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Effect of *Cannabis sativa* supplement in organic laying hen diet on production performance and egg quality

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Abstract The result showed that using *Cannabis sativa* supplement significantly increased production performance ($p<0.01$). Dietary treatment with 1.0 and 1.5 % of cannabis sativa supplement offered the highest rate of production performance at 76.79 and 76.43 % respectively. However, egg weight, eggshell thickness, eggshell weight, yolk weight, albumen weight, albumen height, yolk color and haugh units did not significant difference ($p>0.05$). In conclusion, *Cannabis sativa* supplement can be used in dietary for organic laying hens at 1.0 %.

Keywords: Cannabis sativa, Organic, Laying hen, Production performance, Egg quality

Introduction

Organic egg production is one of the options in raising laying hens with a concept to avoid synthetic substances both in plant materials and raising process; for example, chemical fertilizer, pesticides, antibiotics, hormones and GMOs vegetation (Medina-Cruz *et al.,* 2024). Raising laying hens by using chemical substances causes severe health problems for producers and customers and the cost of chemical substances also increases continuously. Moreover, it has terrible effects on ecosystem and environment (Windhorst, 2005). Therefore, in order to solve these problems, knowledge of organic agriculture and modern innovation in livestock are combined together and become a path for organic livestock. It is an option of animal husbandry that rely on organic agriculture and concerning of animal safety to reduce stress (National Bureau of Agricultural Commodity and Food Standards, 2018). Nowadays, many farmers constantly pay attention in organic livestock including applying knowledge and modern technology to improve production system such as adding herbal supplement in hen diets.

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Adding herbal supplement in laying hen dietary treatments instead of antibiotics is one of the interesting options for organic egg production because Thailand has abundant local herbs which varieties in species and biochemical interactions such as cannabis sativa (*Cannabis sativa indica*). Cannabis sativa especially from its leave and flower has important various bioactive compound like delta-9, tetrahydrocannabinol (THC) and cannabidiol (CBD) (Al-Khazaleh *et al.,* 2024). Besides, cannabis sativa also helps to increase animal's appetite (Boonprakob *et al.*, 2024). Using parts of cannabis sativa such as leave, branch, trunk and root which contain low level of THC, theTHC decreases in the order of inforescences (10–12%), leaves (1–2%), stems (0.1–0.3%), roots (<0.03%), and seeds (generally absent) (Roy *et al*., 2014). Therefore, this present study aimed to evaluate the effect of cannabis sativa supplement in organic laying hen diet on production performance and egg quality to improve production performance and egg quality.

Materials and methods

This research was conducted by completely randomized design (CRD). One hundred and twenty commercial laying hens were allotted into 4 dietary treatments, 3 replications with 10 hens per replication. The dietary treatments were combined with cannabis sativa (branch and trunk) supplement at a level of 0 %, 0.5 %, 1.0 % and 1.5 % respectively.

This study was conducted by using 15-week-old commercial laying hens. The hens were allowed for adaption period for 6 weeks. They were fed by organic diets with 18 % protein which generated 2,800 Kcal/kg. Experiment period started when the hens were 24 weeks old. The hens were fed following the dietary treatment program and food ingredients were produced in accordance with the standard of organic livestock (National Bureau of Agricultural Commodity and Food Standards, 2018). All diet formulation contained 2,800 Kcal/kg. Each hen consumed 120 g of feed/day for 16 weeks. Feed and water were available for *ad libitum* consumption and all hens were vaccinated in accordance with the suggestion from Department of Livestock Development. The hens were kept in free-caged condition in an opened 2 x 5 m^2 housing. The floor was covered by 2 inches thickness of organic chaff. The hens were exposed to a free-range area in 5 x 10 m^2 field from 6 a.m. to 6 p.m.

Data collections

The total number of eggs including number of broken and cracked eggs and egg weight were recorded everyday in order to analyse production performance. Eggs from each treatment were randomly stored in 5 ^{o}c

refridgerator. The egg quality such as egg weight, eggshell weight was recorded in order to calculate for percent of eggshell. Yolk weight, yolk color and albumen height were inspected to calculate to find percent of yolk, percent of albumen and Haugh Unit (HU) in accordance with the following formula;

$$\text{Haugh Unit (HU)} = 100 \log (H - 1.7W^{0.37} + 7.57)$$

when H is albumen height (mm.)
W is egg weight (g)

Statistical analysis

Data collected from the study were analyzed using analysis of variance (Proc. GLM) and compare averages of each treatment.

