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# EFFECT OF OREGANO ESSENTIAL OIL ON THE PERFORMANCE AND MICROBIOLOGICAL STATUS OF SUCKLING PIGLETS

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***Abstract***

*Essential oils are gaining more and more importance in the animal nutrition, due to their antimicrobial and immunostimulant properties. This experiment aimed to assess the effect of oregano essential oil (OEO) on feed intake growth performance, health and microbiological status of suckling piglets. A total of 71 piglets, originated from 8 sows, were randomly allocated to two treatments - control (C - 35 piglets) and experimental (OEO - 36 piglets). Sows from C group received an antibiotic-free diet, and sows from the OEO group - control diet + 0.500 g/kg feed OEO. Piglets from the OEO group were treated orally by 1 cm$^3$/day by an emulsion of OEO from day 3 till day 14 of age and by 1 g/kg oregano powder added to creep feed from day 14 till day 35 of age. Application of OEO significantly increased sows' feed intake during the third and fifth weeks of lactation ($P<0.01$). In general, the use of oregano emulsion and powder had no effect on the growth performance and health status of suckling pigs. No significant difference was found in the results of bacteriological tests for the isolation of E. coli and Salmonella spp. of rectal swab samples between the OEO and C groups.*

***Key words**: ADG, E. coli, growth performance, oregano essential oil, piglets*

## INTRODUCTION

Pork production is associated with a large number of antibiotic applications, especially in the pig weaning process and immediately after. However, overuse of veterinary antimicrobials has led to the development of antibiotic resistance, a serious risk for both animals and humans. The growing concern of the public about health made it necessary the European Union to officially ban growth promoters by a directive (Council Directive 70/524/EC - ECR, 1998, entered into force after 1.01.2006). In view of the total ban on the use of antibiotics after 2018, a variety of alternatives are constantly being sought that aim to stabilize the intestinal ecosystem by encouraging the development of beneficial bacteria and reducing the number of pathogenic microflora. Over the past twenty years, a large body of research in monogastric nutrition has been conducted, involving probiotics, prebiotics, acidifiers, plant extracts, nutrients such as copper and zinc, as well as the more unconventional antimicrobial peptides, recombinant enzymes, soil materials, egg protein, rare earth elements, and others. (Thacker, 2013; Pirgozliev et al., 2019a). The success of using such means is still unproven and controversial. For this reason, establishing the concentrations and the combinations of various herbs, herbal extracts, essential oils, probiotics and other substances is the subject of a number of studies both abroad (Bravo et al., 2014; Karadas et al., 2014; Pirgozliev et al., 2015; 2019b) and in our country (Zapryanova and Ignatova, 2020; Ivanova-Peneva et al., 2010; Ivanova-Peneva, 2012; Ivanova et al., 2016; Ivanova et al., 2018; Ivanova and Nikolova, 2021; Ivanova et al., 2022). Special attention is paid to essential oils, which are capable of increasing food intake, stimulating growth and digestion. In addition, they possess antimicrobial, antioxidant and immunomodulating properties (Pirgozliev et al., 2019c; Valdivieso-Ugarte et al., 2019). The herb oregano and its varieties (*Oreganum vulgare*, *Oreganum heracleoticum*) have been used successfully as growth stimulants (Bankova et

al., 2019; Neil et al., 2004), and for the control of post-weaning diarrhea in pigs, due to the high antimicrobial activity to *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumoniae* (Stamenic et al., 2014) as well as immunostimulating effect (Camps, 2005; Walter & Bilkei, 2004). Hofmann et al. (2021) also demonstrated that oregano essential oil (OEO) altered the expression of genes related to the adaptive immune response in the small intestine of pigs. This experiment aimed to assess the effect of OEO on growth performance, health and microbiological status of suckling piglets.

