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IMPACT OF EARLY FEED RESTRICTION AND CONTINUED SUPPLEMENTATION WITH COATED ORGANIC ACID AND ESSENTIAL OILS ON SUSTAINABILITY OF LAYING PERFORMANCE, EGG QUALITY, FERTILITY, HATCHABILITY, IMMUNITY STATUS, AND GUT MICROBIOTA OF JAPANESE QUAIL HENS

Hayam M. Abo El-Maaty[1♦], Youssef A. Attia[2♦], Rashed A. Alhotan[3], Mohamed A. Al-Banoby[4], Fulvia Bovera[5♦], Sara Kh. Sherif[1♦]

[1]Poultry Production Department, Faculty of Agriculture, Mansoura University, Egypt
[2]Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour 22516, Egypt
[3]Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[4]Al-Shamel Animal Feed Factory, Industrial Area, Hail, Saudi Arabia
[5]Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, via F. Delpino 1, 80137 Napoli, Italy
♦Corresponding authors: F. Bovera: bovera@unina.it; Y.A. Attia: attia0103753095@gmail.com; H.M. Abo EL-Maaty: hayam151@yahoo.com; S.Kh. Sherif: sarasherif349@yahoo.com
ORCID:
Youssef A. Attia: https://orcid.org/0000-0001-6505-3240
Rashed A. Alhotan: https://orcid.org/0000-0002-7789-4722

Abstract

Three hundred and sixty, 42-day-old Japanese quail (JQ) hens were randomly divided into six experimental groups with 5 replicates/group. Each replicate consisted of 12 birds (8 females and 4 males). During the growing period, chickens were fed restricted (FR) during only the second week of age at days 7, 9, 11, and 13. Three levels of FR were applied: 0.0 (control), 12.5 (12.5FR), and 25.0% (25FR) of the *ad libitum* intake of chickens measured on the previous day (groups 1, 2 and 3). In the other three groups (4, 5 and 6) the control, 12.5FR and 25.0FR diets were supplemented with coated organic acid + essential oils (COAWEO) at 100 g/ton feed. The trial lasted from 6 to 18 weeks of age. Feed intake was significantly reduced by 12.5% FR compared with the control group. FR improved all external and internal egg quality of JQ hens except for egg weight, while albumen % decreased in the group fed 12.5FR diet. Coated organic acid with essential oils did not affect all JQ hens' external and internal egg quality. Feed restriction did not affect fertility, pipped and dead chicks, and hatchability, as total and fertile egg percentage. COAWEO increased fertility and hatchability percentage. FR did not affect blood serum biochemistry and immunity characteristics except for high density lipoprotein (HDL) and hemagglutination inhibition test for infectious bursal disease virus (HI IBDV). Coated organic acid with essential oils did not affect quail blood characteristics but significantly decreased total lipids and increased IL6 at 18 weeks of age. Dietary COAWEO decreased *E. coli, Clostridia*, and *Salmonella* while increasing *Lactobacillus* counts. It can be concluded that 25.0% FR supplemented with COAWEO considerably improved the sustainability of productive performance of JQ hens without adverse effects on egg production, quality, fertility and hatchability traits, serum biochemistry and immune markers, and gut microbiota.

Key words: feed restriction, organic acids, essential oils, egg production, hatchability

Recently, there has been an increase in the number of Japanese quails (JQ) farmed in the world (Secci et al., 2022). The hardiness, quick development, and great egg output make this species very interesting (Siyadati et al., 2011). Quails are a good source of high-quality animal protein and have a considerable value for the sustainability of poultry industry considering both eggs and meat production (Mahrose et al., 2020).

Even if poultry meat production is, in general, characterized by a fast growth rate, the feed restriction (FR) has been applied in broilers also the last years (Ebeid et al., 2022; El-Sabrout et al., 2022 a; Ogbuagu et al., 2023). This nutritional strategy, which reduces the feed consumed by the animals, can be considered also a valuable way to reduce the negative effects on the ingredients availability due to the recent global crisis (COVID pandemic and Russia−Ukraine war) affecting feed supply and chain (Hafez and Attia, 2020). However, FR is a tool that improves animal tolerance and productivity when applied to chickens (Attia et al., 1995; Saleh et al., 1996).

Various feeding regimens enhance the generation of viable eggs under heat-stress conditions, and applying an 8-hour restricted feeding schedule had a beneficial impact on Japanese quails' body weight (BW), fertility, hatchability, egg production, egg-specific quality, and ovipositional time (Hassan et al., 2003 b). Because there is a link between excess body fat and decreased egg production, fertility, and hatchability, FR techniques are, in general, used to prevent excessive fat deposition (Wilson and Harms, 1986; Anene et al., 2023). Studies on the effects of early FR on laying performance are poorly rep-

resented in the literature. In addition, under FR, animals are subjected to starvation, and this can affect directly (by modification of intestinal microbiota) or indirectly (through the ingestion of litter) the birds' health (Artdita et al., 2021). In this context the use of organic acids and essential oils may improve birds' tolerance to FR due to their antimicrobial influence. According to recent evidence, essential oils have shown potential effects on growth performance, gut health, and control of pathogens in poultry (Attia et al., 2019; Irawan et al., 2021; El-Sabrout et al., 2022 b).

Therefore, the present study investigated the influence of feed restriction during the 2nd week of age and continuous supplementation of coated organic acid with essential oils and organic acids on sustainability of laying performance, egg quality, egg fertility and hatchability, immunity, antioxidants status, and gut microbiota of female Japanese quail.

Material and methods

Experimental design

The trial was conducted at the Poultry Research Unit, Faculty of Agriculture, Mansoura University, Egypt. Three hundred and sixty, 1-day-old Japanese quail (JQ) were divided into six homogeneous groups, each consisting of 5 replicates of 12 birds (8 females and 4 males). Three groups were subjected to feed restriction (FR) starting from the second week of age, specifically on days 7, 9, 11, and 13. FR was applied at 0% (control), 12.5% (12.5FR) and 25.0% (25FR) of the feed intake recorded in the previous day for the control group. The other three groups received the same diets as control, 12.5FR and 25FR groups but were supplemented with coated organic acid with essential oils (COAWEO) at 100 mg/kg feed during the growing and laying period. All the groups were fed the same basal diet in mash form up to 18 weeks of age. The COAWEO is manufactured by Biofeed (https://www.biofeedtech.com/en/products.html produced by the DACIDS G2P; Idena, Sautron, France) and its composition was: coated citric acid at 115 g, coated lactic acid 72 g, coated malic acid 83 g, coated sorbic acid 72 g, coated formic acid 184 g, and thymol extract 25 g, berberine extracts 30 g and 5'-methoxyhydnocarpin and hydrogenated palm oil up to 1000 g. The COAWEO replaced the same amount of yellow corn and was well mixed with the feed every week. The additive was added at the top of the diet and mixed well with the diet to assure homogeneity. The ingredients and chemical-nutritional characteristics of the diets are reported in Table 1. Chemical analysis (crude protein, ether extract and crude fiber) were performed according to AOAC (2012) official procedures. The other nutritional characteristics reported in Table 1 were calculated according to CVB (2018).

The experimental diets were fed without antibiotics or supplementation of anticoccidial drugs.

