Effect of dietary supplementation of Melissa officinalis and combination of Achillea millefolium and Crataegus oxyacantha on broiler growth performance, fatty acid composition and lipid oxidation of chicken meat

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Abstract

This experimental study investigated the effect of feeding of lemon balm (Melissa officinalis L.) and combination of hawthorn (Crataegus oxyacantha L.) and yarrow (Achillea millefolium L.) on growth performance of chicken meat, meat composition, fatty acid profile and oxidative stability. Ninety one-day-old commercial broiler chicks (ROSS 308) were divided into 3 groups, and fed 41 days, as follows: control (C) with standard diet without antioxidants supplementation; second group (L) with standard diet supplemented with ground lemon balm (2%) and third group (HY) with standard diet supplemented with ground hawthorn (1%) and yarrow (1%). Final body weight and total feed intake were not influenced by plant supplementation. However, feed conversion ratio was the lowest (P<0.05) at HY. Carcass yields were not affected (P>0.05) by plant supplementation, but the proportions of various carcass parts (breast and leg) were higher in L and HY groups. Feeding of plants had no effect on the chemical composition of thigh meat. However, in breast meat fed by HY diet higher content of dry matter and crude protein (P<0.05) was found. In breast (L) the proportion of monounsaturated fatty acids was increased and PUFA was decreased compared with control. Results of thiobarbituric value method showed that supplementation with lemon balm, and mainly combination of hawthorn and yarrow in the diet significantly influenced reduction of lipid oxidation processes in thigh during chilling storage (4°C, 11 days).

Introduction

The increasing pressure on the livestock industry to reduce or eliminate feed antibiotics as growth enhancers has initiated new research to find safe and efficient natural alternatives. This new generation of feed additives includes herbs and their essential oils (Brenes and Roura, 2010). Herbs used as feed additives have a significant antibacterial effect, thereby suppress pathogenic microflora in the gastrointestinal tract of animals and thus reduce mortality during the fattening period, especially in stressful situations (Schone et al., 2006). Plant additives are often applied to feedstuff as they improve the taste and smell and thus increase income and growth performance of animals (Windisch et al., 2008). Herbal additives contain substances which increase also appetite and digestion (Barreto et al., 2008). Several plants or their essential oils has been used for fattening of poultry, especially plants of the aromatic nature as clove (Barreto et al., 2008; Isabel and Santos, 2009), rosemary (Sperićová et al., 2007), cinnamon (Clifé et al., 2010), anise (Al-Kassie, 2008), but also oregano (Marcinčák et al., 2008a; Fikova et al., 2009; Symeon et al., 2010) and sage (Hernandez et al., 2004).

There has been published many studies have confirmed that the addition of plants (Guo et al., 2004; Bampidis et al., 2005; Florou-Paneri et al., 2006; Al-Kassie, 2008; Al-Beltawi Paneri et al., 2010) or their extracts (Hernandez et al., 2004, Cabuk et al., 2006; Barreto et al., 2008) in the feedstuff has a beneficial effect to improve growth parameters and feed conversion in poultry. On the other hand, there is also a sev-

eral studies in which the beneficial effect of feeding of plants and their extracts as growth promoters was not proved and experimentally supplemented chicks had lower final body weight compared with control (Lee et al., 2003; Botsoglou et al., 2004; Govaris et al., 2004; Sarica et al., 2005; Abdullah et al., 2010).

Broiler chicks have a fat content of about 5 to 7% and approximately 30% of which are saturated fatty acids (SFA) and up to 70% are unsaturated fatty acids (Pipova et al., 1995). Generally, poultry fat contains higher levels of PUFA compared to fat of other slaughter animals. This is due to the relatively high content of phospholipids in the membrane structure of the muscle cells (Bystrický and Dičáková, 1998). Just a higher degree of unsaturation of fatty acids in muscle membranes is related to increasing of their susceptibility to oxidation of meat and meat products (Marcinčák et al., 2010). PUFA are subjected to rapid oxidative changes, which impair the organoleptic characteristics and shelf life of food (Korimová et al., 2000). Lipid oxidation is one of the primary mechanisms of quality deterioration in food, especially in meat products (Gorelik et al., 2008). Therefore, there is a need to increase the antioxidant capacity of muscles, what can be achieved by feeding of antioxidant active substances. Using of natural antioxidants in poultry fattening is a simple method to achieve higher antioxidant stability, improve sensory properties and prolongate the storage of poultry meat (Marcinčák et al., 2008a). Important source of natural antioxidants is plant material; lemon balm, yarrow and hawthorn are common among herbs with high proportion of antioxidant active substances (Marcinčák et al., 2008b).

