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Effects of ajwain seed powder supplementation on chemical composition and meat quality of Pratapdhan broiler

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Abstract
The present experiment was carried out the poultry farm of the S.K.N. College of Agriculture, Jobner, Rajasthan (India) in 2016 to determine the impact of Ajwain seed powder as a feed supplement on carcass composition and meat quality of Pratapdhan broiler. A total of 120 day-old Pratapdhan chicks were weighed and distributed randomly to four feeding treatment groups, with each treatment having three replicates of ten birds each. The birds were fed diet supplemented with different proportions of Ajwain seed powder that was fairly isocaloric and isonitrogenous. The quantities of Ajwain seed powder included in diet control, T_1, T_2, and T_3, were 0, 0.1, 0.2, and 0.3%, respectively. The chicks were reared using standard management approaches, i.e. feeding, watering, vaccination, and disease prevention during the experimental period. Results revealed that Ajwain seed powder inclusion in the diet also improved water holding capacity and the lipid oxidation stability of breast meat. Thus, it was concluded that dietary inclusion of Ajwain seed powder is quite effective in improving chemical composition and oxidative stability of breast meat.

Keywords: Ajwain seed powder, chemical composition, meat quality and Pratapdhan

Introduction
Over the last few decades, the Indian poultry sector has undergone major structural and operational changes. Additionally, the poultry industry has grown from a simple backyard enterprise into a significant commercial agro-based enterprise (Meena *et al*., 2012) [1]. The poultry sector is one of the agricultural industries in India that is expanding the fastest, with a growth rate of approximately 8% year^{-1} (APEDA, 2021) [2]. Between the 19th and 20th livestock censuses in India, the number of poultry birds increased from 729.2 million to 851.8 million, an increase of over 17% (DAHD, 2019) [2].
Numerous antibiotics have been used globally in chicken feed as growth and health promoters for the last sixty years (Butaye *et al*., 2003) [4]. The use of antibiotic growth stimulants in chicken feeds has raised concerns about pathogen cross-resistance and the presence of chemical residues in meat (Arsène *et al*., 2022) [5]. Moreover, inclusion of antibiotic growth promoters in poultry diets led to the emergence of bacterial resistance in humans (Saleh, 2014) [6]. Therefore, there is increased demand for food products free from antibiotic residues and chemical growth promoters (Castillo-López *et al*., 2017) [7]. The addition of antibiotics as growth promoters in poultry diets has been banned or restricted in many countries, especially in the European Union, since 2006 (Kumar *et al*., 2018) [8]. This resulted in attempts to find alternative natural growth stimulants to improve the growth performance, laying ability, carcass characteristics and meat quality of chickens. Feed additives in poultry diets alter the intestinal microbiota, which enhances the bird's performance and health condition (Dawood *et al*., 2018) [9]. They also improve feed digestibility, feed conversion ratio, reduce stress, minimize the negative effects of dietary alterations, and prevent the action of enzymes generated by microorganisms (Abdel-Moneim *et al*., 2020) [10]. In recent years, the feed industry has become more interested in phytogenic feed additives (Windisch *et al*., 2008) [11]. The aromatic plants and the essential oils (EO) derived from them have gained much importance as feed additive alternatives due to their antibacterial activities and stimulating effects on animals' digestive systems (Pathak *et al*., 2017) [12].
Ajwain, also known as *Trachyspermum ammi*, is an aromatic, spicy, and medicinal plant in the Apiaceae family It originated in Egypt and is now extensively used across the world. Ajwain is widely grown in the Indian states *i.e.* Rajasthan, Gujarat, and Andhra Pradesh.

The phytochemical constitution and bioavailability of Ajwain seeds are attributed to their therapeutic, medical, and pharmacological potential. Furthermore, Ajwain's antibacterial and immuno-modulatory properties play an important role in its beneficial effects on broiler growth performance (Yang *et al*., 2009). The effect of Ajwain powder on poultry growth performance and serum biochemical indicators has been found in a number of studies (Samadian *et al*., 2015 [13], Mahala *et al*., 2023 [14]); however, only scanty findings are available on chemical composition and meat quality (Rathor *et al*., 2022) [15]. Therefore, this experiment was conducted to determine the effectiveness of various levels of Ajwain seeds as a feed additive on the chemical composition and meat quality of Pratapdhan broiler chickens.