Ingredient composition of diets

The ingredients and composition of organic basal diet and cannabis sativa supplement diet was showed in Table 1.

Table 1. Ingredients and composition of basal diet (%)

Ingredients	Organic basal diet
Organic broken-milled rice	38.00
Organic corn	20.00
Organic rice bran	12.00
Fish meal	9.50
Organic soybean	12.00
Shell flour	7.00
Rice bran oil	1.00
Premix	0.50
Total	**100.00**

Chemical analysis

Chemical substances in the diets were analyzed using proximate analysis (Table 2) in accordance with AOAC (1975). the analysis of chemical substances included humidity, protein, fat, dietary fiber, calcium, phosphorus and gross energy.

Table 2. Nutrient composition of the diets

	Organic basal diet (%)	Cannabis sativa
Moisture	-	14.47
Crude ash	-	0.48
Nitrogen free extracts	-	12.31
Ether extract	-	1.67
Crude protein	16.37	7.76
Crude fiber	6.37	12.43
Ca (%)	3.44	-
P (%)	0.56	-
Gross energy (Kcal/Kg.)	2,839.85	-

Results

Effects of combining Cannabis sativa supplement in organic laying hen diets on production performance

After comparing 4 dietary treatments with supplement level of *Cannabis sativa* as 0 %, 0.5 %, 1.0 % and 1.5 %, the results showed significant difference of production performance from 4 treatments ($p<0.01$) at a level of 58.93 %, 69.64 %, 76.79 % and 76.43 % respectively. It could be assumed that adding *Cannabis sativa* supplement in the diets significantly showed a better result for production performance ($p<0.01$). The level of *Cannabis sativa* supplement added into the diets at a level of 1.0 % had the highest rate of production performance (76.79 %) as showed in Table 2.

Egg weight were not significantly affected by cannabis sativa supplement in the diets ($p>0.05$), then the average was 55.59 %, 55.56 %, 52.08 % and 53.86 % respectively (Table 3). Results showed in that mixing *Cannabis sativa* supplement in the diets did not affect egg weight; however, it increased the performance of egg production (Table 2).

Table 3. Production performance and egg weight from 4 dietary treatments

Factor	Percentage of cannabis sativa supplement				P-value
	0	0.5	1.0	1.5	
Production performance (%)	58.93±18.92^{a}	69.64±15.51^{ab}	76.79±14.16^{b}	76.43±11.93^{b}	0.001**
Egg weight (g)	55.59±2.39	55.56±0.94	52.08±1.44	53.86±1.00	0.071

Remark: different English letter in horizontal line means significant difference 0.05 ($p<0.01$)

Effects of Cannabis sativa supplement in organic laying hen diets on egg quality

Egg quality were not significantly affected by cannabis sativa supplement in the diets ($p>0.05$), and the average were 6.58 g, 6.67 g, 6.31 g and 6.54 g respectively. Results of egg quality were consistent with results of eggshell thickness ($p>0.05$) from the samplings taken from the hens that consumed 4 dietary treatments as showed in Table 4.

Regarding the quality of albumen and yolk, results in Table 3 showed that feeding the laying hens with 4 dietary treatments did not affect albumen weight ($p>0.05$), and the average were 32.63, 31.88, 29.34 and 31.62 respectively. Also, yolk weight was not significantly affected by *Cannabis sativa* supplement in the diets, and the average were 16.38, 16.92, 16.42 and 15.69 respectively. Yolk color showed no difference ($p>0.05$) with the average as 6.22, 5.11, 5.22 and 5.67 respectively. Moreover, albumen height and haugh unit had no difference ($p>0.05$). The average of albumen height was 8.09, 8.08, 8.08 and 8.08 respectively. The average of haugh unit respectively showed as 91.05, 90.99, 91.98 and 91.45 respectively.

In conclusion, the addition of *Cannabis sativa* supplement in laying hen diets did not affect the quality of eggshell thickness, albumen and yolk.