The effect of feeding of lemon balm (Melissa officinalis L.) and combination of hawthorn (Crataegus oxyacantha L.) and yarrow (Achillea millefolium L.) on growth perform-

Key words: Feed additives, Hawthorn, Lemon balm, Lipid oxidation, Yarrow.

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Materials and methods

Experimental design and birds

The study was carried out at approved menagerie of Clinic of birds, exotic and wildlife animals, University of Veterinary Medicine and Pharmacy in Košice. Ninety one-day-old unsexed hybrid broiler chicks ROSS 308 were purchased from a commercial hatchery. Chicks were randomly divided into 3 groups (n=30) in 3 replications, each containing 10 birds. Each group was kept in pen with wood shavings.

The dietary treatments consisted of the same commercial starter and finisher diets (from d 1 to 24 and from 25 to 41, respectively). The ingredients and chemical composition of the diets are shown in Table 1. Experimental group (L) was fed using commercial standard diets with addition of ground lemon balm (Agrokarpata Plavnica, Plavnica, Slovakia) in dose 20 g per 1 kg of commercial diet. Experimental group (HY) was fed using commercial standard diets with addition of ground hawthorn and yarrow (Agrokarpata Plavnica) in dose 10 g per 1 kg of commercial diet. Control group (C) was fed commercial standard diets without any supplementation.

Temperature was gradually decreased from 33°C on day 1 to 22°C on day 21 and then kept constant. The lighting regimen provided 24 h of continuous light per day. The humidity of environment was 70%. None of the birds in the treated groups died during the experiment. The experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice.

Chicks were weighed at arrival and the average weight was recorded as one-day-old weight (40±3 g). The average live body weight (BW) of broilers in each group was recorded weekly. The total feed intake (TFI) as well as the feed conversion ratio (FCR) was calculated at the end of the experiment on the day 41. The carcass yield of broilers (% of final BW) and weight of broilers before slaughtering and carcass weight without offal was recorded. Carcass yield was calculated as a proportion of carcass weight after evisceration to body weight of broilers before slaughtering. Leg and breast muscles used for determination of their proportion to total carcass weight were deboned and weighted. Proportions of leg and breast muscles were calculated as weights of individual parts to carcass weight after evisceration. To determine level of fat degradation the samples (n=30 from each group) of thigh muscles was deboned, skin removed and packed into polyethylene bags. Samples were stored 11 days in refrigerator at 4°C.

Chemical composition of meat

Dry matter was determined by oven drying at 105°C (AOAC, 1997). The crude protein content was determined using a Kjeltec Auto 1030 (Tecator Co., Höganäs, Sweden). Lipids were isolated in ground samples (thigh, breast muscles) with petroleum ether with Soxhlet apparatus and were determined gravimetrically (Folch et al., 1957).

Fatty acid analysis

Fatty acids of total lipids were analysed as their methyl esters using gas chromatography (GC-6890 N, Agilent Technologies, Santa Clara, CA, USA) with a programmed 60 m DB-23 capillary column (Agilent Technologies) according to Čertlík et al. (2006). The fatty acid methylester peaks were identified by authentic standards of C4 to C24 fatty acid methylsters mixture (Supelco, Bellefonte, PA, USA) and quantified by an internal standard of heptadecanoic acid (C17:0, Supelco).

Evaluation of thiobarbituric acid assay

To determine the lipoxidation changes of thigh meat, the method of thiobarbituric value (TBA) determination, expressing the degree of secondary damage of lipids, contingent upon the oxidation of unsaturated fatty acids, was used. Examination of samples was carried out on 1, 7, and 11 days of storage at chilling conditions (4°C). The extent of lipoxidation was evaluated as thiobarbituric acid reactive substances (TBARS) by the method of Marcinčák et al. (2004). TBARS values were measured spectrophotometrically at 532 nm (Helios γ, v. 4.6, Thermo electronic, Cambridge, UK). Results were quantified as malondialdehyde (MDA) equivalents (mg MDA·kg⁻¹ muscle).