Table 1. Ingredients and chemical-nutritional characteristics of the basal diet

Ingredients (%)	
Yellow corn	56.1
Soybean meal	26.5
Corn gluten	8.50
Dicalcium phosphate	1.70
Limestone	6.00
Salt	0.50
Premix[1]	0.30
Methionine	0.10
Lysine	0.30
Total	100.0
Chemical-nutritional characteristics	
Metabolizable energy (ME) (kcal/kg)[2]	2801
Crude protein (%)[3]	22.04
Ether extract (%)[3]	2.56
Crude fiber (%)[3]	3.19
Calcium (%)[2]	2.72
Available phosphorus (%)[2]	0.45
Lysine (%)	1.18
Methionine (%)[2]	0.49
Methionine + Cystine (%)[2]	0.86

[1]Each 3 kg of premix contained: Vit. A, 12,000,000 IU; Vit. D_3, 3,500,000 IU; Vit. E, 20 g; Vit. K_3, 3 g; Vit. B_1, 3 g; Vit. B_2, 8 g; Vit. B_6, 3 g; Vit. B_{12}, 15 mg; Ca pantothenate, 12 g; Niacin, 40 g; Folic acid, 1.5 g; Biotin, 50 mg; Choline chloride, 600 g; Mn, 80 g; Zn, 75 g; Fe, 40 g; Cu, 10 g; I, 2 g; Se, 0.3 g; Co, 0.25 g and $CaCo_3$ as a carrier.
[2]Calculated according to CVB (2018).
[3]Determined value according to AOAC (2012).

The chickens were housed in battery brooders, and each cage/replicate was equipped with one tube feeder and two still-stain nipple cup waterers. The chicks were offered 16:8 light-dark cycles during the experimental period.

Quails performance and egg quality

Feed intake (FI), final body weight (FBW), and body weight changes (BWC) were recorded at the end of the experimental period.

From 6 to 18 weeks of age, the eggs number and weight (to the nearest g) per replicate were recorded daily and egg mass was estimated by multiplying the number of eggs in each cage by the average egg weight. The feed conversion ratio (FCR) was calculated every four weeks and for the whole experimental period (6−18 weeks of age) as follows: FCR = feed intake (g)/egg mass (g).

Egg quality measurements (egg shape index, specific gravity, shell weight and percent, shell thickness, shell weight per unit of surface area – SWUSA, yolk and albumen weight and height, and Haugh unit score) were determined at 17th week of age using 15 eggs randomly sampled from each treatment according to Attia et al. (1994).

Fertility and hatchability percentage

At the 10, 12, 14, and 16 weeks of the experimental period 132 eggs per treatment were collected for a total of 3,168 eggs (132 eggs × 4 weeks × 6 treatments). The eggs were incubated and hatched in a Chick Master incubator and hatching machine (Chick Master Incubator Co., 945 Lafayette Rd., Medina, OH 44256, USA) according to Attia et al. (1994). The hatched chicks were counted, and non-hatched eggs were broken to determine the percentage of fertile eggs and hatchability. Fertility and hatchability percentages were calculated as follows: Fertility % = number of fertile eggs (total eggs set – unfertile eggs)/total eggs set × 100

Hatchability % = number of hatched chicks/total number of fertile eggs × 100

Carcass traits

A total number of 10, 18-week-old JQ hens were randomly selected from each group to represent all treatment replicates and slaughtered after 8 h of feed removal. JQ were slaughtered according to the Islamic method. The feathers were bulked and carcasses eviscerated to calculate the yield, total edible and inedible parts, and dressing percentage relative to live body weight. The internal organs were separated, weighed and expressed relative to live body weight.

Blood biochemistry

At slaughter (18 weeks of age), 10 blood samples were randomly collected from each replicate of each group. Serum was separated by centrifugation at 4,000 rpm for 10 minutes and stored at -18°C for analysis. Total serum protein, TP (Henry, 1964) and albumin, Alb (Doumas et al., 1971) were determined, while globulin (Glb) was calculated by subtracting Alb from TP; thus the albumin/globulin ratio. Various kinds of globulin (α-, β- and γ-globulin) were estimated as reported by Elias (1981).

Total lipids (TL) were determined using the method of Chabrol and Charonnat (1973). Serum lipid profile such as triglycerides, TG (Tietz, 1995), total cholesterol, TC (Allain et al., 1974), and high-density lipoprotein cholesterol, HDL (Sawle et al., 2002), were determined using specific diagnostic kits (Diamond Diagnostics Kft. 1044 Budapest, Hungary, Óradna Street 6). Serum low-density lipoprotein cholesterol (LDL-C) was also assessed by Friedewald et al. (1972). LDL-C = total cholesterol – (HDL-C vLDL), where very low-density lipoprotein vLDL = serum triglycerides/5. Liver enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were evaluated according to Reitman and Frankel (1957). Total antioxidant capacity (TAC), malondialdehyde (MDA), and superoxide dismutase (SOD) were determined by Koracevic et al. (2001), Mihara and Uchiyama (1978) and Yi-Sun and Li (1988), respectively. The corticosterone (Cort) concentration in blood serum was determined (Jezová et al., 1994). Alkaline phosphatase, erythrocyte catalase, triiodothyronine (T3) and thyroxine (T4) levels, immunoglobulin G (IgG), M (IgM) and A (IgA) were determined using an automated analyzer with commercial kits from Bio-Diagnosis Company (Giza, Egypt), according to the manufacturing procedure. The hemagglutination inhibition (HI) test for infectious bursa disease (IBD) and avian influenza (AI) were determined according to Cosgrove (1962). The assay is designed to measure IBD antibody bound to influenza antigen coated plates. HI for NDV and avian influenza was evaluated according to Takatsy and Hamar (1956).

Interferon gamma was determined according to Abiko et al. (2015). Interleukin 1 (IL1) was measured using ELISA kits (Kamiya Biomedical Co, WA, USA), while interleukin 6 (IL6) was measured using ELISA kits purchased from Genorise Scientific, Inc. (Berwyn, PA, USA) according to Wajant et al. (2003).

Gut microbiota

The intestinal microbiota was evaluated using ten samples of each treatment collected from ceca of the slaughtered hens at week 18 of age. For *Salmonella* isolation and identification, 1 g of each fecal sample was homogenized in 9 ml of buffered peptone water (BPW) and incubated at 37°C for 20 h; then the incubated samples were gently shacked and added to Rappaport-Vassiliadis broth (Oxoid, Basingstoke, UK) at 1:100 and incubated at 42°C for further 24 h followed by streaking on Xylose Lysine Desoxycolates at 37°C for 24 h. The plates were examined after incubation for typical and suspected colonies. Up to five colonies from each sample were further identified based on biochemical characterization (indole test, motility, nitrate reduction, methyl red, Voges-Proskauer, citrate utilization, and urease) according to ISO 6579: 2002 guidelines. Biochemically typical *Salmonella* isolates were sero-typed according to White–Kauffmann–Le Minor technique using slide agglutination technique with polyvalent somatic (O) and flagellar (H) antisera (Welcome Diagnostic, UK), which were performed at the Faculty of Agriculture, Mansoura University following the White–Kauffmann–Le Minor scheme (Grimont and Weill, 2007).

To evaluate the number of *E. coli*, one gram of each fecal sample was aseptically added to 9 ml of tryptic soya broth (TSB/CM129, Oxoid, Hants, UK) into a stomacher bag for at least 2 min, and then samples were incubated at 37°C for 24 h according to De Boer and Heuvelink (2000). Loopful from the incubated broth was streaked onto the surface of EMB agar (CM69, Oxoid, Hants, UK). After 24 h at 37°C, the colonies of *E. coli* were confirmed biochemically, followed by O-serotyping. However, one gram was aseptically added to 9 ml of 0.1% sterile peptone solution to prepare a ten-fold serial dilution of up to 107 ml of each sample.

