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Research Article

The Effect Supplementation of Squaw Mint Herb on Oxidant Stability and Microbial Count of Quail Meat at Different Times of Storage in the Freezer

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Abstract

This trial was conducted to evaluate effect of squaw mint herb on antioxidant activity and microbial count of quail meat at storing different times. In this experiment, 80 one-day-old Japanese quails were used with 5 treatments in a completely randomized design and then fed with experimental diets containing 0, 0.5, 1, 1.5 and 2% squaw mint herb for 5 weeks. In the end of trial, antioxidant activity and microbial load of meat at 0, 30, 60 and 90 days after freezing at -20°C were measured. The antioxidant activity of meat did not influence on slaughter day by squaw mint ($P > 0.05$). But after 1 month freezing, treatments containing squaw mint decreased the malondialdehyde amount of meat and lowest was for 1.5 and 2% squaw mint. After 2 and 3 months storage in freezer, malondialdehyde amount of meat was the lowest for 2% squaw mint. By increasing the levels of squaw mint herb in the quail diet, a decrease in the microbial count of meat was observed at storage different times. The diet containing 2% squaw mint had the lowest microbial count ($P < 0.05$). It is concluded that the inclusion squaw mint to 2% in Japanese quail diet had beneficial effects on oxidant stability and microbial load of meat at storage different times in the freezer. Therefore, it is recommended that squaw mint to be used at 2% level can be improved the meat quality of quail.

Keywords: *Squaw Mint; Oxidative Stability; Microbial Load; Quail Meat*

Introduction

Medicinal plants contain phenols, polyphenols, terpenoids, essence oils and other compounds and have antimicrobial and antioxidant properties [11]. Squaw mint (*Mentha pulegium* L.,), one of the species of peppermint that grows in the humid plains and the margins of water [27]. The essential oil of this plant is including pulegone, linalool, limonene, dipentene and azulene, that dominant component of the essential oil is pulegone [1].

The herbs species like mint due to the high amounts of monoterpenes, thymol and carvacrol have antioxidant and antimicrobial properties [8]. The researchers reported, this antioxidant effect is related to the induction of antioxidant enzymes in the presence of phenolic compounds which prevent lipid oxidation [14].

Undesirable effects of synthetic antioxidants include motations, poisoning and also they have same effects with natural antioxidants [25]. Antioxidants act as a cleanser of free radicals, which these free radicals damage to the molecules and destroy their function and the primary defense against oxidative degradation is by antioxidants [28]. The effects of herbal supplements or extracts has been studied in the diet of animals and chicks and it has been determined that herbs such as oregano and thyme reduce the oxidation of meat [21,24].

Squaw mint grows widely in most of area and there are little studies on the effects of squaw mint in quail nutrition. Therefore, in this research microbial count and oxidant stability of the quail meat at storage times in the freezer were investigated.

Materials and Methods

Animals and diets

In this experiment, 80 Japanese quails divided into 5 treatments, 2 replicates and 8 replicates in each replicate. Diets were arranged according to the requirements of the poultry diet and were fed for 35 days (NRC, 22). Birds were placed on floor pens in a temperature-controlled house and had free access to feed and water daily. The lighting program consisted of a period of 16h light and 8 h darkness. The ambient temperature in experimental house was maintained at 34 - 35°C on the first day and gradually decreased to 23°C to 35 days and then fixed on 21 - 25°C to end of laying period.

The experimental diets during experiment were including: control diet (without squaw mint herb) and diets containing 0.5, 1, 1.5 and 2% squaw mint herb. The ingredients of the experimental diets are shown in table 1.

Item	Amount
Corn	51.00
Soybean meal	41.26
Bran	3.00
Squaw mint herb	0.00
Vegetable oil	1.56
Oyster meal	1.37
Dicalcium phosphate	0.7
NaCl	0.27
DL-methionine	0.16
Lysine	0.05
$NaHCO_3$	0.08
Mineral permix[1]	0.25
Vitamin permix[2]	0.25
Metabolizable energy (Kcal/Kg)	2792.80
Protein	23.11
E/P	120.83
Ca	0.8
P	0.3
Lysine	1.3
Methionine	0.5
Linoleic acid	1.35

Table 1: *Ingredients and chemical composition of experimental basal diet of Japanese quails (%).*

[1]Mineral premix is containing (per kg of diet): Pantotenate Ca 40 mg; choline chloride 840 mg; ethoxy quin 0.125 mg; MgSo4 100 mg; Fe 50 mg; CuSo4 100 mg; ZnSo4 60 mg; I 1 mg; Se 0.2 mg.