Statistical analysis

All the data were analysed statistically using
GraphPad Prism Software, Version 4.00 (Graphpad Prism, 2003). One-way analysis of variance (ANOVA) with the post hoc Tukey’s multiple comparison test was used to evaluate statistical significance of differences among the control and experimental groups. The results are given as means, standard error of the mean (SEM) and P<0.05 was considered as statistically significant difference.

Results

Broiler growth performance

The effect of lemon balm (Melissa officinalis L.) and combination of hawthorn (Crataegus oxyacantha L.) and yarrow (Achillea millefolium L.) supplementation on body weight, total feed intake, FCR, carcass yield, breast and legs yield at 41 days of age are presented in Table 2. There was no difference between treatments for BW and TFI. However, compared with control lemon balm supplementation decreased FCR (P<0.05).

Result showed that plants supplementation had no influence on the carcass, breast and leg weight (Table 2). However, the percentage yield of breast was higher in the experimental groups compared to control group (23.1 and 23.0 as 22.2%, P<0.05). In the lemon balm group tend to be higher leg percentage yield than in other groups (28.2 vs 27.2 and 26.5%, P<0.05).

Chemical composition and fatty acid analysis of meat

Results of the chemical composition analysis of thigh and breast muscles are shown in Tables 3 and 4. Feeding of plants in experimental groups (HY, L) had no effect on the chemical composition of the thigh muscle of broiler chicks. The chemical composition of breast muscle of HY group (Table 4) showed higher dry matter and crude protein contents compared with control and lemon balm group (27.26 vs 25.98, 24.08 vs 23.03 and 22.95 respectively; P<0.05). The intramuscular fat content was higher in HY group than in other groups (23.0 vs 22.3 and 21.8%, P<0.05).

The results of fatty acids (FA) profile of breast and thigh meat fed three different diets are in Table 5. Feeding lemon balm influenced the increase in the content of PUFA in breast (P<0.05) and also thigh (P>0.05) muscle compared with control. Significantly lower content of MUFA (47.6 vs 50.28 and 49.26%; P<0.05) was reported in the breast muscle compared with other groups. The lower content of MUFA

### Table 2. Effects of supplementation of plants diet on the growth performance of broiler chickens after slaughter (at the 41st day of life).

|                     | Control | Lemon balm | Yarrow and hawthorn | SEM | P     |
|---------------------|---------|------------|----------------------|-----|-------|
| Final body weight, g| 2107    | 2063       | 2087                 | 46  | 0.431 |
| Total feed intake, g| 4122    | 4093       | 4066                 | 18.20 | 0.160 |
| Feed conversion ratio| 2.00     | 2.02       | 1.98                 | 0.004 | 0.0007 |
| Carcass yield, %    | 71.7    | 70.9       | 69.8                 | 2.10 | 0.205 |
| Legs, g             | 400     | 407        | 392                  | 13.78 | 0.441 |
| Legs, %             | 26.5    | 28.4       | 27.2                 | 0.05 | <0.0001 |
| Breasts, g          | 336     | 334        | 332                  | 12.90 | 0.139 |
| Breasts, %          | 22.2    | 23.1       | 23.0                 | 0.08 | <0.0001 |

*Means with different superscripts in the same row are significantly different (P<0.05).

### Table 3. Effects of supplementation of the diet with plants on chemical parameters of thigh.

|                     | Dry matter, % | Fat, % | Crude protein, % |
|---------------------|---------------|--------|------------------|
| Control             | 25.98         | 1.28   | 23.03            |
| Lemon balm          | 25.99         | 1.14   | 22.95            |
| Yarrow and hawthorn | 27.26         | 1.54   | 24.08            |
| SEM                 | 0.17          | 0.04   | 0.03             |
| P value             | 0.0009        | 0.074  | 0.043            |

*Means with different superscripts in the same column are significantly different (P<0.05).