## MATERIALS AND METHODS

An experiment has been performed at Experimental unit of Agricultural Institute – Shumen. A total of 71 Danube White piglets were involved in the study. The pigs originated from 8 sows, which weaned similar number of piglets. The sows were allocated to two groups following randomization - control (35 piglets) and experimental (36 piglets). The animals were randomized to number of parity, number and weight of piglets in previous farrowing, date of conception. During the pregnancy, the sows were reared in group pens and moved to the farrowing crates a week before the farrowing. The animals were fed two antibiotic-free diets following randomization. A basal diet was produced that contained 12.736 MJ Metabolizable Energy and 18.40 g CP (Table 1). The basal diet was split on two batches as one of them was fed as it is (Control - C) and the other was supplemented with 0.5 g per kilogram diet of OEO (DOSTO©Oregano, Dostofarm, Germany). It is 100% pure natural organically certified OEO of *Origanum vulgare* L., subsp. *hirtum* var. *Vulkan* (DOS 00001), at a concentration of 7.5% (Rychen et al., 2017) and is the only one officially certified by EFSA feed supplement.

All diets were fed in a mash form. Feed intake was determined on a daily basis and animals were fed depending on number of piglets in the litter. Piglets obtained creep feed from day 7 until weaning at day 35 of age. From day 3 to day 14 of age each piglet from the experimental group individually received OEO emulsion at dosage of 1 ml per day. From day 14 to day 35 of age, all piglets received the same creep feed diet supplemented by 1 g OEO per kilogram feed. During the study, the following variables were measured:

- daily feed intake of sows for the entire study period;
- daily creep feed intake of piglets;
- live weight of piglets at birth, on day 7, 14, 21, 28 and at weaning at 35 days of age;
- average daily gain, calculated on weekly basis and for the whole period of the study;
- health condition of piglets. The evaluation of health condition was made on daily basis according to methodology of Camps (2005). The state of faeces and the presence of diarrhea were evaluated from score 1 to 4, as follows: 1 - were normal dry faeces with brown colour; 2 - faeces with increased water content with yellow-brown colour; 3 - watery faeces with yellow colour, 4 - white or yellow diarrhea.

To carry out the microbiological examinations of the faeces of the suckling pigs from the

Table 1. Chemical composition of the experimental diets

| **Components** | **(%)** |
|---|---|
| Maize | - |
| Wheat | 50.00 |
| Barley | 24.17 |
| Soybean meal | 23.50 |
| Lysine, 98% | 0.08 |
| Calcium carbonate | 1.30 |
| Monocalcium phosphate | 0.30 |
| Vitamin mineral premix* | 0.25 |
| NaCl | 0.40 |
| **Total** | **100.00** |
| Metabolizable Energy, MJ | 12.736 |
| Crude Protein, g | 18.4 |
| Lysine, g | 0.97 |
| Methionine + Cystine, g | 0.65 |
| Treonine, g | 0.68 |
| Triptophan, g | 0.25 |
| Crude fat, g | 1.29 |
| Crude fiber, g | 3.94 |
| Ca, g | 0.75 |
| P, g | 0.60 |

*Content of vitamin mineral premix: Vitamin A - 4,000,000 IU/kg, Vitamin D3 - 800,000 IU/kg, Vitamin E - 50,000 mg/kg, Vitamin K3 - 2,000 mg/kg, Vitamin B1 - 1,000 mg/kg, Vitamin B2 - 2,000 mg/kg, Niacin - 9,000 mg/kg, Calcium D pantothenate - 7,000 mg/kg, Vitamin B6 - 1,500 mg/kg, Biotin - 100 mg/kg, Folic acid - 650 mg/kg, Vitamin B12 - 15 mg/kg, Iron - 70,000 mg/kg, Copper - 6,000 mg/kg, Zinc - 40,000 mg/kg, Manganese - 20,000 mg/kg, Iodine - 100 mg/kg, Selenium - 160 mg/kg, Antioxidant BHA - 60 mg/kg, Antioxidant BHT - 160 mg/kg, Preservative Lemon acid - 160 mg/kg, Preservative Phosphorus acid - 120 mg/kg

control (38 pcs.) and the experimental group (38 pcs.), on day 14 and day 28 days of age, rectal swab samples (RSS) were taken in sterile containers with 0.1% buffered peptone water and 0.85% sodium chloride. The samples (76 pcs.) were examined for the detection of pathogenic *E. coli* and *Salmonella* spp.

