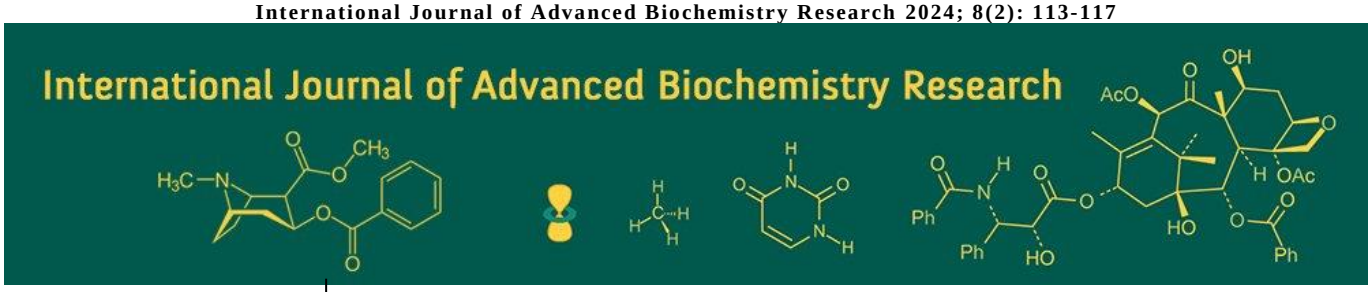

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Evaluation of nutrient metabolizability and intestinal micrometry of layer quail on supplementing different phytoadditives

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Abstract
In order to evaluate the effect of supplementation of different phytoadditives over nutrient intake and metabolizability of layer quails, 360 birds of same hatch (6 weeks old) were randomly distributed into eight groups (n = 45), having three replicas of fifteen quail layers per replica. These eight dietary groups were: Negative control (NC; maize-soya based diet with no additive), positive control (PC; herbal growth promoter- Reproforte plus™ was supplemented @ 500 gm per ton feed), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively. These additives were fed consecutively for 22 weeks and a metabolisability trial was conducted at 20th week of trial. Also, at end of trial, representative sample of duodenum, jejunum and ileum was evaluated for villi length to crypt depth ratio and gland diameter. Results of nutrient intake showed no difference (p>0.05) amongst different dietary groups and was similar in all the treatment groups. The metabolizability of DM, CP, EE, CF, TA, OM and NFE also revealed non-significant differences (p>0.05) on phytoadditive supplementation. Also, villi length to crypt depth ratio was significantly higher (p<0.05) in duodenum for T_2, T_3 and T_4 group in comparison to the NC group, but statistically (p>0.05) similar values was observed for jejunum and ileum. Also, gland diameter (µm) showed no difference in all the three segments of intestine. It may be inferred that 1% phytoadditives supplementation has no effect on nutrient intake and metabolizability in layer quail, but there is improvement in villi length to crypt depth in 1% garlic, fenugreek and cumin supplemented groups.

Keywords: Quail layer, phytogenic feed additives, nutrient intake, villus crypt ratio

Introduction
The use of feed additives in poultry ration improves feed utilization which in turn reduces the input feed cost. But now-a-days use of phytoadditives in poultry ration has gained momentum, as they play a significant role in maintaining optimal gut function, animal health and performance by virtue of active principles present in them. Phytogenic feed additives (PFAs) and/or Herbal feed additives are botanical products with their source belonging to herbs, spices, essential oils, or oleoresins (Ogbuewu *et al*., 2020) [17]. Herbs and spices are commonly used as whole plant or their parts to improve animal performance. Prophylactic antibiotic usage as feed additive in poultry ration improves the utilization of feed, but has resulted in antibiotic resistance, antibiotic residues in meat/eggs and also results in declining the diversity of normal gut microflora (Abd El-Hack *et al*., 2022) [2]. PFA has the potential to serve as an alternative of antibiotic growth promoters and are non-toxic and natural in nature besides being cost-effective (Abou Kassem *et al*., 2021) [3]. A milieu of bioactive chemicals such as curcumin, anethole, allicin, allyl-isothiocyanate, lecithin, choline, trigonelline, cuminaldehyde, carvacrol, and thymol are present in PFA that accounts for different properties *viz*. antioxidant, antimicrobial, anti-inflammatory, digestive stimulant, etc and possess numerous beneficial properties over poultry health and growth performance (Yatoo *et al*., 2012; Alinian *et al*., 2016; Batiha *et al*., 2020; Chowdhary *et al*., 2021; Behnamifar *et al*., 2023) [22, 4, 8, 10, 9].
Quail (*Coturnix coturnix*) is a small and robust bird, reared intensively for egg and meat production.

