

EFFECT OF ESCHERICHIA COLI VACCINATION ON PRODUCTIVE PERFORMANCE OF JAPANESE QUAILS (*COTURNIX JAPONICA*)

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ABSTRACT

This study evaluated the effects of heat-inactivated (HI) and proteinase K-inactivated (PK) *Escherichia coli* vaccines on productive parameters of Japanese quails (*Coturnix japonica*). Thirty-five female quails were randomly assigned to five groups (n=7 per group): HI *E. coli* ro071a, HI *E. coli* ro025, PK *E. coli* ro071a, PK *E. coli* ro025, and a phosphate-buffered saline control. Birds were vaccinated intramuscularly on days 0, 14, and 28. Data on feed intake (FI), body weight (BW), feed conversion ratio (FCR), egg production (EP), body temperature (BT), and mortality rate (MR) were collected over eight weeks. One-way ANOVA revealed no significant differences in FI, BW, FCR, or BT among treatments. However, egg production differed significantly, with PK vaccine groups showing improved performance compared to HI ro071a. Mortality was 14.3% in PK ro025, while other groups recorded 0%. Results indicate that *E. coli* vaccination did not adversely affect growth or physiological stability and may enhance egg production under non-challenge conditions. These findings provide baseline data supporting the integration of *E. coli* vaccination into quail health management systems in Lesotho.

Keywords: Escherichia coli vaccine, Japanese quail, egg production, feed conversion ratio, vaccination, productive performance.

1. INTRODUCTION

Japanese quail (*Coturnix japonica*) production is expanding globally due to their rapid growth, early maturity, and high egg yield (Minvielle, 2004; Ahmed & Al-Barzinji, 2020). Despite their reported disease resilience, quails remain susceptible to bacterial infections including avian pathogenic *Escherichia coli* (APEC), which causes colibacillosis, leading to reduced productivity

and increased mortality (Thenmozhi et al., 2010; Abd El-Ghany, 2019). *Escherichia coli* is a Gram-negative bacterium classified based on O, H, and K antigens, with numerous pathogenic serotypes affecting poultry (McClure, 2005). Colibacillosis is associated with septicemia, peritonitis, and decreased egg production (Barnes et al., 2008). Vaccination represents a sustainable alternative to antibiotics for disease control and antimicrobial resistance mitigation (Marangon & Busani, 2007). Although *E. coli* vaccination has been studied extensively in chickens, limited information exists regarding its effects on productive parameters in quails, particularly in Lesotho. This study aimed to determine the effects of HI and PK-inactivated *E. coli* strains ro071a and ro025 on feed intake, body weight, feed conversion ratio, egg production, body temperature, and mortality in Japanese quails.

2. MATERIALS AND METHODS

2.1 Experimental Site and Climatic Conditions

The study was conducted at the National University of Lesotho Farm, which is located at Roma in the Maseru district, the capital city of Lesotho. It is approximately 35 kilometers south of the capital city, Maseru. Lesotho is a mountainous landlocked country in South Africa with a total area of 30,355 km. Lesotho is divided into four agroecological zones, including lowlands, which lie between altitudes of 1500 meters and 1800 meters and occupy 15% of the country's mass. The average rainfall in Lesotho is about 753 mm. Temperatures vary according to the season, with the highest record of 37°C in summer.

2.2 Experimental Design

A completely randomized design (CRD) was used with this experiment, which lasted for eight weeks (week 1 to week 8). A total of 35 Japanese quails (*Coturnix japonica*) were randomly assigned to five groups (four treatment groups and one control group). Each treatment consisted of seven tagged quails.