Materials and Methods

Experimental designs and treatments

A total of 120 day-old, unsexed, and relatively healthy broiler chicks (Pratapdhan) were procured from Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India under "Aangan me Murgi Palan" initiative supported by Rashtriya Krishi Vikas Yojana. The experiment was carried out at the poultry farm of the S.K.N. College of Agriculture, Jobner, Rajasthan, India during 2016-2017. Before beginning the trial, each individual broiler chick was weighed and had its wings banded for identification. The chicks were randomly distributed over four treatment groups each having 30 chicks. Each of the treatment was replicated three times, with ten birds per replicate in a Completely Randomized Design (CRD). The chicks were fed a diet with various amounts of Ajwain seeds that was quite isocaloric and isonitrogenous. The dietary levels were control (0%), T_1 (0.1%), T_2 (0.2%), and T_3 (0.3%). The basal diet was developed in line with the BIS (2007) recommendations for chicks. All chicks completed their scheduled doses of Ranikhet and infectious bursal disease vaccine. The chicks were maintained during the experimental period using standard management approaches, including as feeding, watering, and disease prevention. Dry and fresh wheat straw was used as bedding material.

Analysis of proximate composition

The proximate composition (moisture, protein, fat, and ash contents) of chicken was determined by standard methods using hot air oven, Kjeldahl assembly, Soxhlet extraction apparatus, and muffle furnace, respectively, as per AOAC (2000) [16].

Moisture estimation

Five gm of breast muscle were transferred into a pre-weighed flat bottom aluminium moisture cup. These aluminium cups without lids were placed in hot air oven. The samples were dried in a hot air oven for 14-16 hours at 100±3 ºC. The dried sample was then placed in a desiccator with silica gel as the desiccant. After 1 h, the cup containing the dried sample was weighed. Moisture content was calculated by applying the following formula:

$$\text{Moisture (\%)} = \frac{\text{Weight before drying} - \text{Weight after drying}}{\text{Weight before drying}} \times 100 \ldots\ldots (2)$$

Fat estimation

Fat content in the sample was extracted in Soxhlet extraction unit. Soxhlet extractor was set with reflux condenser and oil flask which was previously dried and weighed. Meat sample (3–4 g) was taken into fat free extraction thimble, dried in oven for 6 h at 100–102 ºC and placed in Soxhlet extraction apparatus. The 150 ml of petroleum ether (BP: 60–80 ºC) was then poured into extraction flask and condenser was joined and placed on electric heater in order to boil the solvent gently. Extraction was carried out for 16 h. Fat content was calculated by using the following formula:

$$\text{Fat (\%)} = \frac{(\text{Weight of oil flask + fat}) - \text{Weight of empty oil flask}}{\text{Weight of sample taken}} \times 100 \ldots (3)$$

Protein estimation

The sample (2–2.2 g) was digested using Micro-Kjeldahl digester in the presence of digestion mixture that acts as catalyst (sodium sulfate/potassium sulfate: copper sulfate, 5:1) and 40 ml sulfuric acid. The flask was placed in an inclined position and heated gently until frothing ceased, then boiled rapidly until the solution became clear. The sample was then cooled, and distilled water was added to bring the volume up to 250 ml. The diluted sample (10 ml) was distilled with 10 ml of 40% NaOH using Micro- Kjeldahl distillation unit. Steam was distilled over 2% boric acid (25 ml) containing mixed indicator (1 part 0.2% methyl red + 2 parts 0.2% bromocresol green dye) for 30 m. The amount of ammonia trapped in boric acid was determined by titrating with 0.1N sulfuric acid. The nitrogen percentage was calculated using the following formula:

$$\text{Nitrogen (\%)} = \frac{(\text{Titrated value (sample} - \text{blank}) \times 0.0014 \times \text{Total volume made}}{\text{Weight of sample taken} \times \text{Volume of distillate}} \times 100 \ldots\ldots (4)$$

Protein percentage was determined by conversion of nitrogen percentage to protein by using conversion factor (6.25) assuming that all the nitrogen in meat was present as protein i.e. protein percentage = N% ×6.25.