Viable counts of total aerobes, coliforms, and lactobacilli were performed using the spread-plate technique. Total aerobes were enumerated on nutrient agar (Oxoid, Hants, UK), and coliform was enumerated on MacConkey agar; the plates were incubated aerobically at 37°C

for 24 h. For lactobacilli, deMan, Rogosa, and Sharpe (MRS) agar (Biolife, Milan, Italy) was used; the plates were incubated in 5% CO^2 for 48 h. The media plates were inoculated with 0.1 ml of the sample dilutions. After incubation, colonies were counted according to colony morphology. Numbers of CFU per gram of the digesta content were recorded (Mahdavi et al., 2010).

For the isolation of *Clostridium*, the inoculation of fecal samples was carried out in a cooked meat medium (Becton, Dickinson and Company, USA). The samples were then anaerobically incubated at 37°C for 24 h in an anaerobic jar (Oxoid Limited, Thermo Fisher Scientific Inc., UK) containing GasPakTM (Becton, Dickinson and Company, USA) according to Dar et al. (2017). Enriched samples were streaked on sulphite polymixin sulphadiazine (SPS) agar plates (Hi-Media Laboratories, Mumbai) and anaerobically incubated as previously mentioned. Suspected colonies were stained with Gram's stain and subcultured on brain heart infusion (BHI) agar plates until they were free from contaminating bacteria. The pure colonies, suggestive of *Clostridium*, were further streaked on the 5% sheep blood agar and egg yolk agar plates and anaerobically incubated for 24 h. The colonies producing characteristic double zone of hemolysis around them on blood agar and producing zone of opalescence around the colonies on egg yolk agar were tentatively identified as *Clostridium*.

Statistical analysis

Statistical data processing was performed with an analysis of variance (ANOVA) using the GLM procedure of the Statistical Analysis System (SAS, 2004). Data on *in vivo* performance, carcass traits, egg quality, blood profiles, and microbial count were processed by a two-way ANOVA according to the following model:

$$Y_{ijk} = m + F_i + A_j + FA_{ij} + e_{ijk}$$

where: Y_{ijk} = observed characteristics; m = overall mean; F_i = effect of feed restriction (i = 0, 12.5 and 25.0%); A_j = effect of feed additive (j = 0, 1); FA_{ij} = interactive effect of feed restriction and nutritional additives; e_{ijk} = experimental random error.

Data on fertility and hatchability were processed using a three-way ANOVA, according to the following model:

$$Y_{ijkl} = m + F_i + A_j + W_k + FA_{ij} + FW_{ik} + AW_{jk} + FAW_{ijk} + e_{ijkl}$$

where: Y_{ijkl} = observed characteristics; m = overall mean; F_i = effect of feed restriction (i = 0, 12.5 and 25.0 %); A_j = effect of feed additive (j = 0, 1); W_k = week of age (k = 10, 12, 14 or 16); FA_{ij} = interactive effect of feed restriction and nutritional additives; FW_{ik}: interactive effect of feed restriction and week of age; AW_{jk} = interactive effect of additive and week of age; FAW_{ijk} = interactive effect of feed restriction, additive and week of age; e_{ijkl} = experimental random error.

The experimental unit was the replicate. Mean differences were tested using Tukey post-hok at $P<0.05$.

Results

Table 2 showed that FR during the growing period and continuous feed additive supplementation during the growing and laying periods did not affect FBW, BWC, egg production (EP), egg weight (EW), egg mass, and FCR of birds. However, feed intake (FI) of quails was significantly affected by FR as the control group consumed more feed than 12.5% FR group. The 25.0% groups showed an intermediate feed intake. Feed additive did not affect the FI of quails. The interaction between FR and feed additive was independent for all laying performance of quails.

Table 2. Effects of feed restriction and additive on performance of Japanese quail laying hens (6−18 weeks of age)

Treatments	IW g	FW g	BWC g	EP %	EW g	EM g	FI g/hen	FCR g/g
1	2	3	4	5	6	7	8	9
Feed restriction								
0.0 (%)	240	276	35.9	68.4	12.3	705	3319 a	4.76
12.5 (%)	246	277	30.9	65.4	12.2	669	3259 b	4.92
25 (%)	241	276	35.0	68.9	12.2	707	3302 ab	4.70
COAWEO								
Uns	243	274	31.8	67.7	12.2	694	3304	4.81
Sup	242	278	36.1	67.4	12.2	693	3283	4.78
Interactions								
0.0 × Uns	239	273	33.5	66.6	12.3	689	3326	4.91
0.0 × Sup	240	279	38.3	70.3	12.2	721	3311	4.61
12.5 × Uns	246	274	27.4	67.3	12.2	688	3279	4.80

Table 2 – contd.

1	2	3	4	5	6	7	8	9
12.5 × Sup	246	281	34.4	63.5	12.2	650	3239	5.04
25.0 × Uns	242	276	34.4	69.3	12.1	706	3306	4.73
25.0 × Sup	240	276	35.7	68.5	12.3	708	3299	4.68
RMSE	8.81	11.20	13.38	6.73	0.232	72.8	43.6	0.517
P values								
Feed restriction	0.242	0.946	0.676	0.457	0.745	0.427	0.015	0.624
COAWEO	0.902	0.338	0.381	0.889	0.603	0.964	0.208	0.853
Interaction	0.929	0.710	0.889	0.472	0.459	0.561	0.675	0.505

IW: initial weight; FW: final weight; BWC: body weight changes; EP: egg production; EW: egg weight; EM: egg mass; FI: feed intake; FCR feed conversion ratio; a–b: means within a column with different letters are significantly different; RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 3. Effects of feed restriction and additive on carcass traits of Japanese quail laying hens (18 weeks of age)

Treatments	Carcass %	Liver %	Gizzard %	Heart %	Giblet %	TEP %	NEP %
Feed restriction							
0.0 (%)	70.4	2.12	1.43	0.897	4.44	74.8	25.2
12.5 (%)	71.7	2.19	1.19	0.873	4.25	76.0	24.0
25.0 (%)	70.8	2.09	1.38	0.939	4.41	75.3	24.7
COAWEO							
Uns	70.2	2.02	1.31	0.910	4.24	74.5	25.5
Sup	71.7	2.24	1.36	0.896	4.50	76.2	23.8
Interactions							
0.0 × Uns	70.4	2.03	1.47	0.814	4.31	74.7	25.3
0.0 × Sup	70.4	2.21	1.39	0.980	4.58	75.0	25.0
12.5 × Uns	71.6	2.06	1.04	0.874	3.98	75.5	24.5
12.5 × Sup	71.8	2.31	1.35	0.872	4.53	76.4	23.6
25.0 × Uns	68.8	1.97	1.42	1.042	4.43	73.2	26.8
25.0 × Sup	72.9	2.20	1.35	0.836	4.39	77.3	22.7
RMSE	2.9752	0.3889	0.2773	0.1707	0.6136	3.0984	3.0984
P values							
Feed restriction	0.613	0.838	0.150	0.686	0.761	0.720	0.720
COAWEO	0.184	0.130	0.594	0.824	0.258	0.136	0.136
Interaction	0.240	0.984	0.224	0.070	0.556	0.347	0.347

TEP: total edible parts; NEP: non edible parts; RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 3 showed that FR, feed additive, and their interaction did not affect carcass and organs weight percentage of JQ.