For the isolation of *E. coli*, the following media were used: meat peptone broth (MPB) and selective differentiation media to determine the fermentation activity of the isolates - lactose decomposition: brilliant green phenolroth agar, MacConkey agar and Lewin's, Endo's media. For biochemical identification of the isolates, we applied Hi *E. coli* test. To determine the serogroup affiliation of *E. coli*, agglutinating sera were used - O8, O9; O20, O74, O78, O138; O139, O141, O149 and O157. To detect the fimbrial factors, we used Minka agar and slide agglutination with specific sera, respectively F4, F5 and 987P. Blood agar was used to prove hemolyticity of the isolated *E. coli*. The tests for the isolation of *E. coli* were performed according to the Standard of SIV 2789-88, Farm animals, Methods for the laboratory diagnosis of colibacteriosis, Group With 79, in calves and pigs.

To isolate *Salmonella* spp. we used Buffered Peptone Water Granulated; Semisolid Rappaport Vassiliadis Medium Base Modified; Novobiocin supplement; Xylose-Lysine Deoxycholate Agar; Brilliant Green Agar Base Phosphates; Nutrient Agar; Brain Heart Infusion Broth; kit for biochemical identification HiSalmonella Identification kit -KBO11. We developed the samples according to Standard EN/ISO 6579-1:2017.

Data were statistically analyzed by one way ANOVA (Minitab 17). Statistically significant differences were determined at $P < 0.05$.

## RESULTS AND DISCUSSIONS

The results of the sow feed intake are shown in Table 2. No statistically significant differences were found in the sow feed intake for the entire lactation. However, as the lactation progressed, in the OEO treatment group in comparison to control, an increase in the feed intake was observed. In the third week, the difference between the two groups was highly significant ($P = 0.007$), as well as in the last week of lactation ($P = 0.001$). This is most likely due to the appetite stimulating properties of oregano essential oil. Our results are in agreement with those obtained by Amrik & Bilkei (2004), in whose study multiparous sows in the experimental group showed higher feed intake compared to the control group. They suggest that the particular aroma of oregano enhances feed palatability, leading to higher *ad libitum* feed intake and weight gain of piglets. Khajarern & Khajarern (2002) reported in sows, oregano essential oils not only act as alternative antibacterial stimulants, digestive aids and appetite enhancers, increasing daily feed intake ($P<0.05$), but when used as natural feed additives, also act as growth enhancers, reproductive ability and milk production.

The results of the piglets' growth performance, average piglet weight and average daily gain (ADG) are shown in Figure 1 and Table 3.

Birth weight was not statistically affected by treatment (3.88% - $P>0.05$), however, there was an increase in piglet body weight in the control group at week one of age compared to the experimental OEO group by 12.23% ($P>0.05$), resulting in statistically significant increase in ADG by 28.57% ($P = 0.020$). This result could be due to the stress of the application of oregano emulsion every day manually to every pig from the OEO group, from the third day of age. The opposite effect in piglet weight development was observed during the second week of age. Repeated measures ANOVA showed a trend for increased ADG in piglets from OEO group by 15.58% ($P = 0.071$).

It is possible that the initial stress of the application of the emulsion is overcome and the effect of the etheric oil as a growth stimulant is manifested. There was no significant effect at the end of the study at weaning of piglets and the ADG was practically equal (0.204 kg/piglet/day in the control and 0.214 kg/piglet/day in the OEO treatment group). It is necessary to notice that the creep feed intake by piglets was very small and it was very difficult to be measured as a big part of it was used as bedding into the piglet compartment.

Table 2. Sow feed intake during lactation per week

| Items | Control | | Treatment (OEO) | | *P*-value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Sow feed intake week 1 (kg/day) | 1.786 | 0.568 | 1.929 | 0.690 | 0.401 |
| Sow feed intake week 2 (kg/day) | 3.250 | 1.590 | 3.732 | 1.450 | 0.241 |
| Sow feed intake week 3 (kg/day) | $5.196^{A}$ | 1.442 | $5.964^{B}$ | 0.131 | 0.007 |
| Sow feed intake week 4 (kg/day) | 5.625 | 1.267 | 6.018 | 0.095 | 0.123 |
| Sow feed intake week 5 (kg/day) | $6.304^{A}$ | 0.254 | $6.482^{B}$ | 0.094 | 0.001 |
| Sow feed average overall (kg/day) | 4.468 | 2.053 | 4.786 | 1.849 | 0.174 |