Ash estimation

The freshly minced sample (5–10 g) was placed in a pre-weighed crucible and placed in a muffle furnace at 550 °C for 4–5 h. The ash sample was placed in a desiccator with silica gel as a desiccant. After 1 hour, the crucible was weighed. The ash content was calculated by the following formula:

$$\text{Ash (\%)} = \frac{\text{Weight of ashed sample}}{\text{Weight of the sample taken}} \times 100 \ldots\ldots\ldots\ldots (5)$$

Analysis of meat quality and lipid-oxidation

Analysis of pH

The pH of breast meat was assessed by a method described by Troutt *et al.* (1992) [17]. The Ultra Turrax tissue homogenizer (IKA T-18, Ultra Turrax, Germany) was used to homogenize 10 g of meat for 1 min with 50 ml of distilled water. The pH of the homogenate was recorded by immersing a combined electrode of a digital pH meter.

Analysis of water holding capacity

The water holding capacity (WHC) was assessed using a 1 g sample of breast muscle. Briefly, a circular filter paper (Whatman No. 4) was used to hold the sample of minced breast meat. The paper was centrifuged at 6,000 x g for 10

min. The weight of the water absorbed by the filter paper was used to determine the moisture content of the breast meat.

Thiobarbituric acid reactive substance (TBRAS) value

The determination of TBA value was based on the procedure of Witte *et al.* (1970) [18]. Trichloroacetic acid (TCA) extract was prepared by homogenizing 5 g of meat sample with 25 ml of pre cooled 10% TCA solution for 2 min in mortar and pestle. The contents were allowed for extraction for 10 m and then filtered through a Whatman filter paper no. 42. 3 ml of TCA extract was mixed with an equal amount of 0.1% TBA reagent. The mixture was boiled in water bath for 35 m, then cooled and absorbance was measured at 532 nm in spectrophotometer. For blank, the same procedure was followed as described above except that 3 ml 10% chilled TCA solution was added instead of TCA extract. TBA value was calculated as mg malonaldehyde/kg of sample by referring the standard graph.

Statistical analysis

A statistical method using analysis of variance (ANOVA) was used for analysis of data obtained from different experiments. Results were compared with Tukeys'b at $p<0.05$ level of significance with SPSS package (SPSS 16.0 for Windows, SPSS Inc., USA) as per the procedure of Snedecor and Cochran, (1995) [19].

Results and Discussion

Analysis of proximate composition

The percentages of crude protein in breast meat significantly increased with supplementation of Ajwain in diet compared with the control group (Table 1). However, non-significant increases were observed in moisture (%), ether extract (%), and ash contents (%). Only few research studies were found to compare with the results of these findings. Al-Beitawi and El-Ghousein (2008) [20] revealed that concentrations of dry matter and crude protein in breast meat were improved with supplementation of the broiler diet with 15 g of black cumin seeds kg^{-1} of diet. Higher percentages of dry matter and crude protein in breasts might be due to increased intake of digestible crude protein and ether extract (Kumar *et al*., 2018) [8]. Many studies have found that feeding Ajwain seed powder increases feed intake as well as total nutrient retention in the intestinal tract (Shroha *et al*., 2019) [21]. In this light, protein contents in diets change the chemical composition (dry matter, crude protein, and ether extract) of chicken meat (Bregendhal *et al*., 2002) [22]. This might be due to Ajwain increasing blood thyroid hormone levels, which are linked to protein synthesis and energy metabolism in the body. Additionally, crude protein content in chicken meat improved with the addition of a high-protein diet compared to a low-protein diet (Gheorghe *et al*., 2014) [23].

Table 1: Chemical composition of breast muscle of Pratapdhan broiler supplemented with Ajwain seed powder

Particular	C	T_1	T_2	T_3
Moisture (%)	74.02±0.49	73.23±0.31	72.68±0.39	73.20±0.25
Dry matter (%)	25.98±0.49	26.77±0.31	27.32±0.38	26.80±0.25
Crude Protein (%)	21.79±0.38^{b}	22.34±0.32^{ab}	22.92±0.19^{a}	22.52±0.19^{ab}
Ether extract (%)	1.30±0.09	1.44±0.08	1.55±0.06	1.56±0.07
Ash (%)	2.89±0.35	3.01±0.47	2.84±0.45	2.72±0.31

(a) Each value is a mean of three replicates. (b) Means bearing different superscripts, differ significantly ($P<0.05$) row wise.