Table 4 shows that feed restrictions and/or feed additives did not markedly affect egg weight. However, the egg shape index was significantly decreased by only 12.5% FR compared to the control groups. All shell quality measurements were improved considerably due to FR levels during 2nd week of the growing period compared to the control group, except for shell thickness which increased only due to 12.5% FR level.

Table 5 showed feed restriction did not influence internal egg quality traits of Japanese quail hens except for albumin percentage. The albumen % of the control group was significantly higher than only those FR at 12.5%. Feed additive and/or its interaction with FR did not affect all internal egg quality traits.

Table 6 showed that FR did not influence percentage fertility, pipped %, dead chick, hatchability, and hatchability of fertile eggs. Feed additives significantly affected fertility, pipped, and hatchability of fertile eggs % except for dead % and total hatchability %. Where fertility % and pipped % of the control group were higher than those of the group fed diet supplemented with coated organic acid with essential oils, as well as the hatchability of fertile eggs was higher.

Table 4. Effects of feed restriction and additive on external egg quality traits of Japanese quail laying hens (17 weeks of age)

Treatments	EW g	ESI %	ST mm	SW g	Shell %	SWUSA, mg/cm^2	ESG
Feed restriction							
0.0 (%)	13.7	78.2 a	0.225 b	1.53 b	11.2 b	60.8 b	1.09 b
12.5 (%)	14.0	75.9 b	0.242 a	1.72 a	12.3 a	67.4 a	1.10 a
25.0 (%)	14.1	77.5 ab	0.230 ab	1.71 a	12.2 a	66.7 a	1.10 a
COAWEO							
Uns	13.8	77.2	0.232	1.64	11.9	64.8	1.10
Sup	14.1	77.2	0.233	1.67	11.9	65.1	1.10
Interactions							
0.0 × Uns	13.7	78.4	0.227	1.54	11.3	61.1	1.09
0.0 × Sup	13.6	78.0	0.227	1.51	11.2	60.5	1.09
12.5 × Uns	13.8	76.1	0.241	1.73	12.6	68.3	1.10
12.5 × Sup	14.3	75.8	0.243	1.72	12.1	66.5	1.10
25.0 × Uns	13.8	77.1	0.227	1.65	11.9	65.0	1.10
25.0 × Sup	14.3	77.9	0.234	1.78	12.5	68.5	1.10
RMSE	1.20	3.43	0.023	0.14	1.05	5.09	0.006
P values							
Feed restriction	0.339	0.038	0.015	0.0001	0.0001	0.0001	0.0002
COAWEO	0.258	0.939	0.749	0.259	0.994	0.718	0.997
Interaction	0.488	0.759	0.635	0.058	0.198	0.111	0.180

EW: egg weight; ESI: egg shape index; ST: shell thickness; SW: shell weight; SWUSA: shell weight per unit surface area; ESG: egg specific gravity; a–b: means within a column with different letters are significantly different; RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 5. Effects of feed restriction and additive on internal egg quality traits of Japanese quail laying hens (17 weeks of age)

Treatments	Yolk %	YI %	Yolk color score	Albumen %	HU
Feed restriction					
0.0 (%)	31.9	45.8	7.13	56.9 a	96.0
12.5 (%)	32.9	45.6	7.10	54.8 b	96.0
25.0 (%)	32.4	44.8	7.23	55.4 ab	96.0
COAWEO					
Sup	32.7	45.8	7.18	55.3	95.7
Uns	32.0	45.0	7.13	56.0	96.3
Interactions					
0.0 × Uns	32.3	45.2	7.13	56.5	94.7
0.0 × Sup	31.5	46.4	7.13	57.4	97.2
12.5 × Uns	33.7	46.7	7.00	53.8	96.3
12.5 × Sup	32.2	44.5	7.20	55.7	95.8
25.0 × Uns	32.3	45.6	7.27	55.7	96.1
25.0 × Sup	32.5	44.1	7.20	55.0	95.8
RMSE	2.45	3.47	0.75	2.73	1.43
P values					
Feed restriction	0.251	0.524	0.774	0.009	0.981
Feed additive	0.184	0.250	0.779	0.233	0.067
Interaction	0.387	0.153	0.774	0.159	0.0001

YI: yolk index; HU: Haugh score; a–b: means within a column with different letters are significantly different; RMSE: root mean square error COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 6. Effects of feed restriction, feed additive, and age on egg fertility and hatchability traits of Japanese quail laying hens at 10, 12, 14, and 16 weeks of age

Treatments	Fertility %	Pipped %	Dead %	Total hatchability %	Hatchability of fertile eggs %
Feed restriction					
0.0 (%)	60.0	3.38	3.41	53.2	88.7
12.5 (%)	59.1	4.35	2.84	51.9	88.0
25.0 (%)	54.3	3.69	2.66	48.0	88.1
COAWEO					
Uns	59.8 a	4.56 a	2.88	52.3	87.6 b
Sup	55.8 b	3.05 b	3.06	49.7	88.9 a
Week of age					
10	53.7 b	2.99 b	2.86 ab	47.9 bc	89.1 ab
12	64.2 a	5.35 a	4.35 a	54.5 ab	84.9 c
14	48.2 b	3.67 b	2.00 b	42.6 c	88.2 b
16	65.0 a	3.22 b	2.67 b	59.1 a	90.8 a
Interaction Feed restriction × COAWEO					
0.0 × Uns	63.8	3.83 ab	3.54	56.5	88.3
0.0 × Sup	56.2	2.93 b	3.28	50.0	89.0
12.5 × Uns	61.6	5.79 a	2.24	53.6	87.2
12.5 × Sup	56.5	2.92 b	3.43	50.2	88.8
25.0 × Uns	53.8	4.08 ab	2.86	46.9	87.2
25.0 × Sup	54.8	3.30 b	2.46	49.0	89.0
Interaction Feed restriction × Week of age					
0.0 × 10	57.1	3.46	3.47	50.2	87.8
0.0 × 12	64.5	4.04	3.84	56.7	87.9
0.0 × 14	51.3	2.33	3.33	45.7	88.9
0.0 × 16	67.0	3.67	3.00	60.3	90.1
12.5 × 10	51.9	2.53	2.57	46.8	90.2
12.5 × 12	68.3	7.22	4.44	56.7	82.9
12.5 × 14	50.7	4.67	1.33	44.7	88.2
12.5 × 16	65.3	3.00	3.00	59.3	90.6
25.0 × 10	52.2	2.98	2.54	46.7	89.3
25.0 × 12	59.7	4.77	4.77	50.2	84.0
25.0 × 14	42.7	4.00	1.33	37.3	87.4
25.0 × 16	62.7	3.00	2.00	57.7	91.6
Interaction COAWEO × Week of age					
Uns × 10	54.3	3.35	2.82	48.1	88.6
Uns × 12	70.1	6.68	4.04	59.3	84.7
Uns × 14	49.3	4.44	2.00	42.9	86.9
Uns × 16	65.3	3.78	2.67	58.9	90.0
Sup × 10	53.2	2.62	2.90	47.7	89.6
Sup × 12	58.3	4.02	4.66	49.6	85.2
Sup × 14	47.1	2.89	2.00	42.2	89.4
Sup × 16	64.7	2.67	2.67	59.3	91.5
RMSE	8.18	1.58	1.88	7.90	2.54
P values					
Feed restriction	0.053	0.101	0.361	0.066	0.623
COAWEO	0.047	0.0002	0.696	0.170	0.028
Week of age	0.0001	0.0002	0.004	0.0001	0.0001
Interaction FR×C	0.184	0.044	0.280	0.170	0.774
Interaction FR×W	0.773	0.018	0.517	0.908	0.022
Interaction C×W	0.148	0.298	0.951	0.192	0.680

a–b: means within a column with different letters are significantly different; RMSE: root mean square error COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 7. Effects of interaction among feed restriction, feed additive and age on egg fertility and hatchability traits of Japanese quail laying hens

