indicating minimal treatment effects on these parameters during this period. All values are presented as mean ± SEM.

Egg component weights

For eggs from 30–33-week-old hens (Table 6), the BE group showed significantly higher albumen weight compared to the A group ($P < 0.05$), while no other significant differences in component weights (albumen, yolk, shell) were observed between the groups. In 34–37-week-old hens (Table 7), none of the feed additives signifi-

Table 5. Effect of dietary treatments on egg quality parameters in 34–37-week-old laying hens

Parameter	C	L	A	BE	BP	SEM	*P*-value
Breaking strength, N	5.545	5.622	5.602	5.592	5.713	0.04	0.898
Thickness, mm	0.382[a]	0.390[ab]	0.394[b]	0.395[b]	0.395[b]	0.01	0.001
Yolk colour	3.633	4.000	3.666	3.663	3.666	0.05	0.102
Haugh units	75.77	78.43	79.00	76.72	77.53	0.42	0.110

C – control group; L – group fed a basal diet with a mixture of probiotic bacterial yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium–divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) at 0.1%; A – group fed a basal diet with the addition of medium-chain fatty acids in the form of lactate (an ester combination of lactic acid with C12 lauric acid and C14 myristic acid) at 0.2%; BE – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 24.6–28.3% + calcium lactate and bentonite as carriers) at 0.1%; BP – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 80–87%), at 0.05%; SEM – standard error of the mean; [ab] means with different superscripts within the row are significantly different at $P > 0.05$

Table 6. Effect of dietary treatments on the weight of individual egg components in 30–33-week-old laying hens, g

Parameter	C	L	A	BE	BP	SEM	*P*-value
Shell	8.750	9.110	9.050	8.850	9.050	0.06	0.277
Yolk	16.16	15.83	15.58	14.99	15.68	0.133	0.075
Albumen	39.53^{ab}	40.37^{ab}	38.51^{a}	40.87^{b}	40.37^{ab}	0.26	0.039

C – control group; L – group fed a basal diet with a mixture of probiotic bacterial yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium–divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) at 0.1%; A – group fed a basal diet with the addition of medium-chain fatty acids in the form of lactate (an ester combination of lactic acid with C12 lauric acid and C14 myristic acid) at 0.2%; BE – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 24.6–28.3% + calcium lactate and bentonite as carriers) at 0.1%; BP – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 80–87%), at 0.05%; SEM – standard error of the mean; ^{ab} means with different superscripts within the row are significantly different at $P > 0.05$

Table 7. Effect of dietary treatments on the weight of individual egg components in 34–37-week-old laying hens, g

Parameter	C	L	A	BE	BP	SEM	*P*-value
Shell	8.503	8.686	8.660	8.646	8.593	0.0521	0.8216
Yolk	16.36	16.90	16.97	16.29	16.92	0.115	0.160
Albumen	39.69	39.68	39.69	40.62	40.33	0.306	0.801

C – control group; L – group fed a basal diet with a mixture of probiotic bacterial yeast strains (*Lactococcus lactis* B/00039, *Carnobacterium–divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) at 0.1%; A – group fed a basal diet with the addition of medium-chain fatty acids in the form of lactate (an ester combination of lactic acid with C12 lauric acid and C14 myristic acid) at 0.2%; BE – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 24.6–28.3% + calcium lactate and bentonite as carriers) at 0.1%; BP – group fed a basal diet with the addition of calcium butyrate encapsulated in refined vegetable fats (calcium butyrate content 80–87%), at 0.05%; SEM – standard error of the mean; $P > 0.05$

cantly affected the weights of egg components (albumen, yolk, shell), with no statistically significant differences found between the treatment groups. All values are presented as mean ± SEM.