### Table 4. Effects of supplementation of the diet with plants on chemical parameters of breast.

|                     | Dry matter, % | Fat, % | Crude protein, % |
|---------------------|---------------|--------|------------------|
| Control             | 21.95         | 1.37   | 21.62            |
| Lemon balm          | 21.62         | 1.28   | 21.30            |
| Yarrow and hawthorn | 21.30         | 1.28   | 21.09            |
| SEM                 | 0.56          | 0.17   | 0.03             |
| P value             | 0.0009        | 0.074  | 0.043            |

*Means with different superscripts in the same column are significantly different (P<0.05).

### Table 5. Content of selected fatty acids (g/100 g of total detected fatty acids) in breast and thigh samples.

| Fatty acid          | C    | HY  | L    | SEM  | C    | HY  | L    | SEM  |
|---------------------|------|-----|------|------|------|-----|------|------|
| C 16:0              | 21.95| 25.98| 1.37 | 21.62| 21.30| 1.28| 21.09| 1.28|
| C 16:1              | 5.98 | 5.98| 5.10 | 5.10 | 5.10 | 5.10| 5.10 | 5.10|
| C 18:0              | 5.57 | 5.57| 5.62 | 5.62 | 5.62 | 5.62| 5.62 | 5.62|
| C 18:1              | 40.25| 39.43| 37.03| 37.03| 37.03| 37.03| 37.03| 37.03|
| C 18:2              | 2.67 | 2.68| 3.15 | 3.15 | 3.15 | 3.15| 3.15 | 3.15|
| C 18:3              | 18.67| 18.67| 18.79| 18.79| 18.79| 18.79| 18.79| 18.79|
| C 18:4              | 0.20 | 0.21| 0.23 | 0.23 | 0.23 | 0.23| 0.23 | 0.23|
| C 18:5              | 1.56 | 1.59| 1.53 | 1.53 | 1.53 | 1.53| 1.53 | 1.53|
| C 18:6              | 0.08 | 0.09| 0.09 | 0.09 | 0.09 | 0.09| 0.09 | 0.09|
| C 20:1              | 0.46 | 0.43| 0.46 | 0.46 | 0.46 | 0.46| 0.46 | 0.46|
| C 20:2              | 0.22 | 0.20| 0.13 | 0.13 | 0.13 | 0.13| 0.13 | 0.13|
| C 20:3              | 0.15 | 0.13| 0.10 | 0.10 | 0.10 | 0.10| 0.10 | 0.10|
| C 20:4              | 0.40 | 0.28| 1.27 | 1.27 | 1.27 | 1.27| 1.27 | 1.27|
| C 20:5              | 0.06 | 0.05| 0.12 | 0.12 | 0.12 | 0.12| 0.12 | 0.12|
| C 22:1              | 0.03 | 0.03| 0.07 | 0.07 | 0.07 | 0.07| 0.07 | 0.07|
| C 22:2              | 0.09 | 0.06| 0.30 | 0.30 | 0.30 | 0.30| 0.30 | 0.30|
| C 22:3              | 0.06 | 0.04| 0.20 | 0.20 | 0.20 | 0.20| 0.20 | 0.20|
| C 22:4              | 0.06 | 0.05| 0.20 | 0.20 | 0.20 | 0.20| 0.20 | 0.20|
| C 22:5              | 0.06 | 0.05| 0.20 | 0.20 | 0.20 | 0.20| 0.20 | 0.20|
| SFA                 | 28.35| 28.91| 28.64| 28.64| 28.64| 28.64| 28.64| 28.64|
| MUFA                | 50.28| 49.24| 47.50| 47.50| 47.50| 47.50| 47.50| 47.50|
| PUFA                | 21.37| 21.85| 23.80| 23.80| 23.80| 23.80| 23.80| 23.80|

*Means with different superscripts in the same row are significantly different (P<0.05); C, control; HY, yarrow and hawthorn; L, lemon balm; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; content of SFA, MUFA and PUFA is calculated from all fatty acids.
in chicks’ breast muscle from group L was caused by a significant decrease in oleic acid (C18:1). In contrast, a higher content of C18:2, C20:2, C20:3, C20:4, C20:5, C22:5, C22:6 PUFA (P<0.05) have been found in breast muscle. In the thigh muscle of chicks HY significantly increased proportion of SFA and decreased PUFA proportion compared with the control (P<0.05). In comparison with control the share of stearic acid (C16:0, 23.47 (P<0.05)) increased, however the proportion of arachidonic acid (C20:4, P<0.05) decreased.