Treatments	Fertility %	Pipped %	Dead %	Hatchability %	Hatchability of fertile eggs %
Interaction feed restriction × COAWEO × week of age					
0.0 × Uns × 10	59.1	4.05	3.28	51.8	87.4
0.0 × Uns × 12	73.5	3.92	4.90	64.7	88.0
0.0 × Uns × 14	49.3	2.67	3.33	43.3	87.7
0.0 × Uns × 16	73.3	4.67	2.67	66.0	90.0
0.0 × Sup × 10	55.1	2.87	3.66	48.6	88.1
0.0 × Sup × 12	55.5	4.17	2.78	48.6	87.7
0.0 × Sup × 14	53.3	2.00	3.33	48.0	90.1
0.0 × Sup × 16	60.7	2.67	3.33	54.7	90.2
12.5 × Uns × 10	53.1	2.71	2.75	47.6	89.7
12.5 × Uns × 12	73.3	11.11	2.22	60.0	81.7
12.5 × Uns × 14	55.3	6.00	1.33	48.0	86.8
12.5 × Uns × 16	64.7	3.33	2.67	58.7	90.7
12.5 × Sup × 10	50.7	2.34	2.38	46.0	90.7
12.5 × Sup × 12	63.3	3.33	6.67	53.3	84.2
12.5 × Sup × 14	46.0	3.33	1.33	41.3	89.7
12.5 × Sup × 16	66.0	2.67	3.33	60.0	90.5
25.0 × Uns × 10	50.6	3.30	2.43	44.9	88.6
25.0 × Uns × 12	63.3	5.00	5.00	53.3	84.4
25.0 × Uns × 14	43.3	4.67	1.33	37.3	86.3
25.0 × Uns × 16	58.0	3.33	2.67	52.0	89.4
25.0 × Sup × 10	53.7	2.65	2.65	48.4	90.0
25.0 × Sup × 12	56.0	4.55	4.55	47.0	83.6
25.0 × Sup × 14	42.0	3.33	1.33	37.3	88.5
25.0 × Sup × 16	67.3	2.67	1.33	63.3	93.8
RMSE	8.18	1.58	1.88	7.90	2.54
P values					
Interaction FR×COAWEO×week	0.428	0.01	0.226	0.419	0.674

RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 8. Effects of feed restriction and additive on serum blood parameters of Japanese quail laying hens at 18 weeks of age

Treatments	TP g/dl	Albumin g/dl	Globulin g/dl	A/G	α-Glb g/dl	β-Glb g/dl	γ-Glb g/dl
Feed restriction							
0.0 (%)	5.48	2.61	2.87	0.929	1.69	10.2	0.859
12.5 (%)	5.51	2.60	2.91	0.915	1.72	10.6	0.829
25.0 (%)	5.50	2.69	2.82	0.984	1.71	10.3	0.869
COAWEO							
Uns	5.48	2.65	2.83	0.950	1.67	10.2	0.823
Sup	5.51	2.61	2.90	0.935	1.74	10.5	0.881
Interaction							
0.0 × Uns	5.45	2.51	2.94	0.864	1.63	9.9	0.745
0.0 × Sup	5.52	2.71	2.81	0.994	1.75	10.5	0.972
12.5 × Uns	5.42	2.70	2.72	1.004	1.72	10.8	0.819
12.5 × Sup	5.59	2.50	3.09	0.826	1.71	10.4	0.839
25.0 × Uns	5.59	2.75	2.84	0.982	1.67	9.9	0.905
25.0 × Sup	5.42	2.63	2.79	0.985	1.75	10.7	0.832
RMSE	0.29	0.26	0.41	0.20	0.14	1.37	0.14
P values							
Feed restriction	0.980	0.725	0.883	0.718	0.903	0.805	0.805
COAWEO	0.797	0.707	0.679	0.839	0.179	0.486	0.273
Interaction	0.412	0.209	0.361	0.234	0.544	0.572	0.071

TP: total protein; A/G: albumin to globulin ratio; α-Glb: alpha globulin; β-Glb: beta globulin; γ-Glb: gamma globulin; RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Tables 8, 9, 10, and 11 showed that serum blood characteristics and immune blood were not affected by feed restriction except for HDL and HI IBD. Serum blood HDL in the control group was high value without difference with 25% restricted feed group, followed by 12.5% feed restriction group (Table 9). Serum blood HI IBD in the control group was increased without difference with the 12.5% feed restriction group, followed by the 25.0% feed restriction group (Table 11). Feed additives did not affect serum blood characteristics except for decreased total lipids and increased IL 6. Interaction between feed restriction and coated organic acid with essential oils supplementation did not affect serum blood characteristics.

Table 9. Effects of feed restriction and additive on serum blood parameters of Japanese quail laying hens at 18 weeks of age

Treatments	TL mg/dl	Tri mg/dl	Chol mg/dl	HDL mg/dl	LDL mg/dl	HDL/LDL	vLDL mg/dl	ALT U/l	AST U/l	ALT/AST
Feed restriction										
0.0 (%)	5.95	180	210	41.3 a	91.5	0.453	36.0	67.3	58.1	1.16
12.5 (%)	5.90	178	209	37.5 b	91.0	0.415	35.6	66.3	57.9	1.15
25 (%)	6.04	180	205	39.3 ab	91.0	0.435	36.0	66.3	57.5	1.16
COAWEO										
Uns	6.30 a	177	206	38.6	91.1	0.425	35.5	66.8	57.9	1.16
Sup	5.63 b	181	209	40.2	91.2	0.443	36.3	66.5	57.8	1.15
Interactions										
0.0 × Uns	6.25	181	214	40.2	88.9	0.454	36.3	68.0	58.2	1.17
0.0 × Sup	5.66	179	206	42.3	94.0	0.452	35.7	66.7	58.1	1.15
12.5 × Uns	6.29	177	204	37.5	91.0	0.415	35.3	66.3	57.9	1.15
12.5 × Sup	5.51	179	213	37.5	91.0	0.415	35.9	66.3	57.9	1.15
25.0 × Uns	6.37	174	200	37.9	93.5	0.407	34.8	66.1	57.6	1.15
25.0 × Sup	5.72	186	209	40.6	88.6	0.462	37.3	66.5	57.4	1.16
RMSE	0.67	6.75	9.41	2.80	6.12	0.050	1.36	1.476	2.67	0.061
P values										
Feed restriction	0.895	0.716	0.420	0.024	0.982	0.256	0.707	0.212	0.870	0.916
COAWEO	0.011	0.115	0.355	0.131	0.965	0.351	0.109	0.558	0.909	0.929
Interaction	0.947	0.063	0.062	0.537	0.211	0.379	0.067	0.451	0.997	0.857

