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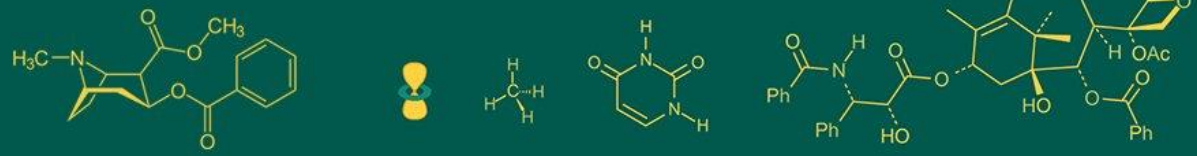

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Impact of amoxicillin, synbiotic and thyme essential oil on nutrient utilization in commercial broilers

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Abstract
A feeding trial was conducted to investigate the effects of amoxicillin, synbiotic and thyme essential oil as feed additives on nutrient utilization in commercial broiler chickens. A total of 200 Cobb broiler chicks of one week of age were randomly distributed into 5 dietary treatment groups: T_0 (Basal diet only); T_1 (Basal diet + amoxicillin @ 200 mg/kg); T_2 (Basal diet + synbiotic @ 100 mg/kg), T_3 (Basal diet + thyme essential oil @ 2ml/kg) and T_4 (Basal diet + synbiotic @ 50 mg/kg + thyme essential oil @ 1ml/kg). Each group contained 4 replicates with 10 birds each for 35 days period. The results revealed a significant (p<0.05) increase in the utilization of ether extract (EE) and crude fibre (CF) in T_4 treatment group than control group (T_0). In antibiotic (amoxicillin) supplemented group (T_1) there was significant (p<0.05) increase in dry matter (DM) and crude protein (CP) utilization compared to control group (T_0). In conclusion, supplementation of synbiotic and thyme essential oil combination can be beneficial for nutrient utilization in broiler chickens.

Keywords: Antibiotic, broilers, digestibility, essential oil, synbiotic

Introduction
Administration of antibiotics at sub-therapeutic levels has been used widely to increase weight gain, improve feed efficiency, reduce morbidity and mortality in poultry birds (Zeng *et al.*, 2015) [20] and reduce poultry and human food borne pathogens (Sims *et al.*, 2004) [15]. Although they have been applied in poultry for over 50 years, the use of antibiotic growth promoters (AGPs) has declined (Sneeringer *et al.*, 2015) [17] due to consumer preferences (Brewer and Rojas, 2007) [4] and regulations (Castanon, 2007) [5]. Additionally, there are concerns over the development of antibiotic resistance in bacteria (Forgetta *et al.*, 2012) [6] and antibiotic residues in meat and other livestock products which pose a risk to public health and the environment (Jazi *et al.*, 2018) [10]. This has led many countries to restrict the use of antibiotics in animal feed. Accordingly, it is necessary to identify cost-effective, safe, and eco-friendly alternatives to antibiotics. India has abundant herbal and medicinal plant resources, inclusion of thyme essential oil (*Thymus vulgaris* L.) in poultry diets could be a great idea to find alternatives to antibiotic growth promoters (AGP). Probiotics, synbiotics, enzymes are commonly used to improve the growth performance of poultry
The synbiotic, considered as an alternative, is a mix of probiotics and prebiotics. Synbiotic supplementation decreases negative effect of heat stress on broilers by improving foot and skeletal health (Hu *et al*., 2022) [8].
Thyme (*Thymus vulgaris* L.) is a prominent plant of Mediterranean countries which has unique medicinal properties. It is one of the herbaceous plants that has recently got a lot of attention because of its antioxidant and antibacterial properties. Antibacterial activity against a wide spectrum of harmful microbiological organisms has also been reported for the herb (Varel, 2002) [19]. The major bioactive components of thyme essential oil, thymol and carvacrol have antibacterial and antifungal properties (Basilico and Basilico, 1999) [3].