Table 1: Experimental group allocation and treatments

Treatment	Vaccine description	No. of birds	Dosage	Vaccination route	Primary vaccination Day 0	1 st booster Day 14	2 nd booster Day 28
T1	HI E. coli ro071a in LPS/PBS	7	500µl (½ on each side)	Intra pectoral muscle	✓	✓	✓
T2	HI E. coli ro025 in LPS/PBS	7	500µl (½ on each side)	Intra-pectoral muscle	✓	✓	✓
T3	PK E. coli ro071a in LPS/PBS	7	500µl (½ on each side)	Intra-pectoral muscle	✓	✓	✓
T4	PK E. coli ro0251a in LPS/PBS	7	500µl (½ on each side)	Intra-pectoral muscle	✓	✓	✓
Control	PBS	7	500µl (½ on each side)	Pectoral muscle	✓	✓	✓

2.3 Management and feeding

Thirty-five female quails (5 weeks old) were housed in seven groups of five under controlled conditions. Birds were individually tagged and kept in well-ventilated cages (55 × 90 × 30 cm) with wood shavings bedding, refuge boxes, and sand baths to promote natural behavior and minimize stress. A lighting schedule of 16 hours light and 8 hours darkness was maintained. Quails were allowed a one-week acclimatization period and monitored daily for health status. Primary vaccination was administered at six weeks of age, followed by booster doses on days 14 and 28. Birds were fed broiler starter feed up to six weeks and layer feed thereafter. Feed and clean water were provided ad libitum throughout the study.

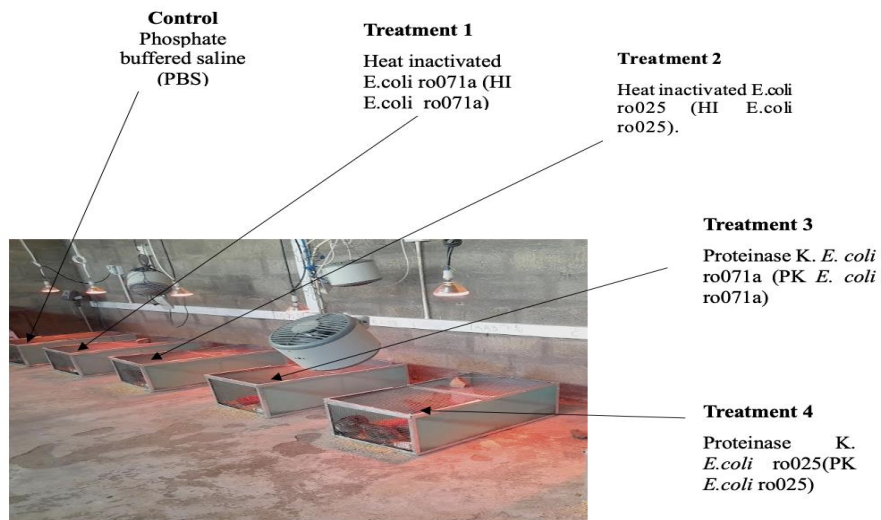


Fig. 1: Experimental cages and treatment groups

2.4 Vaccination

Two *Escherichia coli* vaccine formulations were evaluated: heat-inactivated (HI) lipopolysaccharide (LPS) and proteinase K-inactivated (PK) LPS cells in phosphate-buffered saline (PBS), prepared from strains ro07a and ro025. The vaccine concentration was 4×10^9 CFU/ml (0 CFU/ml for controls) and was emulsified with Freund's complete adjuvant for the primary dose and Freund's incomplete adjuvant for boosters. Each 500 μ l dose contained 1×10^9 CFU/ml equivalent. Quails received primary intramuscular vaccination on day 0, followed by booster doses on days 14 and 28, while controls received PBS.

2.5 Data Collection

Body weights of quails were measured weekly over 8 weeks using a digital scale (0.01 g). Weekly weight gain was calculated as the difference between current and previous week's weights.

Feed intake (FI) = Total feed supplied – Feed refused by Sung & Adeola (2022).

Whereby the feed was supplied every day and on the last day of the week all consumed feeds readings for each day were compiled together to give the total feed consumed for the week.



Fig. 2: Quail being weighed on sf 400 digital scale

Feed conversion ratio (FCR) = Feed intake (g) / Body weight gain (g) by Abdelaty *et al.* (2025).

Egg production (%) = (Total number of eggs produced / Number of birds \times Number of days) \times 100

Mortality rate (%) = (Number of dead birds / Total number of birds) \times 100

Body temperatures for individual birds were measured using an infrared thermometer, with the readings taken directly from the axillary area. Mortality rates that occurred during the experimental period were computed using this formula and the answers were presented in percentage.