Discussion

Performance metrics

The present investigation assessed the impact of probiotics, MCFAs, and organic acid salts on the efficacy of laying hens over a 56-day period. Based on the findings, the incorporation of these feed additives can influence specific production parameters, particularly egg weight and FCR. Hens supplemented with MCFAs (A group) laid significantly heavier eggs compared to controls by the end of the trial period, suggesting improved nutrient absorption and utilisation. This result is consistent with other studies showing that MCFAs exert a beneficial effect on feed efficiency and egg production in poultry. Additionally, the lower FCR observed in the A group supports the view that MCFAs can improve feed efficacy, a critical factor in poultry production that directly affects economic viability. Despite these positive outcomes, egg production rates were similar among all groups, and no statistically significant differences were observed. This result aligns with previous studies reporting that the implementation of non-antibiotic feed additives did not significantly influence overall production rates (Świątkiewicz et al., 2010; Ricke et al., 2020). It is possible that the duration of the experiment was insufficient to detect potential improvements in egg production, as the effects of such additives may be noticeable over longer periods of time.

The advantages of MCFAs over organic acid salts demonstrated in this study may be attributed to several factors. First, MCFAs do not need the formation of bile acid micelles to enter the aqueous phase since they are extremely soluble, similarly to short-chain fatty acids (SCFAs) Their absorption occurs through simple passive diffusion, as their relatively low affinity for enzymes that activate and esterify acyl-CoA, as well as for fatty acid binding proteins, limits esterification in enterocytes (Greenberger et al., 1966). This unique absorption mechanism enables efficient cellular uptake of MCFAs, providing enterocytes with a direct energy source (Playoust and Isselbacher, 1964; Bach and Babayan, 1982; Lamot et al., 2016). SCFAs and MCFAs have lower pKa values, thus they remain mostly undissociated in the acidic environment of the upper gastrointestinal tract (Ahsan et al., 2016; Moquet et al., 2016). In addition, MCFAs can penetrate the cell membranes of pathogens (Çenesiz and Çiftci, 2020) under these conditions,

exerting a strong antimicrobial effect (Bach and Babayan, 1982; Papamandjaris et al., 1998; Marten et al., 2006). It is well established that pathogenic bacteria compete for nutrients, produce toxins, and thus compromise intestinal integrity, leading to reduced nutrient absorption and, consequently, a decline in animal performance. MCFA supplementation inhibits pathogenic growth through antimicrobial activity, preserving gut function and increasing nutrient availability for egg production.

In contrast to the current study, Gama et al. (2000) reported an increase in egg production following the inclusion of organic acids. Although this study did not demonstrate an effect of organic acids on FCR, Gama et al. (2000) suggested that specific combinations of organic acids may improve production parameters. On the other hand, Ricke et al. (2020) incorporated formic acid and propionic acid into the diets of laying hens and, consistent with the current findings, found no effect of organic acids on feed intake and FCR; however, these authors observed an improved egg production. Soltan (2008) also documented a positive effect of specific organic acid combinations (formic acid and salts of butyric, propionic and lactic acids) on average egg production. Świątkiewicz et al. (2010) tested various feed additives such as inulin, oligofructose, volatile fatty acids (VFAs) and MCFAs. and similarly to the present study, did not observe a significant effect on egg production. However, in contrast to the current findings, they also reported no impact of the additives on egg weight and FCR. Zhang et al. (2012) demonstrated that specific probiotic combinations could enhance production efficiency. Their study showed that groups receiving inactivated *Lactobacillus* with *C. butyricum* and *B. subtilis*, or inactivated *Lactobacillus* with sodium butyrate, produced significantly more eggs with improved FCR compared to controls. Similarly, *B. subtilis* supplementation alone or combined with sodium butyrate significantly reduced FCR. These results suggests that carefully selected probiotic combinations may improve laying performance and overall production efficiency in laying hens. Xiang et al. (2019) demonstrated that dietary supplementation with *C. butyricum* significantly improved feed efficiency ($P < 0.05$) while reducing feed intake, though it showed no significant effects on egg production or average egg weight. Variations in the results between the studies may be attributed to the use of different probiotic strains, application of multiprobiotic preparations, as well as methodology, and the duration of supplementation.