Table 6 shows results of the determination of TBA value measured in thigh muscles stored in refrigerator (4°C, 1, 7 and 11 days). On the first day of samples storage were levels of TBA products (TBARS), expressed as the amount of MDA, generally low in all groups. However, already on the first day of storage, the amount of TBARS in both experimental groups (HY, L) was lower compared to control (P<0.05). Following storage of samples, caused that TBARS values in all groups were gradually increased (P<0.05), but the amount of TBARS in the control group was significantly higher in comparison with experimental groups (P<0.05). The lowest levels of TBARS and thus the lowest fat damage throughout the storage period were recorded in samples HY.

### Discussion

#### Broiler growth performance

The effect of herbs and their essential oils on the final weight of chicks has been described by Barreto et al., 2008. However, their effect on increasing total weight of chicks is inconsistent (Marcinčák et al., 2011). In our experiment better feed conversion ratio was found in HY group and body weight and feed intake didn’t show any changes. Our results are similar to those of Brenes and Roura (2010) and Nasir and Grashorn (2010), which stated if plant extracts or essential oils were administered to poultry reduced feed intake has been recorded and no considerable changes in the gain or final body weight were found, which resulted in better feed conversion. Yarrow and Hawthorn have a number of pharmacologically active substances (phenolic compounds, flavonoids, proanthocyanidins) that are supposed to enhance feed digestion and absorption by stimulating secretion of digestive enzymes leading to better feed utilization and feed conversion ratio. A wide range of species, herbs and their extracts are known from medicine to exert beneficial actions within the digestive tract. Stimulation of digestive secretions (e.g. saliva), bile and mucus and enhanced enzyme activity are proposed to be a core mode of nutrition action (Chrabusik et al., 2005; Windisch et al., 2008). The resulting growth promoting effect of plants or plant extracts used as feed additives depends on their proper concentrations, composition of basal diet and management and husbandry conditions (Barreto et al., 2008, Nasir and Grashorn, 2010). Our study was carried out at ideal experimental conditions, which could affect the degree of growth promotion.

Results of carcass characteristics show that broilers chicks in experimental groups have lower carcass percentage as compared with control group. However, chicks in experimental groups had significantly higher breast and leg meat percentage, which indicates a probable positive effect of lemon balm and combination of yarrow and hawthorn on protein metabolism. Similarly, Nasir and Grashorn (2010) indicate that feeding *Nigella sativa* and a combination *Nigella sativa* and *Echinacea purpurea* extract did not affect carcass yield, however the yield of breast was seen in both treatments higher than control.

### Chemical composition and fatty acid analysis of meat

The feed additives have been reported to influence physical and chemical characteristics as well sensory quality of produced meat. In the present study HY diets showed significantly higher dry matter and crude protein content as compared with control. It may be due to synergistic effects of active ingredients of both plants leading to better protein metabolism. The effect of herbs on the chemical composition of meat is ambiguous. Several authors indicate not significant effect of added herbs on the composition of meat products (Gardzielewskā et al., 2003; Koreleski and Swiatkiewicz, 2007). In contrast, our results are consistent with the results Nasir and Grashorn (2010), when after feeding a combination of *Nigella sativa* and *Echinacea purpurea* extract reported a higher content of crude protein content in poultry breast muscle. The impact of administered plants into feeds stuff on fatty acid profile of breast and thigh was studied. Feeding the different herbs had different effects on the composition of fat in chicken breast and thighs muscle. After feeding of hawthorn and yarrow in the breast, but especially in thigh muscle occurred the increase in SFA content and in thigh muscle also more pronounced decrease in PUFA content. In contrast, when lemon balm was applied, a lower proportion of MUFA and higher proportion of PUFA was recorded in breast muscles compared with control and HY groups. The positive is mainly increase in the proportion of EPA and DHA, which are essential for the human organism and their lack is in the diet.