2.6 Statistical Analysis

Data were analyzed using IBM SPSS Statistics 23 and are presented as mean ± standard deviation (SD). Significance was set at $p < 0.05$. The effects of treatments on body weight, body temperature, feed conversion ratio (FCR), egg production, and feed intake (FI) were evaluated using one-way analysis of variance (ANOVA). Egg number showed a statistically significant difference ($p < 0.05$), and Tukey’s HSD post hoc test was applied to identify differences among treatment means. Mortality rates, due to the low incidence, were analyzed descriptively using cross tabulation and chi-square was employed to separate the association.

3. RESULTS

3.1 Feed Intake

Table 2: Effect of treatment on mean feed intake and body weight of experimental birds

Treatment	Feed intake of experimental birds			
	Min	Max	Mean ± SD	P-value
HI <i>E. coli</i> ro071a,	765	2119	1711 ±420 ^b	0.89
HI <i>E. coli</i> ro025,	756	2481	1914±537 ^b	
PK <i>E. coli</i> ro025	751	2185	1837±446 ^b	
PK <i>E. coli</i> ro025	751	2330	1874±515 ^b	
Control (PBS)	790	2342	1945±532 ^b	
	Body weight on the experimental birds			
HI <i>E. coli</i> ro071a,	341.83	367.83	351.61±9.54 ^b	0.989
HI <i>E. coli</i> ro025,	341.83	367.83	350.40±9.28 ^b	
PK <i>E. coli</i> ro025	339.24	368.38	350.83±10.02 ^b	
PK <i>E. coli</i> ro025	336.84	367.72	348.74±10.93 ^b	
Control (PBS)	336.68	368.68	349.62±11.56 ^b	

No significant difference between the treatment means (P>0.05, Min- Minimum, Max -Maximum)

The results showed that treatment had no significant effect on feed intake of the experimental birds ($P = 0.89$). Birds in the Control (PBS) group recorded the highest mean feed intake (1945 ± 532 g), followed closely by HI *E. coli* ro025 (1914 ± 537 g), PK *E. coli* ro025 (1874 ± 515 g and 1837 ± 446 g), while HI *E. coli* ro071a had the lowest mean feed intake (1711 ± 420 g). However, all treatment means carried the same superscript, indicating that the differences observed were not

statistically significant ($P > 0.05$). This suggests that administration of both heat-inactivated (HI) and pathogenic (PK) *E. coli* strains did not adversely affect feed consumption compared to the control group. Similarly, there was no significant difference in body weight among the treatment groups ($P = 0.989$). Mean body weights ranged narrowly from 348.74 ± 10.93 g (PK *E. coli* ro025) to 351.61 ± 9.54 g (HI *E. coli* ro071a). The control group had a mean body weight of 349.62 ± 11.56 g. The uniform superscript across treatments confirms that body weight was not significantly influenced by the treatments ($P > 0.05$). This indicates that exposure to the different *E. coli* strains did not negatively affect growth performance of the birds.

3.2 Feed Conversion Ratio

Table 3: Effects of treatment feed conversion ratio on experimental birds

Feed conversion ratio (FCR)	
Treatment	
HI <i>E. coli</i> ro071a	0.72
Hi <i>E. coli</i> ro025,	0.85
PK <i>E. coli</i> ro071a	0.76
PK <i>E. coli</i> ro025	0.80
Control (PBS)	0.81
	Mortality (%)
HI <i>E. coli</i> ro071a	0
HI <i>E. coli</i> ro025,	0
PK <i>E. coli</i> ro071a	0
PK <i>E. coli</i> ro025	14.3
Control (PBS)	0

Table 3.2 presents the effects of different treatments on feed conversion ratio (FCR) and mortality in experimental birds. Feed conversion ratio is an important indicator of efficiency, showing how effectively birds convert feed into body mass. Lower FCR values indicate better feed utilization and improved performance. Birds treated with HI *E. coli* ro071a recorded the lowest FCR of 0.72, indicating the most efficient feed utilization among all groups. This suggests that this treatment had a positive influence on growth performance and nutrient conversion. The PK *E. coli* ro071a group followed with an FCR of 0.76, which also reflects good feed efficiency, though slightly lower than the HI *E. coli* ro071a group. The PK *E. coli* ro025 group had an FCR of 0.80, while the control group treated with PBS recorded an FCR of 0.81. These values are relatively similar, suggesting moderate feed efficiency with no major improvement compared to the control. The HI *E. coli* ro025 group showed the highest FCR of 0.85, indicating the least efficient feed conversion among the treatments. A higher FCR implies that birds required more feed to gain the same amount