Meat quality and lipid-oxidation stability

The effects of dietary supplementation with Ajwain seed powder on pH are illustrated in Figure 1. The results revealed that no significant effect ($p>0.05$) was observed in the pH of breast meat after supplementation with Ajwain seed powder. Sikder *et al*. (2012) [24] observed that the pH of the thigh and breast meat of control group was significantly higher han that of the treatment groups, which received restricted feeding. The muscle pH is a vital index that reflects the speed of muscle glycogen degradation after slaughter. However, Abdulla *et al*. (2017) [25] showed a decrease in the pH of breast muscle in broiler chickens after probiotic treatment. The pH of meat has an indirect effect on its color. The extent of the reaction between myoglobin, oxygen, and enzymes, particularly when pH changes, is the most important factor influencing meat color (Yang *et al*., 2015) [26].

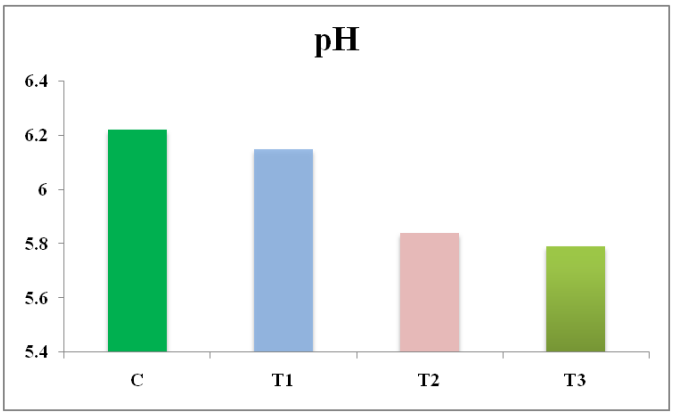

Fig 1: Graphical representation of pH of Pratapdhan breast muscle supplemented with Ajwain seed powder

Figure 2 depicts the effect of dietary inclusion of Ajwain as growth promoter on water holding capacity of breast muscle of Pratapdhan chicken. The supplementation of Ajwain seed powder to broiler diets significantly ($p<0.05$) increased the water holding capacity of breast meat. The highest water holding capacity was observed in T_2 groups;

thereafter, a non-significant decrease was observed in the T_3 group. Yang *et al*. (2015) [26] reported that one of the main quality attributes of fresh meat is its water-holding capacity because it influences consumer acceptance and final product quality. The current findings showed that inclusion of Ajwain seed powder in Pratapdhan broiler diets increased water holding capacity, in agreement with other trials where peppermint had the same effect (Mehri *et al*., 2015) [27]. However, Sikder *et al*. (2012) [24] observed that the non-significant effect of water holding capacity on the thigh and breast meat of the control group was significantly higher than that of the treatment groups that received restricted feeding.

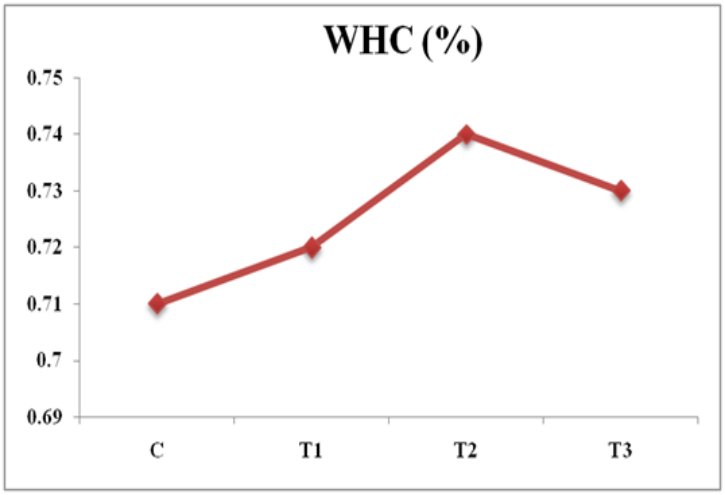

Fig 2: Graphical representation of WHC of Pratapdhan breast muscle supplemented with Ajwain seed powder