TL: total lipids; Tri: triglycerides; Chol: cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein-cholesterol; VLDL: very low-density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; a–b: means within a column with different letters are significantly different; RMSE: root mean square error. COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 10. Effects of feed restriction and additive on serum blood parameters of Japanese quail laying hens at 18 weeks of age

Treatments	AP U/100ml	CBR U/ml	TAC Umol/l	MAD Umol/l	Antioxidant balance	SOD U/mg	T_3 Ug/100ml	T_4 Ug/100ml	T_3/T_4 ratio	Corticosterone pg/ml
1	2	3	4	5	6	7	8	9	10	11
Feed restriction										
0.0 (%)	7.58	38.7	424	1.09	395	3125	0.1712	7.83	2.19	3.91
12.5 (%)	7.40	38.2	425	1.04	415	3123	0.1737	7.86	2.21	4.17
25.0 (%)	7.63	38.8	425	1.09	397	3131	0.1732	7.63	2.27	4.15
COAWEO										
Uns	7.49	38.2	426	1.06	405	3126	0.1732	7.75	2.23	4.00
Sup	7.58	38.9	424	1.07	399	3127	0.1722	7.79	2.21	4.16
Interactions										
0.0 × Uns	7.53	38.5	427	1.07	406	3126	0.1718	7.75	2.22	3.45 b
0.0 × Sup	7.63	38.9	421	1.11	384	3123	0.1706	7.90	2.16	4.36 a
12.5 × Uns	7.40	37.8	425	1.06	405	3123	0.1818	7.86	2.31	4.17 ab
12.5 × Sup	7.40	38.6	425	1.01	425	3123	0.1656	7.86	2.11	4.17 ab
25.0 × Uns	7.54	38.2	425	1.06	405	3128	0.1660	7.64	2.18	4.36 a
25.0 × Sup	7.71	39.3	425	1.11	389	3133	0.1804	7.62	2.36	3.95 ab
RMSE	0.28	2.02	5.65	0.13	48.7	12.2	0.036	0.42	0.44	0.45

Table 10 – contd.

1	2	3	4	5	6	7	8	9	10	11
P values										
Feed restriction	0.192	0.781	0.933	0.616	0.601	0.378	0.987	0.451	0.912	0.358
COAWEO	0.399	0.330	0.314	0.849	0.736	0.894	0.940	0.764	0.865	0.328
Interaction	0.799	0.932	0.373	0.652	0.576	0.752	0.640	0.877	0.629	0.011

AP: alkaline phosphatase; CBR: catalase in red blood cell; TAC: total antioxidant capacity; MAD: malondialdehyde; SOD: superoxide dismutase; T3: triiodothyronine, T4: thyroxine; antioxidant balance = total antioxidant capacity/malondialdehyde; a–b: means within a column with different letters are significantly different; RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 11. Effects of feed restriction and additive on immunity of Japanese quail laying hens at 18 weeks of age

Treatments	IgG, mg/100 ml	IgM, mg/100 ml	IgA, mg/100 ml	HI IBDV Log^2	HI AIV Log^2	Interferon gamma Pg/ml	Interleukin-1 Pg/m	Interleukin-6 Pg/m
Feed restriction								
0.0 (%)	999	253	83.8	3.40 a	3.50	5.40	18.2	11.0
12.5 (%)	999	257	82.1	2.60 ab	3.10	5.52	17.6	10.7
25.0 (%)	997	253	82.4	2.50 b	3.40	5.75	17.8	10.9
COAWEO								
Uns	998	257	80.6	2.60	3.53	5.54	17.5	10.6 b
Sup	1000	254	85.0	3.07	3.13	5.58	18.2	11.1 a
Interactions								
0.0 × Uns	1001	251	82.1	2.80	3.80	5.34	17.3	10.9
0.0 × Sup	997	254	85.6	4.00	3.20	5.46	19.1	11.1
12.5 × Uns	998	260	79.5	2.60	3.40	5.54	17.0	10.4
12.5 × Sup	1001	254	84.7	2.60	2.80	5.51	18.2	11.0
25.0 × Uns	994	260	80.2	2.40	3.40	5.73	18.2	10.6
25.0 × Sup	1001	255	84.6	2.60	3.40	5.76	17.3	11.2
RMSE	9.64	11.24	5.13	0.753	0.764	0.655	2.772	0.443
P values								
Feed restriction	0.890	0.582	0.726	0.026	0.486	0.501	0.894	0.403
COAWEO	0.583	0.477	0.029	0.103	0.164	0.869	0.494	0.009
Interaction	0.480	0.603	0.942	0.183	0.604	0.967	0.521	0.516

a–b: means within a column with different letters are significantly different; RMSE: root mean square error; IgG: immunoglobulin G; IgM: immunoglobulin M; IgA: immunoglobulin A; HI IBDV: hemagglutination inhibition test for infectious bursal disease virus; HI AIV: hemagglutination inhibition test for avian influenza virus; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 12. Microbial count of Japanese quail laying hens

Treatments	TBC, CFU/mL	*E. coli*, CFU/mL	*Clostridium*, CFU/mL	*Salmonella*, CFU/mL	*Lactobacillus*, CFU/mL
Feed restriction					
0.0 (%)	10.36	5.23	4.60	4.27	4.98
12.5 (%)	10.27	5.19	4.46	4.04	5.32
25.0 (%)	10.14	5.03	4.28	4.03	5.15
COAWEO					
Uns	10.41	5.50 a	5.11 a	4.80 a	4.12 b
Sup	10.10	4.79 b	3.79 b	3.42 b	6.17 a
Interactions					
0.0 × Uns	10.73	5.80	5.45	5.02	3.90
0.0 × Sup	10.00	4.66	3.76	3.51	6.06
12.5 × Uns	10.16	5.53	4.95	4.81	4.08
12.5 × Sup	10.11	4.84	3.97	3.27	6.21
25.0 × Uns	10.34	5.18	4.92	4.57	4.39
25.0 × Sup	10.20	4.89	3.64	3.49	6.25
RMSE	0.586	0.274	0.325	0.616	0.449
P values					
Feed restriction	0.693	0.269	0.101	0.635	0.268
COAWEO	0.163	0.0001	0.0001	0.0001	0.0001
Interaction	0.390	0.008	0.068	0.663	0.714

TBC: total bacterial count; a–b: means within a column with different letters are significantly other; RMSE: root mean square error; COAWEO: coated organic acids + essential oils; Uns: unsupplemented; Sup: supplemented.

Table 12 shows the effects of feed restriction and coated organic acid with essential oils on microbial counts of Japanese quails. Feed restriction did not significantly affect total microflora counts, *E coli*, *Clostridia, Salmonella,* and *Lactobacillus*. Dietary coated organic acid with essential oils supplementation has significantly decreased *E coli*, *Clostridia, Salmonella,* and increased *Lactobacillus*. The interaction between feed restriction and coated organic acid with essential oils was not considerably different in total microflora counts.