of weight, which may suggest reduced growth performance under this treatment. Regarding mortality, all treatment groups recorded zero mortality except the PK *E. coli* ro025 group, which showed a mortality rate of 14.3 percent. The absence of mortality in the HI *E. coli* ro071a, HI *E. coli* ro025, PK *E. coli* ro071a, and control groups suggests that these treatments were generally safe and did not negatively affect bird survival. However, the mortality observed in the PK *E. coli* ro025 group indicates a possible adverse effect associated with this treatment, which may have influenced overall performance and efficiency.

3.3 Egg Production

Table 4: Effects of treatments on the egg production and Body temperature of experimental birds

Treatment	Egg production of experimental birds			
	Min	Max	Mean ± SD	P-value
HI <i>E. coli</i> ro071a,	2.75	3.75	3.36±0.41 ^b	0.03
HI <i>E. coli</i> ro025,	2.97	4.09	3.39±0.43 ^{ab}	
PK <i>E. coli</i> ro025	3.22	4.47	3.99±0.46 ^a	
PK <i>E. coli</i> ro025	3.22	4.47	3.99±0.41 ^a	
Control (PBS)	3.27	4.41	3.95±0.40 ^a	
	Body temperature of experimental birds			
HI <i>E. coli</i> ro071a,	36.075	36.725	36.36±0.203 ^a	0.541
HI <i>E. coli</i> ro025,	36.188	36.700	36.41±0.207 ^a	
PK <i>E. coli</i> ro025	35.463	36.913	36.35±0.452 ^a	
PK <i>E. coli</i> ro025	36.233	37.463	36.61±0.410 ^a	
Control (PBS)	36.125	36.938	36.41±0.274 ^a	

Means with different superscripts (a,b) in the same column differ significantly (P<0.05). Min- Minimum, Max -Maximum

Regarding egg production, there was a statistically significant difference among treatments (P = 0.03), indicating that the treatments influenced laying performance. Birds treated with HI *E. coli* ro071a recorded the lowest mean egg production (3.36 ± 0.41), with values ranging from 2.75 to 3.75 eggs. Similarly, those treated with HI *E. coli* ro025 had a slightly higher mean of 3.39 ± 0.43, with a minimum of 2.97 and a maximum of 4.09 eggs. In contrast, birds treated with PK *E. coli* ro025 showed higher egg production, with a mean of 3.99 ± 0.46 in one group and 3.99 ± 0.41 in the repeated group, and production ranging from 3.22 to 4.47 eggs. The control group (PBS) also demonstrated relatively high egg production, with a mean of 3.95 ± 0.40 and a range between 3.27 and 4.41 eggs. The results indicate that the PK *E. coli* ro025 and control treatments supported better egg production compared to the heat-inactivated treatments, particularly HI *E. coli* ro071a,

which had the lowest performance. In contrast, body temperature was not significantly affected by the treatments ($P = 0.541$). The mean body temperatures across all groups were very similar, ranging from 36.35°C to 36.61°C . Birds treated with HI *E. coli* ro071a had a mean temperature of $36.36 \pm 0.203^{\circ}\text{C}$, while those treated with HI *E. coli* ro025 recorded $36.41 \pm 0.207^{\circ}\text{C}$. The PK *E. coli* ro025 groups showed mean temperatures of $36.35 \pm 0.452^{\circ}\text{C}$ and $36.61 \pm 0.410^{\circ}\text{C}$, respectively. The control group had a mean body temperature of $36.41 \pm 0.274^{\circ}\text{C}$. The overlapping ranges and close mean values indicate that none of the treatments caused abnormal thermal responses in the birds.

