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# Influence of green tea powder on the performance, nutrient utilisation, caecal microbiota profile and meat quality of broiler chickens

**D.V. Thomas[1], A.L. Molan[1,2], Y. Singh[1,3] and V. Ravindran[1*]**

*[1]Monogastric Research Centre, School of Agriculture and Environment, Massey University, Palmerston North 4442, New Zealand; [2]Present address: Department of Biotechnology, College of Science, University of Diyala, Diyala, Iraq; [3]Present address: Department of Livestock Production and Management, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University (GADVASU), Ludhiana, Punjab 141004, India; v.ravindran@massey.ac.nz*

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## Abstract

This study assessed the influence of green tea powder diet supplementation on performance, nutrient utilisation, caecal microbiota profile and meat quality in broiler chickens. A total of 144, one-day-old broiler (Ross 308) chicks were allocated to 18 cages (eight broilers/cage) which were randomly assigned to one of three dietary treatments: a wheat-based basal diet or supplemented with one of two types of green tea; normal (N-GT) or selenium-rich (Se-GT) tea, at an inclusion rate of 1%. Bird performance, nitrogen-corrected apparent metabolisable energy (AMEn), and total tract digestibility of fat and starch were measured on d 7, 21 and 35. Effects on microbiota profile on d 7, 21 and 35 were determined in a cohort study. Green tea supplementation reduced ($P<0.05$) the weight gain and feed intake but improved ($P<0.05$) feed efficiency. Supplementation with N-GT increased the AMEn on d 7 and 21, and with Se-GT on d 7 ($P<0.05$). AMEn increased with age for all treatments. Fat digestibility increased ($P<0.05$) in birds fed N-GT on d 21 and Se-GT on d 7 and 21. Starch digestibility increased ($P<0.05$) on d 21 with N-GT supplementation and on d 7 with Se-GT supplementation. Carcass and breast meat yields were unaffected ($P<0.05$) by the dietary treatments. The abdominal fat pad decreased ($P<0.05$) in the N-GT diet and numerically in the Se-GT diet. Drip loss was less in meat from birds ($P<0.05$) fed both green tea treatments. Cooking loss was reduced in the Se-GT treatment ($P<0.05$). Dietary inclusion of green tea powder positively influenced microbiota profile, with increased ($P<0.05$) numbers of beneficial bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) and reduced ($P<0.05$) numbers of pathogenic bacteria (*Clostridium* spp. and *Bacteroides* spp.). Differences were observed between the two green tea types, with Se-GT being more beneficial than N-GT in the positive modulation of intestinal microbiota.

**Keywords:** broilers, green tea, selenium, meat quality, microbiota

## 1. Introduction

Globally, tea is the most popularly consumed beverage and originates from the leaves of *Camellia sinensis*. Tea leaves can be processed in several ways and, depending on the level of fermentation, produce four types: white, green (both unoxidised), oolong (partially oxidised) and black (fully oxidised) teas. Green tea leaves contain 10-30% (dry weight) of polyphenols, including catechins, flavonols, flavanones, phenolic acids, glycosides and the aglycones of plant pigments, which are known to have diverse biological activities (Crespy and Williamson, 2004). In green tea, the major flavonoids present include catechin (flavan-3-ol) such as epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG; Balentine *et al.*, 1997). Green tea is superior to black tea in terms of antioxidant activity, owing to its higher EGCG content (Lee *et al.*, 2002).

Three different green tea products, namely green tea powder (Biswas and Wakita, 2001; Jelveh *et al.*, 2018;

Kaneko *et al.*, 2001; Uuganbayar *et al.*, 2005; Xia *et al.*, 2018), green tea extract (Erener *et al.*, 2011; Jelveh *et al.*, 2018) and green tea polyphenols (Cao *et al.*, 2005), have been evaluated in poultry diets. In some studies, green tea powder was shown to improve poultry performance (Jelveh *et al.*, 2018), which was attributed to its antioxidant activities (Lee *et al.*, 2002; Molan *et al.*, 2009a) and antimicrobial properties (Erener *et al.*, 2011; Lee *et al.*, 2006) of polyphenols. Green tea has been shown in rodent studies (Molan *et al.*, 2010) to modify intestinal microbiota that may impact the digestibility of nutrients and affect growth. The role of commensal intestinal microflora in animal production has attracted attention in recent decades due to the global trend of moving away from in-feed antibiotics. The gastrointestinal tract contains a complex microbial community with potentially beneficial pathogenic bacteria. An unfavourable microflora profile can promote sub-clinical enteric infections, leading to poor animal performance. Consequently, a favourable intestinal microflora is necessary for optimum nutrient utilisation and growth. Despite this importance, studies investigating the effect of green tea on nutrient utilisation and intestinal microbiota in broilers are limited.