Legend: OEO Oregano Essential Oil; SD - Standard Deviation; Superscript letters A-B represent statistical significance at $P < 0.05$

Table 3. Treatment effect on piglet growth performance

| Items | Control | | Treatment (OEO) | | *P*-value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Average birth live weight (kg) | 1.777 | 0.510 | 1.708 | 0.276 | 0.480 |
| Average live weight 1-7 days (kg) | $2.706^{A}$ | 0.849 | $2.375^{B}$ | 0.430 | 0.041 |
| Average live weight 7-14 days (kg) | 4.074 | 1.319 | 3.992 | 0.830 | 0.752 |
| Average live weight 14-21 days (kg) | 5.477 | 1.538 | 5.542 | 1.066 | 0.837 |
| Average live weight 21-28 days (kg) | 7.200 | 1.793 | 7.333 | 1.599 | 0.742 |
| Average live weight 28-35 days (kg) | 8.931 | 1.763 | 9.186 | 1.570 | 0.522 |

Legend: OEO Oregano Essential Oil; SD - Standard Deviation; Superscript letters A-B represent statistical significance at $P < 0.05$

A part of it was also mixed with urine and feces and probably it was not possible to consume the oregano powder added to the creep feed. On the other hand, the intensive smell, due to the high level of essential oil, could presumably reduce the palatability of the diet, leading to poorer intake and growth.

These results are similar to those from a previous study with the addition of oregano to the feed of sows and suckling piglets as a ground oregano herb from the whole plant (Ivanova-Peneva & Kanev, 2014). The results from these two experiments with suckling piglets are different from the results from our earlier study testing the effect of ground dry oregano herb (Ivanova-Peneva et al., 2010). Statistically significant difference in ADG from day 1 to day 40 of age of the piglets in one of the experimental groups was established in comparison to the control ($P$<0.05) and in comparison, to the water solution of oregano herb treatment group ($P$<0.01). Previous studies also did not show consistent results, probably due to the application of a mixture of five herbs including oregano as well (Ivanova-Peneva et al., 2006).

Results from the study made in Wageningen University of testing the effect of DOSTO©Oregano powder on sow and piglet performance added to the sow's feed in the quantity of 750 mg/kg feed reveal the functional mechanism of the essential oil (Auge, 2013). Its basic component carvacrol was found in milk, urine and feces of piglets (58% of dietary intake). The discovery of carvacrol in milk probably shows that the piglets could receive the essential oil directly from the mother's milk. This can improve the health of their gastrointestinal tract due to the antimicrobial effect of oregano essential oil, as well as to a better condition of the sows' udder, expressed in a lower somatic cell counts in milk. In this study, there was an effect on the birth weight of the piglets in the OEO sow group and this effect could be due to a stimulation of the production of more endogenous enzymes and better digestion of the feed. The total weaning weight of the piglets also showed a trend to increase,

although an effect on the weight growth performance, expressed as ADG and average weight at weaning was not established, similarly to the results from this study and from other studies with suckling and weaning pigs (Neill et al., 2006; Ariza-Nietto et al., 2011; Hall et al., 2021). Hall et al. (2021) found that the supplement (1% essential oil of *Origanum*