Similarly, Koreleski and Sviatkiewicz (2007) report the different effect of plant extracts feeding (sage and coneflower in the dose 560 mg) to the profile of fatty acids in breast muscle. Supplementation of the diet with sage extract increased the levels of stearic acid, arachidonic acid and n-3 PUFA and decreased the level of MUFA in comparison with control. However, the supplementation of the diet with coneflower extract increased the levels of SFA and decreased the levels of PUFA compared with control. In general, we observed that the addition of plants into feedstuff of poultry had only minimal interest in improving the profile of fatty acids in produced meat. Important role in fatty acid composition of meat had FA composition in used feeding mixtures. The proportion of health beneficial n-3 PUFA in meat, using supplementation of feedstuff, can be increased by addition of linseed (Bečková and Václavková, 2010) or fish oil (Muriel et al., 2002). However, higher PUFA content could make the meat more susceptible to oxidation and reduce the shelf life of produced meat (Karami et al., 2003).
Lipid oxidation of stored meat

The results of the present study indicate that lemon balm and combination of hawthorn and yarrow has a positive effect on the oxidative stability of thigh meat during chilling storage. Antioxidant activity of added lemon balm is underlined by the fact that in the meat is higher proportion of PUFA compared with the control. However, throughout storage period, higher oxidative stability was in meat from group L compared to control. The best oxidative stability during storage was observed in HY group, when except effective combination of herbs also the lowest amount of PUFA in meat was recorded.

The lower lipid oxidation of experimental groups could be related to the antioxidant characteristics of plants. Lemon balm, yarrow and hawthorn are rich in phenol compounds that exhibit antioxidant properties (Sokol-Letowska et al., 2006; Marcinčák et al., 2008b). Studies have shown that phenol compounds (flavonoids, proanthocyanidins) have the capacity to act powerful antioxidant activity by scavenging free radicals and terminating oxidative reactions (Sayago-Aerdi et al., 2009). Luna et al. (2010) fed thymol and carvacrol (the main phenol components of oregano) in a dose of 150 mg.kg⁻¹ for 42 days and monitored the effect on the oxidative stability of meat during storage in a refrigerator (4°C, 10 days). They noted that the addition of the extracts had no effect on the oxidative stability of breast muscle, but oxidative stability during storage of thigh muscle was significantly positively affected. Similar results were also recorded by Šperliková et al. (2007) after feeding of powdered rosemary and Govaris et al. (2004) after feeding of oregano. Florou-Paneri et al. (2006) investigated the effect of feeding oregano and oregano extract on oxidative stability of turkey meat. They also noted that the administration of 10 g.kg⁻¹ ratio of oregano or 200 mg.kg⁻¹ of oregano extract reduced fat oxidation, while at higher dosages oxidation is lower. The positive effect of reducing the oxidation of fats in poultry meat as a result of application of combination of extracts of oregano, anise and clove was found (Ertas et al., 2005).

Lipid oxidation in meat and meat products (apart from microbial spoilage) is the primary process by which quality loss occurs. Recently, scientific research has been focused on the use of antioxidant properties of natural plants and their extracts in animal nutrition due to the stabilization of fat in produced meat (Lahucky et al., 2010; Marcinčák et al., 2008a; Luna et al., 2010). Considering the character of lipid oxidation, the antioxidants effect is higher the earlier they are applied; ideally, as fats are protected immediately from the moment of receiving them, as soon as possible after slaughtering.

It is therefore necessary that the plants or their extracts, such as lemon balm, hawthorn and yarrow, be fed to the animals; present fats in meat will be protected from the beginning of oxidation process. This reduces the oxidative damage of fats in meat, extends shelf life; but not less important is meat safety.

Conclusions

Based on our results we can conclude that feeding a combination of herbs hawthorn and yarrow had an impact on improving feed conversion and increased percentage proportion of breast and leg muscle. Thigh muscle of chicks from group HY had a higher content of dry mater and protein. Feeding hawthorn and yarrow to chicks had also an effect on fatty acid composition in meat. In the thigh muscle of groups HY significantly increased proportion of SFA and decreased PUFA proportion compared to control. In contrast, feeding of lemon balm caused an increase of PUFA and decrease of MUFA in thigh, but especially in breast muscle of produced chicks compared to control.

Oxidative stability of meat during storage at refrigeration temperatures (4°C) was in both groups of chicks after feeding of herbs significantly higher in comparison with oxidative stability of meat in control. Oxidation stability is supported by components of herbs that exhibit strong antioxidant activity.

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