Selenium (Se) is an essential trace mineral that is required for a range of functions in animals, including antioxidant defence systems, thyroid function and reproduction (Surai, 2002). In particular, organic forms of Se offer advantages over the traditionally used inorganic forms of Se, such as improved Se bioavailability and uptake into tissues, and positive effects on production traits of broilers, including feed efficiency, breast meat yield and meat quality (Olivera *et al.*, 2014; Ravindran and Elliot, 2017; Surai, 2002).

In recent years, interest in using natural ingredients as a source of antioxidants in broiler diets is growing. Interestingly, green tea manufactured from plants grown in some regions of China is enriched with trace minerals, particularly Se. Because of their complementary antioxidant properties, it may be possible to achieve additive or synergetic effects by combining green tea and Se.

The present study was designed to assess the effect of two types of green tea (normal, N-GT, and selenium-rich, Se-GT) on performance, carcass characteristics, meat quality, apparent metabolisable energy (AME), total tract nutrient utilisation and caecal microbiota profile in broilers.

## 2. Materials and methods

The study protocol was approved by Massey University Animal Ethics Committee, New Zealand.

### Green tea powder

Green tea (N-GT), of an unknown brand, was procured from a local retailer while selenium-rich green tea (Se-GT) was obtained from a tea plantation located close to a selenium mine, in Hubei province, China. The Se-GT and N-GT samples were ground to pass through a 0.5-mm sieve. Representative samples of both teas were analysed for minerals at the Nutrition laboratory, Massey University, New Zealand.

### Diet and birds

A completely randomised design with three dietary treatments was employed. A wheat-based basal diet or the basal diet containing one of the two types of green tea, namely N-GT or Se-GT, at an inclusion rate of 1%, were used. This inclusion rate was selected on previous published data (Shomali *et al.*, 2012; Yang *et al.*, 2003). The diets were formulated to meet or exceed the requirements recommended by the breeding company (Ross, 2019). The ingredient composition and analysis of diets are shown in Table 1. All diets contained titanium dioxide (0.3%) as an indigestible dietary marker and were cold pelleted at 70 °C.

One-day-old broiler (Ross 308) chicks from a commercial hatchery were randomly allocated to 18 brooder cages (eight birds/ cage, six replicates/ treatment) in three-tier batteries housed in an environmentally controlled room. Cages were then assigned randomly to one of the three dietary treatments. Birds were transferred to grower cages in an environmentally controlled room on d 14. Room temperature was maintained at 32±1 °C during the first week and gradually reduced to 24 °C by the end of the third week. Twenty hours of fluorescent lighting per day was provided. Diets were offered *ad libitum* and water was available throughout the 35 d trial.

### Growth performance

Body weight and feed intake were recorded on a cage basis on d 7, 21 and 35 and mortality was recorded daily. Any birds that died were weighed, included in weekly weight gain data and used to adjust feed per gain.

### Metabolisable energy and total tract nutrient digestibility measurements

Feed intake and excreta output of each cage were measured quantitatively for 4 d, between d 4 to 7, d 18 to 21, and d 32 to 35 post-hatch. Total excreta output was pooled within a cage, mixed well and subsampled, which were then lyophilised, ground to pass through a 0.5 mm screen, and stored in airtight plastic containers at 4 °C until analysis. Excreta and diet samples were analysed for the dry matter (DM), gross energy (GE) and nitrogen (N). The