They are resistant to environmental constraints and need no vaccination and medication (ICAR, 2013) [14]. Quail possess characteristics of fast growth, early sexual maturity and high rate of egg production. But, a range of phyto-additives are available in the market to counter microbial resistance and improving feed efficiency *viz*. turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder. With this background, a study has been carried out to compare different phytoadditives as a feed supplement by evaluating the nutrient intake/nutrient metabolizability and villus crypt ratio in layer quails.

Material and Methods

The present trial was carried out at Division of Animal Nutrition, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Jammu, India. Three hundred sixty Japanese quail (*Coturnix coturnix japonica*) layers of same hatch (6 weeks old) were randomly distributed into eight groups (n=45), having three replicas of fifteen quail layers per replica. A maize-soya based basal diet for layer quails was formulated as per specifications given by ICAR (2013) [14] (Table 1). The eight dietary groups were: Negative control (NC) group contains no additive, positive control (PC) is supplemented with commercial growth promoter (Reproforte plus™ containing *Adhatoda vasica* - 20%, *Asparagus officinalis*- 15%, *Leptadenia reticulate* - 15%, *Zingiber officinalis*- 10%, *Rubia cordiolia*- 10%, *Tribulus terrestris*- 10%, *Solena amplexicaulis*- 10%, *Punica granatum*- 10% and was supplemented @ 500 gm per ton feed, supplied by Arvind Herbal Labs, Saharanpur, UP), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented as 1% dietary additive with turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively. All the phytoadditives used except *aloe vera* and oregano powder were purchased raw from local market. These were dried and grinded to powder form before mixing in the basal feed whereas *Aloe vera* powder was bought from AMORVET, UK, India. At the 20th week of feeding trial, layer quails were transferred to battery cages for conduction of metabolism trial. A metabolism trial of four days was carried out and three birds per group *i.e.* nine birds per group were transferred to the battery cages. Birds were weighed at start and end of metabolism trial for getting initial, final and average body weight (g) of layer quails. Feed and faecal samples were collected daily for assessment of proximate composition as per method described by AOAC (2012) [5] for estimating the nutrient intake and nutrient metabolizability. At the end of feeding trial (*i.e*. 22 weeks of trial), nine birds per group were slaughtered and duodenum, jejunum and ileum tissue specimens were collected and then fixed in 10% Neutral Buffered Formaline solution (Luna, 1968) [16] for histo-morphological processes. The micrometrical observations were recorded (Haematoxylin and Eosin-stained sections) by using ocular micrometry which was duly calibrated with stage micrometer and villus length to crypt depth ratio and gland diameter (µm) was determined.

Statistical analysis

The data pertaining to different parameters were subjected to statistical analysis as per Snedecor and Cochran (1994) [19]. The means in different treatments were subjected to Duncan's multiple range test (1995) [11] for ranking (p<0.05).

Results and Discussion

Results for nutrient intake and nutrient metabolisability of layer quail at 20th week of trial are presented in Table 2 and 3, respectively. It was found that supplementation of phytoadditives @ 1% in layer quail diet had no effect on the body weight of layer quail during initiation and completing the metabolizability trial. Similarly, no effect was observed on metabolizability of different nutrients *i.e.* dry matter, crude protein, ether extract, crude fibre and organic matter on supplementation of phytoadditives at the rate of 1%. Similar to our findings, Silva *et al*. (2017) [18] revealed that turmeric supplementation at the rate of 0, 0.5, 1, 1.5 and 2% did not affect the dry matter; crude protein and ether extract metabolizability in layer quails**.** Likewise, Abaza (2007) [1] and Yatoo *et al*. (2012) [22] reported no effect of fenugreek supplementation @ 0.5% and 1% on metabolisability values of nutrients in laying hens and broilers, respectively. In line to our results, Kichloo *et al*. (2023) [15] also reported that *aloe vera* supplementation @ 0.4 and 0.6% in meat quail diet had no effect on nutrient metabolizability. Similar findings were also reported by earlier authors also on aloe vera supplementation (Tariq *et al*., 2015 [21]; Barman *et al*., 2019) [6]. On the contrary, Zeweil *et al*. (2011) [23] revealed that the supplementation of 0.75% of dried garlic resulted in a significant ($p\leq$ 0.01) improvements in digestibility coefficients of crude protein and nitrogen free extract (NFE) in layer quails. But this study has been carried out in 12 weeks old quail, however the present study is carried out in 26 weeks old quail (20 weeks of trail). However, EL-Mallah *et al*. (2005) [12] stated that supplementation of fenugreek seeds @ 2% in diet of turkey chicks resulted in significant increase in digestibility of NFE%, and attributed it to saponin content of fenugreek seeds. Species difference and dosage difference might be the possible reason for the difference in these two studies. Similarly, Yatoo *et al*. (2012) [22] reported higher DM digestibility on supplementing 1% cumin in boiler diet. The literature pertaining to the effect of phytoadditive supplementation on nutrient intake and its metabolizability is scanty, thus cannot be discussed.