[2]Vitamin premix is containing (per kg of diet): vitamin A 10000 IU; vitamin D_3 9790 IU; vitamin E 121 IU; vitamin K 2 mg; thiamin 4 mg, riboflavin 41.4 mg; nicotinic acid 22 mg; pantothenic acid 40 mg; vitamin B_{12} 20 mg; folic acid 1 mg; biotin 30 mg; choline 840 mg.

Squaw mint herb powder added at 0. 0.5, 1, 1.5 and 2% to basal diet of Japanese quails.

Samplings

After the 35 days, at end of experiment, birds of experimental units were slaughtered. The skin was bled immediately and after emptying the carcass from the viscera, the samples (chest and thigh) with ice were immediately transferred to laboratory. The amount of fat oxidation of meat at different times of storage (slaughter day, 1, 2 and 3 months freezing at -20°C) was estimated by measuring Thiobarbituric Acid using Botsoglou., *et al* [5].

To evaluate the anti-bacterial properties of squaw mint herb in meat, a spread plate microbial test was performed and the number of all cultured colonies in the culture medium was counted in 10^{-2} dilutions [16].

Statistical analysis

All data were analyzed using the General Linear Model procedures of SAS [26] in a completely randomized design based on the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$. Where Y_{ij} is the observation, μ is the general mean, T_i is the effect of squaw mint herb and e_{ij} is the SE of term. The Duncan's multiple range tests was used to compare the mean difference at $P < 0.05$.

Results and Discussion

The oxidant stability of quails meat did not influence on slaughter day by squaw mint ($P > 0.05$). But after 1 month freezing, treatments containing squaw mint decreased the malondialdehyde amount of meat and lowest was for 1.5 and 2% squaw mint. After 2 and 3 months storage in freezer, malondialdehyde amount of meat was the lowest for 2% squaw mint (Tables 2-5).

Squaw mint herb %	Malondialdehyde (mg/kg)	Microbial count (Log cfu/g)
0	0.46	3.12[a]
0.5	0.44	2.40[b]
1	0.36	1.71[c]
1.5	0.23	1.52[d]
2	0.17	1.21[e]
SEM	0.2	0.01
P Value	0. 23	0.004

Table 2: *Effect of squaw mint herb on malondialdehyde and microbial count of quail's meat at slaughter day. SEM: Standard Error of Means; Means with letters within each column differed significantly (P < 0.05).*

Squaw mint herb %	Malondialdehyde (mg/kg)	Microbial count (Log cfu/g)
0	0.49[a]	4.71[a]
0.5	0.46[a]	2.80[b]
1	0.40[b]	1.92[c]
1.5	0.32[c]	1.61[d]
2	0.20[d]	1.32[e]
SEM	0.022	0.02
P Value	0.03	0.03

Table 3: *Effect of squaw mint herb on malondialdehyde and microbial count of quail's meat after 1 month freezing at -20°C. SEM: Standard Error of Means; Means with letters within each column differed significantly (P < 0.05).*

Squaw mint herb %	Malondialdehyde (mg/kg)	Microbial count (Log cfu/g)
0	0.54[a]	5.61[a]
0.5	0.48[a]	3.2[b]
1	0.39[b]	2.1[c]
1.5	0.35[c]	1.72[d]
2	0.23[d]	1.42[e]
SEM	0.04	0.012
P Value	0.011	0.021

Table 4: *Effect of squaw mint herb on malondialdehyde and microbial count of quail's meat after 2 months freezing at -20°C. SEM: Standard Error of Means; Means with letters within each column differed significantly (P < 0.05).*

Squaw mint herb %	Malondialdehyde (mg/kg)	Microbial count (Log cfu/g)
0	0.62[a]	7.8[a]
0.5	0.51[b]	4.10[b]
1	0.38[c]	2.4[c]
1.5	0.34[d]	1.78[d]
2	0.24[e]	1.48[e]
SEM	0.03	0.03
P Value	0.01	0.04

Table 5: *Effect of squaw mint herb on malondialdehyde and microbial count of quail's meat after 3 months freezing at -20°C. SEM: Standard Error of Means; Means with letters within each column differed significantly (P < 0.05).*

The researchers reported inclusion 2% thyme in quail nutrition had not any effect on oxidation of meat [6]. But it is concluded carvacrol and phenolic compounds of herbs duo to induce antioxidant enzymes delayed the meat fat oxidation of broiler chicks at slaughter day [24]. The plant antioxidants and flavonoids remove of free radicals [23]. The potential of phenolic compounds of medicinal plants to improve the oxidative stability of meat has been proved in numerous studies [17]. The peroxidation of the lipids in the meat, causes undesirable changes in the smell and taste of the meat [18].