Table 1. Composition and analysis of experimental diets, % as fed.[1]

| Ingredient | Control diet | Normal green tea | Se-rich green tea |
|---|---|---|---|
| Wheat | 64.76 | 64.76 | 64.76 |
| Sand | 1.00 | | |
| Normal green tea | - | 1.00 | - |
| Se-rich green tea | - | - | 1.00 |
| Soybean meal | 27.34 | 27.34 | 27.34 |
| Soybean oil | 2.73 | 2.73 | 2.73 |
| Dicalcium phosphate | 2.03 | 2.03 | 2.03 |
| Limestone | 0.34 | 0.34 | 0.34 |
| Salt | 0.14 | 0.14 | 0.14 |
| Lysine.HCl | 0.34 | 0.34 | 0.34 |
| DL-methionine | 0.24 | 0.24 | 0.24 |
| L-threonine | 0.12 | 0.12 | 0.12 |
| Sodium bicarbonate | 0.36 | 0.36 | 0.36 |
| Vitamin and trace mineral premix[1] | 0.30 | 0.30 | 0.30 |
| Calculated analysis | | | |
| AME, MJ/kg | 12.5 | 12.5 | 12.5 |
| Crude protein | 22.0 | 22.0 | 22.0 |
| Lysine | 1.35 | 1.35 | 1.35 |
| Methionine + cysteine | 1.02 | 1.02 | 1.02 |
| Threonine | 0.85 | 0.85 | 0.85 |
| Calcium | 0.95 | 0.95 | 0.95 |
| Non-phytate phosphorus | 0.48 | 0.48 | 0.48 |
| Analysed values | | | |
| Dry matter | 88.6 | 88.9 | 89.0 |
| Crude protein(nitrogen ×6.25) | 21.6 | 20.9 | 21.3 |
| Crude fat | 4.22 | 4.20 | 4.32 |
| Starch | 39.6 | 38.9 | 39.1 |
| Gross energy, MJ/kg | 16.6 | 16.7 | 16.7 |

[1] Supplied per kg diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

samples were analysed for titanium, fat and starch for the determination of total tract digestibility of fat and starch.

## Carcass and meat quality characteristics

On d 35, two birds per replicate cage (12 birds per treatment) were randomly selected, weighed and sacrificed by cervical dislocation. Following exsanguination, feathers, viscera, shanks and neck were removed and, weights of the eviscerated carcass, breast meat and abdominal fat were recorded, and expressed as a proportion of live body weight.

Samples of breast muscle from these birds were collected for the measurement of meat quality. Meat pH was measured in samples chilled for 24 h after slaughter. The muscle was diced with scissors into a plastic vial, and 10 ml of 150 mM KCl, pH 7 was added and homogenised. The pH was measured by inserting a digital electrode tip into the solution. Drip losses were measured as described by Ravindran and Elliot (2017). Briefly, breast muscle was chilled in ice water for 4 h, blotted dry, cut into 3 cm cubes and weighed. The samples were then placed in a plastic netting to allow the escape of any fluid and suspended on a stainless-steel hook in a sealable jar, ensuring that the sample did not make contact with the jar. After 24 and 48 h at chiller temperatures (1-5 °C), the cubes were removed, gently blotted dry and weighed. Drip losses were expressed as a percentage of the initial weight. Cooking losses were determined using samples chilled 24 h. Meat was cut into 3 cm thick cubes, weighed, arranged with cut surface facing out into polythene bags and suspended into a water bath at 70 °C for 60 min. The bags were then drained, and the cooked meat was chilled for 24 h. The cooked pieces were removed from the bag, blotted and the weight was recorded. Cooking losses were calculated by the weight difference between uncooked and cooked samples, and expressed as a percentage of the initial weight.

## Caecal microbiota profile

In addition, a cohort study involving 144 d-old broiler (Ross 308) chicks was undertaken to collect caecal samples for microbiota analysis. The dietary treatments, design and conduct of the cohort study were the same as the main study, except that it started with 12 chicks per replicate cage and four birds per age were removed on d 7, 21 and 35 post-hatch for caecal sampling. On sampling dates, the birds were sacrificed by cervical dislocation and both caeca were excised under sterile conditions. The contents were pooled within a cage, mixed well and representative samples (1 g) were obtained for analysis by fluorescence in situ hybridisation. The probes used were specific for *Lactobacillus*, *Bifidobacterium*, *Clostridium,* and *Bacteroides* spp., which were commercially synthesised and labelled with the fluorescent dye Cy3 (GeneWorks Pty Ltd, Hindmarsh, SA, Australia). The samples were prepared as described by Molan *et al.* (2010) and the analytical procedures have been described in detail by Singh *et al.* (2019). Populations for each bacterial group were expressed as log number of bacterial cells/g caecal content.