The structure of intestinal epithelium have important role in nutrient digestion, absorption, and overall gut health (Soumeh *et al*., 2019) [20]. Among micrometrical observations, significantly higher (p<0.05) villi length to crypt depth ratio was observed in garlic (T_2), fenugreek (T_3) and cumin (T_4) supplemented groups in the duodenum, but in the jejunum and ileum no effect was observed on phytoadditive supplementation. Higher villus height to crypt depth ratio reflects vast area for nutrient utilization and higher absorptive capacities (Ashayerizadeh *et al*., 2023) [6]. Gland diameter (µm) was similar in all three intestinal segments *viz.* duodenum, jejunum and ileum. Similar to our study, Ashayerizadeh *et al*. (2023) [6], revealed that supplementation of turmeric powder at 0.5% in the diet of quail showed no significant difference in terms of villus height and crypt depth ratio in duodenum and jejunum. He *et al*. (2017) [13] revealed that addition of 100 mg/kg oregano essential oils in the diet of layer quail increased villus height to crypt depth ratios (p<0.01) in the duodenum compared with the control group. However, in jejunum, after the addition of oregano essential oils, there was no effect on villus height to crypt depth ratios. The difference can be due to different form of phytoadditive used. Longer and slender villi result in increased surface area which leads to better

absorption of nutrients which is expressed in improved digestibility of nutrients (Chowdhary *et al*., 2021) [10]. Data pertaining to the effect of dietary inclusion of different phytoadditive is scarce; therefore the results cannot be compared.

Table 1: Ingredient and chemical composition of quail layer basal diet.

Attributes	Ingredient composition (%)
Maize	62.66
Meat bone meal	4.02
Soybean meal	24.65
Salt	0.25
Sodium bicarbonate	0.01
Soybean oil	1.30
DL-Methionine	0.10
L-Lysine hydrochloride	0.12
Limestone powder	6.72
Vitamin supplement	0.05
Trace minerals	0.10
Chemical composition (on DMB, %)	
Organic Matter	95.31
Crude Protein	18.62
Ether Extract	5.51
Crude Fibre	4.19
Total Ash	4.69
Nitrogen free extract	66.99
ME (Kcal/kg; calculated value)	2850

Table 2: Effect of supplementation of different phytoadditives on body weight (g) and nutrient intake (g) during metabolism trial of layer quail

Attributes	Groups								Overall mean ± SEM	P- value
	NC	PC	T_1	T_2	T_3	T_4	T_5	T_6		
Initial body weight	209.17±5.74	213.67±6.42	215.33±6.04	213.00±4.77	214.50±2.75	217.33±6.24	218.50±6.00	219.3±5.88	215.10±1.88	*p*>0.05
Final body weight	212.33±5.67	217.67±6.88	218.97±5.85	219.17±4.34	218.17±4.00	223.83±5.55	221.33±5.81	222.33±5.11	219.23±1.85	*p*>0.05
Average body weight	210.75±5.67	215.67±6.63	217.15±5.80	216.08±4.51	216.33±3.31	220.58±5.65	219.92±5.87	220.83±5.47	217.16±1.83	*p*>0.05
Nutrient intake										
Feed	163.88±5.10	167.76±3.93	164.69±4.34	169.05±4.39	169.77±4.01	169.91±4.05	169.96±3.91	168.94±3.64	168.02±1.36	*p*>0.05
DM	152.40±4.74	156.01±3.66	153.35±4.04	157.22±4.08	157.89±3.73	158.02±3.76	158.06±3.64	157.11±3.39	156.26±1.27	*p*>0.05
CP	28.38±0.88	29.05±0.68	28.55±0.75	29.27±0.76	29.40±0.69	29.42±0.70	29.43±0.68	29.25±0.63	29.10±0.24	*p*>0.05
EE	7.71±0.24	7.89±0.19	7.76±0.20	7.96±0.21	7.99±0.19	8.00±0.19	8.00±0.18	7.95±0.17	7.91±0.06	*p*>0.05
CF	6.39±0.40	6.54±0.15	6.43±0.17	6.59±0.17	6.62±0.16	6.62±0.15	6.58±0.14	6.58±0.14	6.55±0.05	*p*>0.05
TA	7.15±0.22	7.32±0.17	7.19±0.19	7.37±0.19	7.40±0.17	7.41±0.18	7.41±0.17	7.37±0.16	7.33±0.06	*p*>0.05
OM	145.26±4.52	148.70±3.49	146.15±3.85	149.85±3.89	150.48±3.55	150.60±3.59	150.65±3.47	149.74±3.23	148.93±1.21	*p*>0.05
NFE	102.78±3.20	105.22±2.47	103.42±2.72	106.03±2.75	106.48±2.51	106.57±2.54	106.60±2.45	105.96±2.28	105.38±0.86	*p*>0.05