Egg quality parameters

Egg quality is a critical factor in poultry production, directly influencing consumer acceptance and commercial value. In this study, after 28 days, eggs from hens fed MCFAs, probiotics, and organic acid salts showed improved structural integrity and freshness, as evidenced by increased eggshell thickness and higher Haugh units. Moreover, eggshells from the group receiving calcium butyrate were significantly thicker compared to the MCFA group. These findings have substantial practical implications, as adequate shell thickness directly reduces breakage rates during handling and transportation. Eggshell thickness remained significantly higher after 56 days in MCFA and organic acid salt groups, indicating long-term effectiveness of these additives in maintaining eggshell quality throughout the production cycle. Similar age-dependent effects were observed by Świątkiewicz et al. (2010), showing minimal egg quality improvements in young hens but significant enhancements in older layers. Their study found that MCFAs and inulin increased eggshell strength by 46 weeks, while combinations of inulin, VFAs and MCFAs improved shell density (4.5%) and percentage (2–3%) by 58–70 weeks. These findings indicate that feed additives may exert a more pronounced effect on egg quality parameters during later laying phases.

The study found no significant differences in yolk colour or breaking strength between treatment groups, suggesting that feed additives selectively improve certain egg quality parameters. These findings align with existing literature demonstrating variable effects of dietary interventions on egg quality traits. While Zhang et al. (2012) found no probiotic effects on egg shape, shell thickness, yolk colour, or cholesterol levels, they observed significantly higher Haugh units ($P < 0.05$) in groups receiving *Lactobacillus*/sodium butyrate or *B. subtilis*/sodium butyrate combinations. In contrast, Xiang et al. (2019) demonstrated that dietary supplementation with *C. butyricum* in laying hens significantly reduced eggshell strength and yolk colour.

Component weights

At 28 days, the analysis of individual egg components showed that albumen weight was significantly higher in the BE group compared to the A group. This difference could be attributed to the effect of organic acid salts on albumin production and protein metabolism. However, no statistically significant differences were observed in shell, yolk,

and albumen weights between the groups after 56 days of the trial. These findings suggest that while feed additives may induce short-term changes in egg composition, their long-term effects remain uncertain.

Implications for poultry production

The current study suggests that MCFAs, probiotics, and organic acid salts might be a suitable substitute for antibiotic growth promoters in layer diets. However, these feed additives should complement, not replace, comprehensive management strategies. Optimal layer performance and welfare require integrated attention to housing conditions, nutritional balance, and strict biosecurity measures. Careful formulation and monitoring of these feed additives is required to ensure their optimal effectiveness when incorporated into poultry production systems.

This research demonstrates that organic acid salts, probiotics, and MCFAs improve egg weight and shell thickness in laying hens, offering sustainable alternatives to antibiotic growth promoters. Further research is needed to evaluate the long-term effects of these supplements on the performance of laying hens, egg quality, and overall flock health, as well as to elucidate the mechanisms underlying their beneficial effects.

Conclusions

This study highlights the beneficial effects of medium-chain fatty acids (MCFAs), probiotics, and organic acid salts on the performance and egg quality parameters of laying hens over a 56-day period. Specifically, dietary supplementation with MCFAs significantly increased egg weight and improved feed conversion ratio, indicating better nutrient absorption and utilisation. Moreover, MCFAs and organic acid salts improved eggshell thickness and Haugh units, both key indicators of egg quality. These findings support the potential of these additives as effective alternatives to antibiotic growth promoters in poultry nutrition. However, their successful implementation requires integration with comprehensive management strategies addressing housing, nutrition, and biosecurity. Future research should focus on optimising formulations, evaluating long-term effects, assessing economic viability, and elucidating the underlying mechanisms of action to facilitate their practical application in sustainable egg production systems.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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