## Laboratory analysis

Dry matter was determined using standard procedures (method 930.15; AOAC, 2005). Gross energy was determined using an adiabatic oxygen calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid. Nitrogen was determined using a FP-428 nitrogen determinator (LECO® Corporation, St Joseph, MI). Titanium was measured on a UV spectrophotometer following the method of Short *et al.* (1996). Starch was measured using the total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Crude fat was determined as petroleum spirit extractable material (40-46 °C) using Soxhlet extraction (Soxtec System HT 1043 Extraction Unit, Höganäs, Sweden). The Se, Zn, Mn and Cu contents were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700 series, Agilent technologies, Stockport, Cheshire, UK). The Ca, P, K, Na and Fe were analysed in an inductively coupled plasma-optical emission spectrometry (ICP-OES, Varian Vista MPX CCD, Agilent technologies).

### Calculations

The AME was calculated using the following formula, with appropriate corrections made for differences in DM content:

$$\text{AME (MJ/kg diet)} = \frac{(\text{Feed intake} \times \text{GEdiet}) - (\text{Excreta output} \times \text{GEexcreta})}{\text{Feed intake}}$$

Nitrogen retention, as a percentage of N intake, was determined as follows:

$$\text{N retention (\%)} = 100 \times \frac{(\text{Feed intake} \times \text{NDiet}) - (\text{Excreta output} \times \text{NExcreta})}{\text{Feed intake} \times \text{NDiet}}$$

Correction for zero nitrogen retention was made as described by Hill and Anderson (1958), using a factor of 36.54 kJ per gram nitrogen retained in the body and N-corrected AME (AMEn) was calculated.

Total tract nutrient digestibility coefficients were calculated using the following formula:

$$\text{Nutrient digestibility coefficients} = \frac{(\text{nutrient/Ti})\text{d} - (\text{nutrient/Ti})\text{e}}{(\text{nutrient/Ti})\text{d}}$$

Where (nutrient/Ti)d = ratio of nutrient to Ti in the diet and (nutrient/Ti)e = ratio of nutrient to Ti in the excreta.

### Data analysis

Cage served as the experimental unit for all measured data. Counts of all bacteria were logarithmically-transformed as log10. Data were analysed using the General Linear Model procedure of the SAS (SAS, 2004). For nutrient utilisation and caecal bacterial count data, repeated measures analysis was used to assess treatment differences over age and treatment × age interactions. The level of significance was set at $P<0.05$.

## 3. Results

The mineral profile of N-GT and Se-GT are summarised in Table 2. The K and Mn were highest among macro and trace minerals, respectively. Selenium concentration of Se-GT green tea powder was 10-fold higher than that of N-GT (1.40 vs 0.13 mg/kg).

During 1-21 d of age, the weight gain of broilers fed the Se-GT diet was unaffected ($P>0.05$) whereas that of broilers fed the N-GT diet was reduced ($P<0.05$; Table 3). Feed intake was reduced ($P<0.05$) and feed per gain was improved ($P<0.05$) by both green tea types. Over the 35-d feeding period, weight gain and feed intake were depressed ($P<0.05$), and feed per gain was improved ($P<0.05$) by both green tea types.

Supplementation with N-GT increased ($P<0.05$) the AMEn on d 7 and 21, and with Se-GT on d 7 (Table 4). Green tea inclusion, however, had no influence ($P<0.05$) on the AMEn on d 35, resulting in a significant interaction ($P<0.05$) between the age and treatments. The AMEn was similar ($P<0.05$) between d 7 and 21, and followed by increase ($P<0.05$) at d 35 (Table 4). An age × interaction ($P<0.05$) was observed for fat digestibility with increase with N-GT on d 21, compared with Se-GT on d 7 and 21, but the differences disappeared on d 35. Fat digestibility increased ($P<0.05$) with advancing age. Inclusion of both green teas increased ($P<0.05$) the starch digestibility on d 7 and 21, but no effect was seen at d 35, as indicated by the age × treatment interaction ($P<0.05$). Starch digestibility increased ($P<0.05$) with advancing age.