Negative control (NC; contains no additive), positive control (PC; herbal growth promoter- Reproforte plus™ was supplemented @ 500gm per ton feed), whereas T_1, T_2, T_3, T_4, T5 and T6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, aloe vera and oregano powder, respectively; DM (Dry matter), OM (Organic matter), CP (Crude protein), EE (Ether extract), CF (Crude fibre), TA (Total ash) and NFE (Nitrogen free extract)

Table 3: Effect of supplementation of different phytoadditives on digestibility (%) of nutrients in meat quail

Attributes	Groups								Overall mean ±SEM	P- value
	NC	PC	T_1	T_2	T_3	T_4	T_5	T_6		
DM	67.59±1.92	72.38±1.36	71.84±1.78	72.65±2.50	72.63±1.47	72.48±1.42	72.15±1.74	72.34±1.53	71.76±0.62	*p*>0.05
OM	68.63±1.99	73.45±1.32	73.02±1.79	73.84±2.61	73.70±1.54	73.70±1.35	73.27±1.76	73.50±1.52	72.89±0.63	*p*>0.05
CP	68.36±2.47	69.94±1.04	70.81±1.45	70.72±2.85	70.68±1.11	70.02±2.23	69.64±2.90	70.37±1.95	70.07±0.67	*p*>0.05
EE	72.04±3.17	74.29±2.05	73.94±3.04	74.40±2.62	73.43±1.48	74.18±1.84	73.46±2.98	73.68±1.89	73.68±0.78	*p*>0.05
CF	50.00±2.23	50.48±3.10	49.06±2.38	48.86±4.00	46.16±2.70	46.08±2.95	50.30±2.18	47.88±3.06	48.60±0.94	*p*>0.05
NFE	69.61±2.13	75.79±1.61	75.04±1.99	76.21±2.53	76.26±1.62	76.40±1.05	75.68±1.48	75.94±1.59	75.12±0.68	*p*>0.05

Negative control (NC; contains no additive), positive control (PC; herbal growth promoter- Reproforte plus™ was supplemented @ 500gm per ton feed), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively; DM (Dry matter), OM (Organic matter), CP (Crude protein), EE (Ether extract), CF (Crude fibre), TA (Total ash) and NFE (Nitrogen free extract)

Table 4: Effect of phytoadditives supplementation on Micrometry of different components small intestine of layer Quails

Groups	Duodenum		Jejunum		Ileum	
	Diameter of gland (µm)	Villi length/crypt depth	Diameter of gland (µm)	Villi length/crypt depth	Diameter of gland (µm)	Villi length/crypt depth

NC	50.09±1.53	8.63^{a}±0.20	45.30±1.38	9.31±0.46	42.87±2.62	4.64±0.21
PC	54.90±3.99	9.56^{abc}±0.33	46.08±1.47	9.34±0.52	42.73±2.01	4.77±0.31
T_1	60.63±2.71	9.14^{abc}±0.32	45.30±1.34	10.09±0.49	47.33±2.24	4.85±0.18
T_2	56.65±2.80	10.01^{b}±0.41	47.32±1.20	10.05±0.76	47.12±1.43	4.96±0.12
T_3	50.82±2.79	9.65^{bc}±0.28	45.86±0.64	9.68±0.27	44.58±1.94	4.63±0.23
T_4	56.45±3.11	9.99^{b}±0.28	47.46±1.31	9.72±0.45	47.57±1.63	4.95±0.22
T_5	54.32±2.28	9.51^{abc}±0.31	47.44±1.17	9.63±0.55	42.32±1.21	4.71±0.25
T_6	55.76±3.02	9.00^{ab}±0.27	45.82±1.48	9.57±0.55	43.91±1.73	5.01±0.40
Overall mean± SEM	54.95±1.02	9.39±0.11	46.32±0.44	9.67±0.18	44.80±0.68	4.82±0.09
P-value	p>0.05	p<0.05	p>0.05	p>0.05	p>0.05	p>0.05

Negative control (NC; contains no additive), positive control (PC; herbal growth promoter- Reproforte plus™ was supplemented @ 500gm per ton feed), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively

Conclusion

It can be concluded that garlic and cumin supplementation in layer quails resulted in increased villus height to crypt depth ratio in duodenum and it resulted in numerically higher nutrient metabolisability.

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