Materials and Methods
Experimental site and material
This experiment was conducted at Instructional Poultry Farm (I.P.F), Govind Ballabh Pant

University of Agriculture and Technology, Pantnagar (U.S. Nagar), Uttarakhand. Amoxicillin, synbiotic and thyme essential oil were used as the supplement in this trial. Amoxicillin was purchased locally, synbiotic was purchased from the Zeus Biotech Private Limited, Mysore and Thyme essential oil was purchased from the Empirical Sciences, Greater Noida (UP).

Experimental birds

A total of 200 day-old commercial broiler chicks procured from reputed dealers. All the chicks were individually weighed on day 8th and 10 were randomly assigned per replicate belonging to 5 treatments of broiler chicks.

Experimental diets

The feeding regime comprised starter diet from 8th day until 21 days age and a finisher diet from 22 to 42 days of age.

The ingredient composition of the basal diet is given in Table 1. The nutrient composition percentage of the basal diet is presented in Table 2. All the diets were prepared with the same batch of ingredients, and all diets within a period had the same composition.

Table 1: Composition of basal diet fed to commercial broilers

Feed ingredients (%)	Broiler starter ration (8-21 days)	Broiler finisher ration (22-42 days)
Maize	50.66	57.45
Soya meal	30.00	25.00
GNC	10.00	8.00
Rice polish	6.00	6.00
DCP	2.40	2.40
Vegetable oil (g)	0.045	0.055
Lysine (g)	0.10	0.10
DL-Methionine (g)	0.10	0.10
Vitamin mix (g)	0.10	0.10
Poultry min (g)	0.20	0.40
Choline chloride (g)	0.05	0.05
Coccidiostate (g)	0.05	0.05
Common salt (g)	0.30	0.30
Total	100	100

Table 2: Nutrient composition of basal diet fed to commercial broilers

Chemical composition (%)	Starter feed	Finisher feed
Moisture	9.32	9.73
Crude protein	22.78	20.31
Crude fibre	4.47	4.26
Ether extract	4.48	4.22
Total ash	6.82	6.53
Acid insoluble ash	1.33	1.27
NFE	62.03	64.03
Calcium	1.28	1.24
Phosphorus	0.80	0.78

Parameters

Moisture, crude protein, ether extract, acid insoluble ash and total ash of feed were determined using AOAC (2003) [2] methods, as were dry matter, crude protein, ether extract, crude fibre and total ash of excreta.

Determination of dry matter

In a pre-weighted Petri dish, a representative sample was taken and kept in a hot air oven at 100 °C for 24 hours. After 24 hours, the weight of the petridish was measured again. The following formula was used to calculate dry matter:

Dry matter (%) = b/a × 100

Where,
a = Fresh weight of the sample (g)
b = Weight of the sample after drying (g)
Moisture (%) = 100 - Dry matter (%)

Determination of nitrogen and crude protein

The Kjeldahl technique was used to determine the crude protein concentration. For this purpose, 2 g of representative sample was weighed and placed in a digestion flask, then 3g of digestion mixture (K_2SO_4:$CuSO_4$, in a 9:1 ratio) and 20 ml of concentrated sulphuric acid were added. After that, the contents were digested until a clear blue/green transparent liquid appeared. After cooling, distilled water was added to make the capacity 100 ml. After a 20 ml aliquot of digested mixture was distilled with excess of 40 percent NaOH solution, liberated ammonia was collected in 20 ml of 2 percent boric acid solution containing 2 to 3 drops of mixed indicator (10 ml of 0.1 percent bromocresol green + 2 ml of 0.1 percent methyl red indicator in 95 percent alcohol). The ammonia entrapped was titrated against 0.1N HCl. In the same method a reagent blank was digested and distilled. The nitrogen content of the sample was calculated using the following formula:

$$\text{Nitrogen } \% = \frac{(\text{Sample Titre - Blank Titre}) \times \text{Normality of HCl} \times 14 \times \text{volume made up}}{\text{Aliquot of digest taken} \times \text{Weight of the sample taken}}$$