**Table 2. Mineral composition of normal and selenium-rich green tea powders.**

| | Normal green tea | Se-rich green tea |
|---|---|---|
| Calcium, g/100 g | 0.40 | 0.48 |
| Phosphorus, g/100 g | 0.33 | 0.41 |
| Potassium, g/100 g | 1.55 | 1.70 |
| Sodium, g/100 g | <0.01 | <0.01 |
| Iron, mg/kg | 155 | 162 |
| Zinc, mg/kg | 80 | 98 |
| Copper, mg/kg | 30 | 34 |
| Manganese, mg/kg | 2,620 | 2,450 |
| Selenium, mg/kg | 0.13 | 1.42 |

**Table 3. Influence of green tea powder supplementation on the weight gain (g/bird), feed intake (g/bird) and feed per gain (g/g) of broilers.[1,2]**

| | Control | Normal green tea | Se-rich green tea | SEM[3] |
|---|---|---|---|---|
| 1-21 days | | | | |
| Weight gain | 942$^{a}$ | 872$^{b}$ | 918$^{a}$ | 13.3 |
| Feed intake | 1,398$^{a}$ | 1,240$^{c}$ | 1,303$^{b}$ | 17.1 |
| Feed per gain | 1.494$^{b}$ | 1.421$^{a}$ | 1.428$^{a}$ | 0.0161 |
| 1-35 days | | | | |
| Weight gain | 2,228$^{a}$ | 2,111$^{b}$ | 2,148$^{b}$ | 22.1 |
| Feed intake | 3,639$^{a}$ | 3,277$^{b}$ | 3,400$^{b}$ | 49.7 |
| Feed per gain | 1.641$^{c}$ | 1.553$^{a}$ | 1.590$^{b}$ | 0.0132 |

[1] Means in the same row with different superscript letters are significantly different ($P<0.05$).
[2] Each value represents the mean of six replicates (8 birds per replicate).
[3] Pooled standard error of mean.

**Table 4. Influence of green tea powder supplementation on the nitrogen-corrected apparent metabolisable energy ($AME_n$, MJ/kg) and, total tract fat and starch digestibility coefficients in broilers.[1,2,3,4]**

| | Control | Normal green tea | Se-rich green tea | SEM[5] |
|---|---|---|---|---|
| $AME_n$ | | | | |
| Day 7 | 10.75[aA] | 11.31[bA] | 11.53[bA] | 0.19 |
| Day 21 | 11.11[aA] | 11.77[bA] | 11.06[aA] | |
| Day 35 | 12.22[B] | 12.35[B] | 12.56[B] | |
| Fat digestibility | | | | |
| Day 7 | 0.38[aA] | 0.42[aA] | 0.56[bA] | 0.032 |
| Day 21 | 0.61[aB] | 0.76[bB] | 0.74[bB] | |
| Day 35 | 0.80[C] | 0.83[B] | 0.84[C] | |
| Starch digestibility | | | | |
| Day 7 | 0.88[aA] | 0.92[bA] | 0.92[bA] | 0.010 |
| Day 21 | 0.86[aA] | 0.90[bB] | 0.91[bB] | |
| Day 35 | 0.93[B] | 0.94[A] | 0.94[A] | |

[1] Means in the same row with different superscript letters are significantly different ($P$<0.05).
[2] Means in the same column with different capital superscript letters are significantly different ($P$<0.05).
[3] Each value represents the mean of six replicates (8 birds per replicate).
[4] Significant age × treatment interactions ($P$<0.05) for all three parameters.
[5] Pooled standard error of mean.

Abdominal fat pad, as a proportion of live weight, was decreased ($P$<0.05) by the inclusion of both green tea types (Table 5). Carcass yield, breast meat yield and pH were unaffected ($P$>0.05) by the dietary treatments. Drip losses were reduced ($P$<0.05) in the N-GT after 24 h storage, and in both N-GT and Se-GT-fed birds after 48 h. Cooking losses were not influenced ($P$<0.05) by the N-GT treatment, but reduced ($P$<0.05) by the Se-GT treatment.