Discussion

Our results on *in vivo* performance agree partially with those of Bossolani et al. (2018), who found that quails reared under feed restriction (6 h/day) did not show differences for EP, FI, and FCR but showed a decreased IgM at 26–28 weeks of age compared with the group exposed to 16 h feeding period per day. Al-Khatib et al. (2017) found that growing quails exposed to FR did not change body weight (BW) and FCR, while the FI was decreased. JQ exposed to FR (15 and 30%) did not impact egg weight (EW) and LR % at 8, 10, 12, and 14 weeks of age (Fidan and Kaya, 2014), and FR at 90, 80, 70, and 60% of daily FI did not significantly affect BW and FI at 14 weeks of age (Ali et al., 2007). Hassan et al. (2003 a) demonstrated that FR (85 and 70% of daily feed intake during 2 to 5 weeks of age did not significantly affect BW and FCR at 13 weeks of age and BWG. In addition, 30% FR during 15–28 days did not affect first EW (Ocak and Erener, 2005). On the contrary, Elsanhoury et al. (2023) showed that quails fed twice and four times a day had substantially increased egg weight at 6–9 weeks of age and, which was similar to the control. Mahrose et al. (2020) indicated a significant positive effect on JQ hens' LR, EW, BWG, and FCR due to FR (30 g/birds/day), but BW decreased compared to the control group. Gholami and Nobakht (2017) showed that FR reduced growth, but improved FCR. Management of feeding time for quails did not significantly affect FCR, EW, but increased BW and EP at 20–24 weeks of age compared to the control group (Farghly, 2011). In addition, quails fed 60% of daily FI requirements significantly decreased egg weight and increased egg mass at 6–14 weeks of age (Ali et al., 2007). Differences in the responses to FR among different experiments could be attributed to breed of birds differences (chickens vs. quail vs. ducks), strain of chickens (broilers vs. layers), methods of FR and severity of FR and the time of applying the feed restriction.

According to Vishwanath et al. (2020), the quails' diets supplemented with butyric acid did not influence EW, BW, FI, or FCR compared with the group supplemented with Phytogrow and probiotics. Tchoffo et al. (2019) observed that ginger essential oil supplemented at 50, 100, 150 µl/kg body weight to quail diets did not significantly impact EP, EM, BW, FI, and FCR. Soltan (2008) reported that the organic acid mixture did not affect the FI and EW of quails at 54–70 weeks of age but improved EP, weight gain, and FCR. On the other hand, BW, FCR, EW, and EP were unaffected by the organic acid mixture supplemented in the quails' diet at 1.5, 3, and 4.5 g/kg diet (Kaya et al., 2015). An essential oil mixture (24 mg/kg feed) did not influence EW and FI but did improve FCR and EP compared to the control group (Çabuk et al., 2014). However, quail diets supplemented with organic acid increased BW, FI and improved FCR (Uddin et al., 2020).

Carcass traits were unaffected by dietary treatments. Al-Khatib et al. (2017) found that exposure of quails to FR did not significantly impact carcass trait percentages. Ocak and Erener (2005) observed that 30% FR during 15–28 days of age did not substantially affect carcass weight and carcass traits of quails. In addition, Uddin et al. (2020) showed that quail diets supplemented with organic acid did not influence carcass traits but increased carcass percentage and liver weight. On the other hand, Silva et al. (2022) showed that FR significantly reduced carcass trait percentages of quails, but carcass weight was not affected. In addition, Rønning et al. (2009) reported that feed restriction decreased effective body mass, liver, and kidney weight of quails.

Regarding the effect of feed additives, Hassan et al. (2015) and Tchoffo et al. (2019) observed that quail diets supplemented with ginger essential oil (50, 100, and 150 µl/kg body weight) did not markedly affect carcass parts percentages compared with the control group. Attia et al. (2013) also reported that quail diets supplemented with acetic acid did not affect carcass traits. A quail diet containing citric (0.6%), malic (0.6%), fumaric acids (0.6%), and a mixture of these acids (0.6%) did not significantly impact carcass and total edible part percentages (Abd EL-Latif et al., 2022). The ongoing discussion indicates the effect of FR and/or feed additives depends on the level of FR and type of additives, strain, and age of chickens.

The results obtained for egg quality indicate that FR has a significant positive carry-over effect on calcium metabolism. This novel finding needs further investigation due to its practical application for layer hens. The improvement of eggshell parameters could be suggestive of an improvement of Ca accumulation: changes in metabolic pathways of Ca under feed restriction conditions must be further investigated. Our results agree partially with those cited by Mahrose et al. (2020), who reported that quails exposed to FR (30 g and 35 g/bird/day) and fed *ad libitum* had increased shell thickness with no influence on shape index and eggshell % compared to other groups. Also, Elsanhoury et al. (2023) demonstrated that feeding quails twice, three, and four times a day did not significantly impact egg shape index and shell thickness compared to the control. However, Gholami and Nobakht (2017) showed that shell thickness was decreased in quail exposed to FR, but BWG and eggshell were enhanced. Farghly (2011) reported that management of feeding time did not significantly impact egg shape index and eggshells but increased shell thickness. However, 15% FR during 2 to 5 weeks of age had a significant positive

impact on egg-specific gravity compared to the quails exposed to 30% feed restriction (Hassan et al., 2003 a). On the other hand, Bossolani et al. (2018) found that rearing quails under FR (6 h/day) did not significantly affect shell quality. And, providing quails with 60% of daily FI requirements did not considerably impact egg shape index and shell quality at 14 weeks of age (Ali et al., 2007). The differences in the described trials can be tied to the species and/or the different FR regimen adopted.

Feed additive and the interaction between feed restriction and additive did not influence Japanese quail hens' external egg quality traits. Kaya et al. (2015) showed that egg shape index and shell quality measurements ESI and ST, were not affected by organic acid mixture supplemented (1.5, 3, and 4.5 g/kg diet) in quails' diet. Çabuk et al. (2014) found that an essential oil mixture (24 mg/kg feed) did not influence shell weight and thickness. In addition, Soltan (2008) reported that an organic acid mixture did not affect SW and ESI for quails at 54–70 weeks of age.

The results on internal egg quality agree with those of Mahrose et al. (2020), who reported that feeding quails restricted (35 g/bird/day) and *ad libitum* had increased albumin percentage but did not affect yolk % and yolk index % compared to other groups. Bossolani et al. (2018) found that rearing quails under FR (6 h/day) did not significantly affect yolk and albumin % at 26–28 weeks of age. Farghly (2011) reported that management of feeding time for quails did not considerably affect yolk %, albumin %, and yolk index % and increased HU. Ali et al. (2007) reported that quails fed 60% of daily FI did not significantly affect yolk weight, yolk index %, albumin weight, and HU at 14 weeks of age. Tchoffo et al. (2019) observed that quail diets supplemented with ginger essential oil did not significantly influence yolk weight. On the other hand, Elsanhoury et al. (2023) illustrated that quails fed four times a day had considerably decreased yolk index compared with other groups, which was similar to the control but increased albumin index.

Following the lack of significant effect of feed additives on the interior egg quality, Kaya et al. (2015) showed that yolk color, yolk index, albumin %, and HU were not affected by organic acid mixture supplemented in quails' diet (1.5, 3 and 4.5 g/kg diet). Also, Çabuk et al. (2014) found that an essential oil mixture (24 mg/kg feed) did not affect yolk color, albumin %, and HU but improved yolk index.