Crude protein % was then calculated as,
Crude protein (%) = Nitrogen (%) × 6.25

Determination of ether extract

The ether extract was calculated using the Soxhlet method. For this purpose, 2 g of dried and pulverized sample was transferred to a thimble and the weight of the empty oil flask was recorded. The thimble was placed in a straight line in Soxhlet's apparatus for 8 hours. The solvent used was petroleum ether (B.P. 40-60 °C), which was then evaporated. After that, it was taken out of the hot air oven, cooled in a desiccator and weighed. The following formula was used to determine the percentage of ether extract:

Ether extract (%) = b/a × 100

Where,
a = weight of the sample
b = (weight of the oil flask after extraction) - (weight of oil flask before extraction)

Statistical analysis
The experimental data acquired in the present study was subjected to relevant statistical analysis (Snedecor and Cochran, 1994) [16] through the employment of general linear model procedures within the SPSS package. Distinctions between treatment means were assessed using Duncan's multiple range test (Kramer, 1957) [11].

Results and Discussion
The significant (p<0.05) nutrient utilization was observed in treatment groups. The highest dry matter and crude protein utilization was observed in treatment T_1 followed by T_4 treatment group. Numerically similar dry matter and crude protein utilization was observed in treatment groups T_2 and T_3 and the lowest dry matter and crude protein utilization was observed in control group (T_0). In treatment group T_4 in which combination of synbiotic and thyme essential oil was given showed maximum nutrient utilization in terms of ether extract and crude fibre. However, ether extract and crude fibre utilization found similar in treatment groups T_2 and T_3 like that of control group (T_0).

Table 3: Nutrient utilization (Mean±SE) of broiler chickens fed with amoxicillin, synbiotic and thyme essential oil

Parameters	Dietary treatments					
	T_0 Control	T_1 Amoxicillin	T_2 Synbiotic	T_3 Essential Oil	T_4 Synbiotic & Thyme Essential Oil	P Value
Dry Matter (%)	74.51^{c}±0.73	78.32^{a}±0.44	76.13^{abc}±0.63	75.52^{bc}±0.66	77.19^{ab}±0.85	0.021
Crude Protein (%)	65.32^{c}±0.55	68.46^{a}±0.40	66.27^{bc}±0.62	67.21^{abc}±0.90	67.73^{ab}±0.61	0.041
Ether Extract (%)	77.46^{c}±0.79	80.12^{ab}±1.00	$77.63^{c±}$0.26	$78.70^{bc±}$0.82	81.47^{a} ±0.47	0.012
Crude Fiber (%)	14.85^{bc}±0.42	13.70^{c}±0.31	14.94^{bc}±0.29	$15.38^{ab±}$0.31	16.07^{a} ±0.47	0.011

Means having different superscript in a row differ significantly (p<0.05)

A significant improvement in crude protein utilization in broiler chickens supplemented with thyme essential oil was reported by Abbasi *et al.* (2020) [1]. Kumar *et al.* (2017) [12] reported improvement in the digestibility of nutrients in broilers by supplementation of phytogenics in the diet. Hernandez *et al.* (2004) [7] studied the effect of essential oil extract from oregano, cinnamon, and pepper and labiatae extract from sage, thyme, and rosemary found that plant extract supplementation improved apparent whole-tract and digestibility of the nutrients and dry matter digestibility. Prasad *et al.* (2009) [4], however, reported that essential oils didn't improve the digestibility of nutrients in the gut of birds. The difference in the results may be attributed to type of essential oils used, inclusion levels, etc., as some oils irritate the wall of the intestinal lining, leading to some inflammation (Su *et al.*, 2020) [18].

Conclusion
On the basis of results of present study, it can be concluded that synbiotic and thyme essential oil supplementation, especially in combination, proved to be a viable strategy as against antibiotic feed additives in improving nutrient utilization in commercial broiler chickens.

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