Significant interactions ($P$<0.05) between the age and treatments were seen for counts of four bacterial species (Table 6). In general, dietary inclusion of green teas had positive effects ($P$<0.05) on the caecal microflora, with increased numbers of beneficial bacteria (*Lactobacillus* and *Bifidobacterium* spp.) and reduced numbers of pathogenic bacteria (*Clostridium* and *Bacteroides* spp.). Differences were observed between the two green tea types, with Se-GT being more beneficial than N-GT in the positive modulation of microflora.

## 4. Discussion

There do not appear to be any previous studies reporting the mineral profile of green tea powder. Minerals were analysed mainly to confirm the difference in Se content between N-GT and Se-GT. The data demonstrated that the Se content of Se-GT was 10 times higher than that of N-GT. Differences in the contents of other minerals were not directly comparable, as the two green tea samples were from different origins. Differences in cultivar, geographical location, agronomy and growing season can contribute to the variation in mineral composition in plant materials. Of particular note was the high content of Mn in both teas and suggested that tea leaves are an accumulator of this mineral.

The unique aspect of the present trial was that, despite the growing interest in the benefits of Se and green tea polyphenols as antioxidants, this was the first study investigating whether combining these two components had any beneficial effects on the performance, nutrient utilisation, carcass characteristics, meat quality and caecal microflora in broilers. The effects of their co-administration was of interest, because of their potentially complementary mechanisms as antioxidants. However, this thesis was not

**Table 5. Influence of green tea powder supplementation on carcass and meat quality characteristics of broilers.[1]**

| | Control | Normal green tea | Se-rich green tea | SEM[2] |
|---|---|---|---|---|
| Carcass yield (g/kg live weight) | 706 | 711 | 723 | 11.8 |
| Abdominal fat pad (g/kg live weight) | 16[b] | 10[a] | 11[a] | 1.6 |
| Breast meat yield (g/kg live weight) | 266 | 268 | 262 | 4.2 |
| Breast muscle pH | 6.18 | 6.13 | 6.13 | 0.060 |
| Drip loss (% initial weight)[1] | | | | |
| 24 hrs | 0.19[a] | 0.16[b] | 0.18[a] | 0.003 |
| 48 hrs | 0.24[a] | 0.21[c] | 0.19[b] | 0.005 |
| Cooking loss (% initial weight) | 0.22[b] | 0.21[ab] | 0.20[a] | 0.007 |

[1] Means in the same row with different superscript letters are significantly different ($P$<0.05).
[2] Pooled standard error of mean.

**Table 6. Influence of green tea supplementation on the enumeration of caecal bacterial counts ($Log_{10}$ cells/g of wet content; mean ± standard error) in broilers.[1,2]**

| | Control | Normal green tea | Se-rich green tea |
|---|---|---|---|
| *Bifidobacterium* spp. | | | |
| Day 7 | 7.12±0.021[a] | 7.13±0.025[a] | 7.20±0.021[b] |
| Day 21 | 6.98±0.040[a] | 7.30±0.044[b] | 7.29±0.029[b] |
| Day 35 | 7.19±0.023[a] | 7.27±0.024[b] | 7.32±0.012[c] |
| *Lactobacillus* spp. | | | |
| Day 7 | 6.06±0.029[a] | 6.06±0.039[a] | 6.16±0.032[b] |
| Day 21 | 5.91±0.062[a] | 5.98±0.045[a] | 6.16±0.040[b] |
| Day 35 | 5.68±0.059[a] | 6.16±0.022[b] | 6.20±0.024[b] |
| *Clostridium* spp. | | | |
| Day 7 | 7.14±0.032[a] | 6.96±0.037[b] | 6.82±0.039[b] |
| Day 21 | 7.00±0.027[a] | 6.77±0.036[b] | 6.77±0.031[b] |
| Day 35 | 7.08±0.020[a] | 6.98±0.058[a] | 6.42±0.043[b] |
| *Bacteroides* spp. | | | |
| Day 7 | 7.20±0.033[a] | 7.10±0.065[a] | 6.82±0.070[b] |
| Day 21 | 7.06±0.031 | 7.03±0.047 | 6.98±0.030 |
| Day 35 | 7.34±0.027[a] | 6.53±0.034[b] | 6.22±0.045[b] |

[1] Means in the same row with different superscript letters are significantly different ($P$<0.05).
[2] Significant age × treatment interactions ($P$ <0.05) for all four bacterial species.

supported by the findings of current work and, in general, there was no notable benefits from feeding the combination.