The researchers reported that oxidation stability of raw meat was not influenced by thyme leaves on the first day, which was similar to that of the present experiment. Also, with increasing storage time up to 7 days, the amount of oxidation of meat increased. The base of researcher's antioxidant activity was due to the presence of phenolic compounds with conjugated ring structures that prevented fat oxidation [24].

Contrary to the present experiment, Khosrawinia., *et al*. [17] stated the addition 200 to 500 mg/l of *Khuzestani satureja* essential oil contain carvacrol to drinking water of broiler chicks reduced peroxidation of breast muscle lipids after slaughter.

Some researchers investigated the effect of medicinal herbs on oxidative stability of meat at different times after slaughter. Djenane., *et al*. [10] examined the antioxidant effects of peppermint and lavender essential oil in minced beef meat during storage, decreased fat oxidation in compared to control treatment.

Peroxidation of lipids begins after slaughter and the primary defense against these oxidative degradations are antioxidants [28].

There are numerous internal and external oxidants that protect cell components against free radicals. Herbal antioxidants strengthen the body's antioxidant system and significantly prevent lipid peroxidation and membrane's vulnerability [13]. Therefore, one of the reasons for the reduction of malondialdehyde after taking squaw mint herb is due to the lower production of free radicals in the electron transfer chain, which is proportional to the increase in oxygen consumption [13,29].

It has been reported that the use of thyme in sheep diet has reduced the sensitivity of fresh meat to oxidation and improved the quality of the final product [24]. Increasing activity of liver antioxidant enzymes and decreasing the concentration of TBARS using purslane herb in mice have been reported [7].

Phenolic compounds of medicinal herbs by increasing the efficacy of body's antioxidant enzymes like glutathione reductase can be benefit for healthy [2].

The defence role of herbal medicine is attributed to induction of the antioxidant enzyme activity [15]. Also, the effects of medicinal plants on the quality and shelf life of meat in cow, goats, lambs and chickens have been investigated [9,21]. Botsuglou., *et al*. [4] reported that oregano essential oils reduced the activity of oxidation in meat and abdominal fat. Also, in the other research, oregano and thyme extract duo to presence of antioxidants of thymol and carvacrol had the greatest effect on the protection of pig fat against oxidation [3].

By increasing the levels of squaw mint herb in the quail diet, a decrease was observed in the microbial count of meat at slaughter day and different storage times. The diet containing 1.5 and 2% mint had the lowest microbial count in all storage times ($P < 0.05$).

Squaw mint essential oil has strong antimicrobial activity, which is due to the presence of Pulegone, piperton and Peritenon compounds. Also, linalool of squaw mint inhibits the growth of pathogenic microorganisms in the digestive system [20].

Medicinal plants and essential oils reduce the levels of clostridium in the ileum, cecum and colon [19]. Djenane., *et al*. [10] concluded peppermint and lavender essential oils in minced beef meat during storage in aerobic conditions at 9°C, reduced the growth of *Escherichia coli* and *Staphylococcus aureus*.

The use of squaw mint herb, especially at high levels, can improve the quality of meat during storage. Considering that increasing meat storage time increases the meat's microbial load, it can be concluded that, at different times after slaughter, squaw mint diets have better results than the control treatment.

Carvacrol and thymol compounds delayed the microbial growth of all aerobic bacteria in raw sheep meat. These degrade the outer membrane of gram negative bacteria and cause the release of lipopolysaccharides and increase the permeability of the cytoplasmic membrane. In addition to carvacrol is also able to inhibit the production of toxin by bacteria [24].

Therefore, mixture of essential oils of thyme and rosemary played a significant role as antimicrobial agents in the quality and conservation time of processed meat in the refrigerator [12].

Conclusion

It is concluded that the inclusion squaw mint herb up to 2% in Japanese quail diet decreased meat oxidant and increase the meat shelf life, also decrease microbial count of meat at different storage times in the freezer. Therefore, it is recommended that squaw mint herb to be used at 2% level as a benefit supplement in Japanese quail diets improved the meat quality.

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