Table 4. quality of albumen and yolk of laying hens that consumed 4 dietary treatments with the addition of *Cannabis sativa* supplement

Factor	Percentage of cannabis sativa supplement				P-value
	0	0.5	1.0	1.5	
Eggshell thickness (mm)	0.38±0.02	0.38±0.01	0.38±0.01	0.37±0.01	0.908
Eggshell weight (g)	6.58±0.08	6.67±0.32	6.31±0.61	6.54±0.48	0.654
Yolk weight (g)	16.38±0.34	16.92±0.45	16.42±1.90	15.69±0.76	0.587
Albumen weight (g)	32.63±2.73	31.88±0.36	29.34±1.72	31.62±0.96	0.179
Albumen height	8.09±0.26	8.08±0.31	8.08±0.26	8.08±0.25	1.000
Yolk color	6.22±1.07	5.11±0.19	5.22±0.84	5.67±0.00	0.258
Haugh unit	91.05±1.98	90.99±1.63	91.98±1.11	91.45±1.48	0.860

Remark: different English letter in horizontal line means significant difference 0.05 ($p<0.05$)

Discussion

As compared with the control diet, the dietary treatments with the addition of *Cannabis sativa* supplement on a level of 1.0 % and 1.5 % showed better results in production performance ($p<0.01$). One percent addition of

Cannabis sativa supplement in the diets showed the best production performance as 76.79 %. When comparing this study, *Cannabis sativa indica*, with hemp (*Cannabis sativa sativa*), it was found that the use of hemp seed in laying hen feed at levels of 5 – 20 % did not affect production performance ($p>0.05$) (Silversides *et al*., 2002; Silversides and Lefrancois, 2005; Neijat *et al.,* 2014; Kanbur *et al.,* 2023). In this study, the results showed that egg weight were not affected by the addition of cannabis sativa supplement in the diets ($p>0.05$), in according to the report of Silversides and Lefrancois (2005), Neijat *et al.* (2014), and Sandison (2017). However, Mierlita *et al.* (2024) revealed in their research that adding cannabis sativa supplement more than 10 % could give negative effects on egg production because the high level of lignified cell walls and tannins could affect bird's digestive. In summary, it could be assumed that the addition of 1.0 % *Cannabis sativa* supplement in laying hen diets involved with better production performance ($p<0.01$).

With respect to measure albumen and yolk quality, the addition of cannabis sativa supplement in 4 dietary treatments showed no significance on albumen weight, yolk weight, yolk color and albumen height ($p>0.05$). According to studies by Neijat *et al.* (2014) and Sandison (2017), the addition of hemp to laying hen diets did not affect egg quality, such as egg mass, feed per egg ratio, albumen weight, yolk weight, yolk color, albumen height, eggshell, albumen percentage, and yolk percentage. Moreover, results of haugh unit also showed no difference. In consideration of egg freshness, in accordance with the standard of Ministry of Agriculture and Cooperatives in Thailand, Pimpen and Nitiya (2022), reported in their research that egg quality is divided into 3 levels; AA (H.U. > 72), A (H.U. = 60 - 71) and B (H.U. < 60). According to results of this study, all eggs were in AA level which mean they had the best quality. The study of Taaif *et al*. (2023) found that adding hemp supplement in Lohmann Brown Classic hen diets significanly improved quality of albumen. Mierlita *et al.* (2024) reported that adding hemp supplement in the diets could increase omega-3 fatty acid in yolk especially alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids could reduce a risk of heart disease, promote immune system and reproductive system. Kanbur *et al.* (2023) also stated in the research that the addition of hemp supplement in hen diets did not showed negative effects on egg quality and production performance.

In summary, adding branches of *Cannabis sativa* supplement which were the residue from production process in the diets showed a good result since it could significantly increase production performance ($p<0.01$). On the other hand, the different level of *Cannabis sativa* supplement added in 4 dietary treatments did not affect on egg weight, eggshell thickness, eggshell weight, yolk weight,

albumen weight, albumen height, yolk color and haugh unit. Adding 1.0 % of cannabis sativa supplement in the diets showed the best result on production performance (76.79%). Compared with the control group, production performance of the laying hens consumed the diets mixed with *Cannabis sativa* supplement showed higher rate (17.86%).

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