In the current work, 1% green tea inclusion reduced the weight gain of broilers, due primarily to reduced feed intake. The magnitude of reductions in feed intake was greater than those for the weight gain, resulting in lowered feed per gain. Similar reduced growth when feeding broilers green tea have been reported by other researchers (Biswas and Wakita, 2001; Chen *et al.*, 2019; Jelveh *et al.*, 2018; Yang *et al.*, 2003). Yang *et al.* (2003) showed that reductions in weight gain and feed intake tended to be dose related, which suggested this may have been due to the high polyphenol and fibre contents. This was confirmed in a recent study by Jelveh *et al.* (2018) whereby feed intake and weight gain were improved in broilers fed green tea extract with a polyphenol content of 10.2%, but depressed in those fed green tea powder with a polyphenol content of 14.9%. The negative effects of sorghum polyphenols on feed intake in poultry are well established (Nyachoti *et al.*, 1997; Ravindran and Blair, 1991). In contrast, Cao *et al.* (2005) showed no effect on bodyweight or feed intake when a semi-synthetic diet was supplemented with 0.4% green tea polyphenols. El-Deek and Al-Harthi (2004) reported no impact on growth performance when broiler chicks were fed diets supplemented with 0.5% green tea powder. The observed discrepancy in published literature reflected the differences in the source (green tea powder, green tea extracts and green tea polyphenols), inclusion level, contents and composition (total, hydrolysable and condensed of polyphenols) and age of the broilers.

The trial data showed an improvement in the feed efficiency when diets were supplemented with green tea. This was consistent with previous studies (Biwas and Wakita, 2001; Erener *et al.*, 2011; Jelveh *et al.*, 2018) although some researchers (Cao *et al.*, 2005; El-Deek and Al-Harthi, 2004; Yang *et al.*, 2003) showed no effect on feed per gain from green tea supplementation. The reason for improved feed efficiency may be attributed, in part, to the improvements in AMEn and digestibility of fat and starch. The shift in intestinal microflora towards beneficial species may have partly contributed to the improved feed efficiency.

Supplementation of N-GT increased the AMEn on d 7 and 21, and of Se-GT on d 7, but no effect was seen at d 35. The changes in total tract utilisation of fat and starch closely paralleled the patterns observed for AMEn. No advantages on nutrient utilisation were seen for the combination of GT and Se. Changes with advancing age revealed three interesting patterns. First, utilisation of fat and energy was compromised in the newly hatched broiler chick, which was consistent with published literature (Ravindran and Abdollahi, 2021). Secondly, starch digestion was not limiting in young chicks, as stated by Moran (1982). Third, the AME and digestion of nutrients increased as the broilers grew older, and similar findings were reported recently by Khalil *et al.* (2021).

The present study showed a proportional decrease in abdominal fat pad, but breast meat yield was unaffected. The reduction of abdominal fat may, in part, have been attributed to the suppressive effect of green tea on feed intake, which could have reduced hepatic lipogenesis and fat accumulation in adipose tissue and muscles (Biswas and Wakita, 2001). This effect was consistent with those of Kaneko *et al.* (2005) who showed a reduction in abdominal fat pad in broilers with the addition of 2.5 and 1.5% green tea powder, respectively. Biswas and Wakita (2001) showed that the relative weight of abdominal fat decreased with green tea supplementation. In addition, Eid *et al.* (2003) reported a reduction in abdominal fat pad with the addition of dietary green tea polyphenols. In contrast, Yang *et al.* (2003) and El-Deek and Al-Harthi (2004) showed an increase in abdominal fat, whereas Jelveh *et al.* (2018) failed to show any effect on abdominal fat deposition in broiler fed diets supplemented with green tea. Previous studies (Biswas and Wakita, 2001; Jelveh *et al.*, 2018) showed no increase in breast meat yield with green tea supplementation, which was consistent with the present results. The inconsistency for the abdominal fat results among studies could be explained by the differences in polyphenol content of the green tea products used in the studies.