The interaction between feed restriction and coated organic acid with essential oils supplementation did not impact fertility and hatchability traits. Our results partially agree with those by Fidan and Kaya (2014) who showed that FR quails (15 and 30%) did not influence fertility and hatchability % at 8, 10, 12, and 14 weeks of age. In addition, Farghly (2011) reported that management of feeding time for quails did not significantly affect hatchability % while increased fertility % compared to the control. Also, 15 and 30% FR during 2 to 5 weeks of age did not substantially influence fertility and hatchability and pipped % and decreased dead % (Hassan et al., 2003 a). Henrich and Marks (1995) found that rearing quails under 30% FR at 1–44 days of age did not significantly impact hatchability %. On the other hand, Elsanhoury et al. (2023) illustrated that quails fed three times a day significantly increased fertility and hatchability % compared to the other group. Mahrose et al. (2020) observed that quails reared under FR (35 g/bird/day) improved fertility and hatchability percentages compared to other groups exposed to FR at 20 g, and 30 g/bird/day, and the control group, too.

Regarding feed additives affecting quail reproductive performance, Fouladi et al. (2017) showed that quail diets supplemented with organic acid increased hatchability percentage compared with the control. Attia et al. (2013) reported that quail diets supplemented with 0, 1.5, 3, and 6% acetic acid did not significantly impact Japanese quails' fertility and hatchability percentages. The effect of essential oils on reproductive performance was studied by Tchoffo et al. (2017) who reported that ginger essential oil added to quail diets (100, 150 µl/kg body weight) enhanced fertility, hatchability, and hatchability of fertile eggs % compared with used (50 µl/kg body weight) essential oil and control group but decreased chick dead %. In the literature, the effect of feed additives depends on breed of chickens (chickens vs. quail vs. ducks), strain of chickens (broilers vs. layers), type of feed additives, feed additive composition, and dose and age of chickens.

The age of quails significantly affected productive performance, resulting in the best fertility, total hatchability, and hatchability of fertile eggs at 16 weeks of age, and these enhancements are associated with significantly lower pipped and dead embryos. There was a significant FR by age interaction of quails in pipped embryos and the hatchability of fertile eggs %, showing that the effect of FR depends on the age of Japanese quail.

There were also three interactions on pipped embryos, showing that the highest incidence was at 12.5% FR supplemented without feed additives at 12 weeks of age, the contrary was shown of the same FR group supplemented with feed additives at 16 weeks. These indicate that the effect of FR and feed additives depends on the age of the chicks. In general, the results indicated that FR during the growing period and coated organic acids with essential oils during the growing and laying periods did not adversely impact the reproductive performance of Japanese quail.

Our results agree partially with Arslan et al. (2022), who demonstrated that a laying hen diet supplemented with essential oil (50, 100, and 200 mg/ kg diet) had increased value of serum blood AST due to higher FI and enhanced EP and did not affect serum blood ALT. Silva et al. (2022) showed that serum blood corticosterone, total protein, and albumin values were not affected by feed rationing for quails, but glucose value was reduced. Sözcü et al. (2021) reported that laying hens reared under feed restriction (120 g/day at 50–51 weeks of age) had a significant decrease in cholesterol, AST, ALT val-

ues but no effect on T3, T4, IgG, IgA, IgM. Mahrose et al. (2020) found quails exposed to feed restriction (35 g/bird/day) had increased total blood protein and albumin values without significant differences from the control. However, feed restriction (35 g/bird/day) of quails significantly reduced blood AST and cholesterol levels and did not affect total globulin, ALT, MDA and cholesterol. Sherif and Mansour (2019) reported that plasma concentration of complete protein, albumin, globulin, triglycerides, cholesterol, HDL, LDL, vLDL, TAC and MDA, AST and ALT were not affected by exposure of broiler chicks to feed restriction but it caused a significant increase in plasma levels of corticosterone. The different results obtained in the different trials could be tied to the species and/or the FR regimen applied.

Also, our results agree with Farouk et al. (2020) who found that the addition of essential oil in quail diets (150 and 300 mg/kg diet) did not significantly affect blood total protein, albumin, globulin, AST, ALT, HDL, LDL, cholesterol, and triglyceride. Kaya et al. (2015) showed that blood total protein, albumin, glucose, cholesterol, HDL, and alkaline phosphatase were not affected by the organic acid mixture supplemented in the quails' diet (1.5, 3, and 4.5 g/kg diet). Attia et al. (2013) reported that supplementing quails' diets with 0, 1.5, 3, and 6% of acetic acid did not significantly impact total plasma protein, globulins, albumin, AST, and ALT at 84 days old.

On the contrary, Rønning et al. (2009) reported that feed restriction had significantly decreased quails' plasma blood T3, and T4 values. Also, Tchoffo et al. (2019) observed that quail diets supplemented with ginger essential oil (50, 100, and 150 µl/kg body weight) had significantly reduced serum blood cholesterol, HDL, LDL, triglycerides, AST, ALT, creatinine, and MDA compared to the control group. Also, Bansod et al. (2020) reported that the addition 0.25% acetic acid + 0.25% butyric acid + 0.25% propionic acid in a quails diet had significantly reduced values of blood total protein, albumin, globulin, and cholesterol and did not affect triglyceride compared with the control group.

Our results agree with Artdita et al. (2021), who reported a pathogenic bacterium was significantly decreased by using feed restriction (20 and 40%) for laying hens and increased *Bacteroides* and *Rikenellaceae* compared to the control group. Wilkinson et al. (2020) found that exposure of the quails' to early inoculation *Lactobacillus* had a positive effect on quail intestines due to improved gut growth and immunity. Vishwanath et al. (2020) showed that butyric acid supplemented in quail diets did not influence *E. coli* and *C. perfringens* in ileum compared to the group supplemented with Phytogrow and probiotic. Mehdipour and Afsharmanesh (2018) reported that the quail diet supplemented with cinnamon oil (200 mg/kg diet) had reduced ileal coliform count, and enhanced ileal *Lactobacillus* count compared with a control group fed an antibiotic and cinnamon powder. Fouladi et al. (2017) showed that quail diets supplemented with organic acid had reduced *E. coli* and *Salmonella* compared to the control group.

However, Aliverdi-Nasab et al. (2023) demonstrated that acidifier supplementation on quail diets did not affect total aerobic, *Lactobacillus*, and *E. coli*. Bansod et al. (2020) showed that 0.25% acetic acid + 0.25% butyric acid + 0.25% propionic acid addition to quail diet significantly increased *E. coli* and *Salmonella* compared to the control group. Hassan et al. (2015) reported that an essential oil mixture in a quail diet did not significantly affect bacterial count, *E. coli*, *Streptococcus*, and *Lactobacillus*.

Conclusion

It can be concluded that 25.0% feed restriction during the 2nd week of the growing period supplemented with coated organic acid and essential oil during the growing and the laying periods considerably improved the sustainability of productive performance of Japanese quails without any adverse effect on egg production, quality, fertility and hatchability traits, serum biochemistry and immune markers, and gut microbiota.

Author contributions

RAA, YAA – funding, and project manager; YAA, HMA, SKhS, MAA and FB – experimental set-up, data collection methodology, statistical analyses, and drafting of the manuscript; HMA MAA and SKhS – lab analyses; RAA, FB, YAA, MAA, HMA and SKhS – proofread the manuscript. All authors have finally approved the submission of the final version of the manuscript for publishing.

Institutional review board statement

The protocol recommends general humane treatment of animals that does not cause pain, suffering, distress, or lasting harm, following the Royal Decree number M59 from 14/9/1431H and institutional approval code ACUC-22-1-2.

Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

The data are available upon official request from the principal investigator upon the permission of the funding agent.

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