4. DISCUSSION

The findings of this study indicate that vaccinating quails with different *E. coli* vaccine strains did not significantly affect feed intake (FI). This aligns with Aida et al. (2022), who reported that *E. coli* vaccination enhanced immunity without influencing feed intake in broilers under non-challenged conditions. Similarly, Ebrahim et al. (2024) found that vaccination improved disease resistance but had minimal impact on appetite or feed conversion. Khan et al. (2022) also observed that feed intake was more influenced by breed and environmental conditions than by vaccination. Conversely, Smialek et al. (2020) reported higher feed intake and improved performance in inoculated broilers due to better gut health and reduced bacterial burden. Some studies, such as Mensour et al. (2023), documented a transient reduction in feed intake post-vaccination in layers, resolving within a week, suggesting that minor short-term effects may occur depending on vaccine formulation. Overall, the present results suggest that under minimal disease pressure, *E. coli* vaccination does not alter feed consumption.

Regarding body weight, no significant differences were observed between vaccinated and non-vaccinated quails ($p=0.989$), indicating that vaccination had no measurable impact. This is consistent with Landman et al. (2025), where subcutaneous vaccination with inactivated *E. coli* vaccines in layers did not affect body weight, egg production, or egg weight. Likewise, Sadeghi et al. (2018) reported similar trends, although vaccinated quails challenged with pathogenic *E. coli* showed improved health parameters, highlighting the vaccine's benefits under disease pressure. Landman & Van Eck (2017) also observed that brown layer hens vaccinated with autogenous *E. coli* vaccines maintained body weight while gaining protection against homologous challenges. These findings underscore that in the absence of disease, growth performance primarily depends on nutrition and genetics, rather than vaccination, which serves primarily for disease prevention. Histopathological observations by Mansour et al. (2023) further confirmed that vaccination prevents systemic infection and reduces lesions in key organs, while non-vaccinated birds exhibited signs of colibacillosis such as hepatic congestion, splenic depletion, and intestinal inflammation.

Feed conversion ratio (FCR) showed numerical variation among treatment groups (0.72–0.85), but vaccination did not significantly affect feed efficiency. This is in agreement with Rawiwet & Chansiripornchai (2009), who reported no significant FCR differences among vaccinated broilers, and Gregersen et al. (2010), who emphasized that the primary goal of vaccination is reducing mortality from colibacillosis rather than improving FCR. Combined approaches, such as parent stock vaccination with autogenous vaccines and dietary supplements like *Enterococcus faecium* DSM7134 and fructo-oligosaccharides, improved health and body weight without affecting FCR (Fuhrmann et al., 2022), whereas Chrétien et al. (2021) found that live *E. coli* vaccines under high disease pressure improved FCR, reduced mortality, and enhanced egg quality.

In this study, *E. coli* vaccination, particularly PK-inactivated strains, was associated with improved egg-laying performance in quails. These results are consistent with Nandre et al. (2013), where vaccination against *Salmonella enterica* serovar Enteritidis reduced internal egg contamination and increased egg production. Similar findings were reported by Lozica et al. (2022) and Gottstein et al. (2022), demonstrating higher egg production and lower mortality after vaccination. However, Ismail & Ibrahim (2017) observed serotype-specific effects, where only certain *E. coli* serotypes post-challenge led to increased egg yield, suggesting that vaccination may provide targeted protection that preserves productivity. No significant differences in body temperature were observed between vaccinated and control groups, indicating that vaccination did not induce hyperthermia or febrile reactions. This aligns with Kromann et al. (2021) and Śmiałek et al. (2020), who reported stable body temperatures and improved health parameters following vaccination, emphasizing disease prevention and homeostasis rather than thermoregulation. Mortality was generally low across all groups, except for the PK *E. coli* RO025 group, which showed a 14.3% mortality rate. This suggests that the vaccines were safe and did not adversely affect survival, though limited replication makes it difficult to attribute mortality directly to treatment.

5. CONCLUSION

E. coli vaccination did not adversely affect feed intake, body weight, feed conversion ratio, or body temperature in Japanese quails. Proteinase K-inactivated vaccines were associated with improved egg production. Mortality remained low across treatments, indicating overall vaccine safety. These findings provide baseline evidence supporting the potential integration of *E. coli* vaccination into quail health management systems in Lesotho. However, further studies involving larger sample sizes and pathogen challenge models are recommended to validate these results.

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