In recent years, the physicochemical, sensory and nutritional characteristics of chicken meat is gaining wider consumer attention. In fast growing modern broilers, breeding for high productivity parameters, such as breast muscle development, weight gain and feed efficiency, has a negative influence on the pH and water holding capacity of meat, thus affecting the flavour, storage and processing properties (Rance *et al.*, 2002). Drip loss is related to pH and reduces the nutritional value, flavour and tenderness of meat (Barbut, 2009). In the present study, broilers fed diets supplemented with green tea powder had a less drip loss compared with control birds, but pH was unaffected. Zou *et al.* (2018) speculated that tea polyphenols may degrade the myofibrillar protein myosin and could enhance the tenderness of meat because of the lower drip loss. To the authors' knowledge, no studies to date have reported the impact of green tea supplementation on cooking losses, which were reduced by supplemental green tea in the present study.

Inclusion of both green teas in the diet resulted in significant increases in the numbers of lactic acid bacteria (*Lactobacillus* spp. *and Bifidobacteria* spp.) and a reduction in the numbers of pathogenic bacteria (*Bacteroides* and *Clostridium* spp.). Although the mechanisms by which green tea promoted the growth of lactic acid bacteria is not known, a possible explanation lies in the ability of polyphenols to act as antioxidant and antiradical agents

(Molan *et al.*, 2009b, 2010; Serafini *et al.*, 1996; Xu *et al.*, 2003), and consequently provide a better environment for the growth and multiplication of beneficial bacteria. Physiological concentrations of green tea polyphenols and extracts have been shown, in *in vitro* studies, to delay or inhibit the growth of a wide range of pathogenic strains of enteric bacteria, including pathogenic strains of *Escherichia coli* (Ciraj *et al.*, 2001; Yam *et al.*, 1997). In contrast, Cao *et al.* (2005) found that feeding green tea polyphenols reduced the counts of *Bifidobacteria*, *Bacteroidaceae*, *Peptococcaceae* and *Lactobacillus* spp. and significantly lowered the total bacteria count. The bacterial diversity and richness of intestinal microbiota were affected by supplementing tea polyphenols in broiler diets (Zou *et al.*, 2018).

The green teas used in the current study were not analysed for polyphenols because of the complexity in such chemistry related to classification, extraction, separation and analytical methods (Bravo, 1998; Tsao, 2010). Despite this limitation, the findings, nevertheless, had implications to the understanding the influence of green tea inclusion on nutrient digestion, meat quality and caecal microbiota.

Inclusion of 1% N-GT and Se-GT lowered the weight gain during the 1-35 feeding period by 5.2 and 3.6%, respectively, compared to the control diet. This effect paralleled reductions of 9.9 and 6.6% in feed intake respectively, which could be attributed to polyphenol content of green teas (Nyachoti *et al.*, 1997). Feed intake is the major factor driving weight gain and modern broilers are highly susceptible to intake stressors (Abdollahi *et al.*, 2018). It appeared that the provision of polyphenols from the 1% green tea exceeded the tolerance threshold of broilers. Green tea increased the energy and nutrient utilisation, but not to the extent sufficient to restore the weight gain equivalent to the control birds. This finding highlighted the importance of feed intake and the fact that growth performance is essentially a result of digestible nutrient intake rather than nutrient digestibility *per se* (Abdollahi *et al.*, 2013).

In the main, feeding diets supplemented with green tea had negative effects on the growth of broilers, but increased the utilisation of energy, fat and starch. Importantly, green tea supplementation promoted the proliferation of beneficial bacteria (*Lactobacillus* and *Bifidobacterium* spp.) and inhibited potentially harmful bacteria (*Clostridium* and *Bacteroides* spp.). Between the two green tea types, Se-GT was more beneficial than N-GT in the positive modulation of intestinal microbiota, but no other advantages were observed in the combination of green tea and Se. Green tea supplementation improved meat quality by reducing drip loss. The present results demonstrated that green tea supplementation can be beneficial in maintaining a healthy gut microbiome in the absence of in-feed antibiotics and to assist in efficient nutrient utilisation in broilers. The negative effects of green tea powder on feed intake and gain, however, highlighted the need to determine the polyphenol content before diets are mixed and to establish the threshold for polyphenols in broiler diets.

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