The effect of dietry inclusion of Ajwain as growth promoter on lipid-peroxidation (as malondialdehyde; MDA) on Pratapdhan chicken is represented in Figure 3. The level of Ajwain dietary treatments significantly reduced the extent of lipid peroxidation in breast meat (p<0.05). The lowest TBRAS was found in the T_2 group supplemented with 0.2% Ajwain, and the highest was observed in the control group. The findings proposed that the Ajwain supplemented diet's improved oxidation stability might be the reason for the reduced water loss from the meat. It has been observed that deterioration of the meat's protein was associated with increased drip loss (Traore *et al*., 2012) [28]. In other words, oxidative processes inhibit proteins' potential to form bonds with water molecules (Mehri *et al*., 2015) [27]. One of the most vital components for consumers is the stability of minced meat following processing, such as cooking or storage. Lipid oxidation of meat affects the product's quality by reducing the sensory quality, i.e., appearance, colour, odour, and flavor. Poultry meat is especially prone to oxidative degradation because of its high proportion of polyunsaturated fatty acids (Brenes and Roura, 2010) [29]. A number of studies have been conducted to examine the positive effects of dietary supplementation with aromatic plants on the oxidative stability of meat derived from poultry (Anilakumar *et al*., 2014) [30]. However, there is insufficient research on the influence of Ajwain on poultry meat quality.

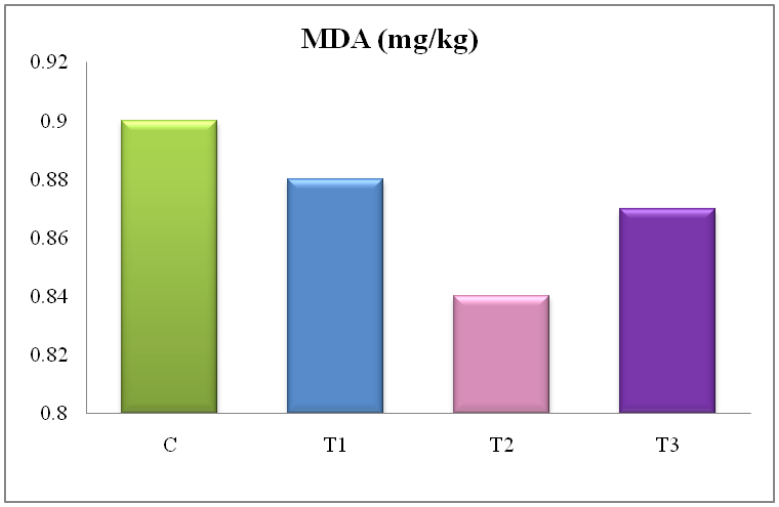

Fig 3: Graphical representation of TBRAS value of Pratapdhan breast muscle supplemented with Ajwain seed powder

Additionally, it has been stated that adding herbs and their extracts to the diet as dietary supplements is a simple and fast way to add natural antioxidants to chicken tissue (Brenes and Roura, 2010) [29]. Moreover, consuming Ajwain can significantly boost broiler productivity by reducing lipid peroxidation and enhancing antioxidant activity. Ajwain seeds fed to broilers in the present investigation had decreased TBARS levels. Other researchers who used peppermint in the diets of Japanese quail saw similar results (Mehri *et al*., 2015) [27]. Vitali *et al*. (2016) [31] observed that the significant

activity of Ajwain oil was due to the antioxidant activity of its primary component, thymol. Thus, Ajwain can be used as an efficient growth promoter and antioxidant agent comparable to other commercial feed additives.

Conclusion

The supplementation of Ajwain in broiler feed had revealed some positive effects on carcass composition and meat quality, resulting in improved livestock productivity and consumer food safety. Ajwain might be a beneficial dietary supplement to chicken feed as a potential antibiotic substitute, helping to facilitate effective production in a sustainable way to robust poultry industry. However, more investigation is needed to understand mechanism of action for using Ajwain seed powder as a supplement in broiler diets.

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Conflict of interests

The authors have declared no conflict of interests exist.

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