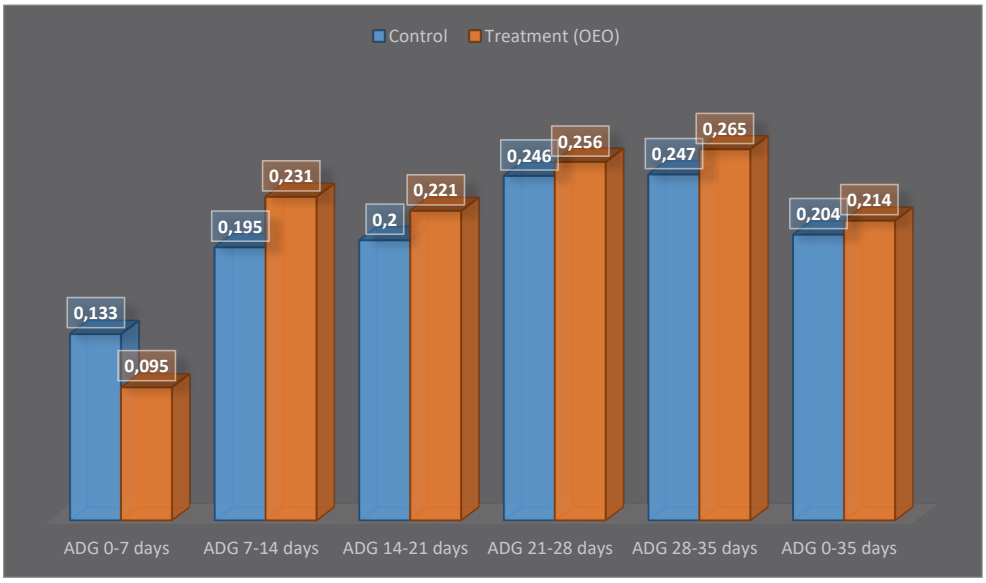


Figure 1. Average daily gain (ADG) of control and treatment (oregano essential oil - OEO) groups, kg/pig/day

*vulgare* ssp. *hirtum*) had a positive impact on the lifetime performance of pigs from birth to slaughter, with reduced medication use recorded, which may be due to changes in the microbiome early in life through maternal transfer. However, no increased intake of the creep feed was observed and and accordingly no better ADG was established in OEO treated piglets.

The most common reason of diarrhea in suckling, weaning and growing pigs is enterotoxigenic *Escherichia coli* (ETEC). ETEC are characterized by the ability to produce two types of virulence factors: adhesins that facilitate binding to specific enterocyte receptors for intestinal colonization and enterotoxins responsible for secretion of fluid (Dubreil et al., 2016). The best characterized adhesins are expressed in the context of fimbriae, such as F4 (also designated K88), F5 (K99), F6 (987P), F17 and F18 fimbriae. Once established in the small intestine of animals, ETEC produce enterotoxins that cause diarrhea. Table 4 shows the *E. coli* RSS positive samples from the control and experimental (OEO-treated) groups. In the researches of RSS from the piglets of the control group (n = 19), taken on day 14, are isolated 5 samples positive for *E. coli*: 2 samples O8:F5+:Hem-, 2 samples O139:F4+:Hem+ and one with *E. coli* O non-typifying (ONT):F-:Hem+. In the tests of RSS (n=19) from the piglets at day 14 day of age from the experimental group, 4 positive samples were detected, 2 samples RSS with *E. coli* O8:F5+:Hem- and 2 samples RSS with *E. coli* O139:F4+:Hem+. In researches of RSS from piglets at day 28 of age, equivalent results were observed in both groups. In both groups 5 samples positive for *E. coli* were isolated: 2 *E. coli* O8:F5+:Hem-, 2 *E. coli* NT:F-: Hem+, and one *E. coli* O139:F4+:Hem+. Investigating the prevalence of enteritis in pigs in 8 pig farms, Petkova (2017) found that ETES that produce fimbriae (O139:F5) can cause enteritis in both suckling and weaned pigs, which is according to the evidence in this research of hemolytic *E. coli* O139:F4+ from RSS of suckling pigs.

Table 4. Results from investigation of rectal swab samples (RSS) from suckling piglets, raised with and without oregano essential oil (OEO)

| Piglets' age | Control group (n = 38) | | | | OEO group (n = 38) | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* | O-sero-group | Fimbrial factor | Hemo-lysis | *E. coli* | O-sero group | Fimbrial factor | Hemo-lysis |
| 14 days | Pos. | ONT | - | + | Pos. | O8 | F5 | - |
| 14 days | Pos. | O8 | F5 | - | Pos. | O8 | F5 | - |
| 14 days | Pos. | O8 | F5 | - | Pos. | O139 | F4 | + |
| 14 days | Pos. | O139 | F4 | + | Pos. | O139 | F4 | + |
| 14 days | Pos. | O139 | F4 | + | - | - | - | - |
| Total | 5 | 5 | 4 | 3 | 4 | 4 | 4 | 2 |
| 14 days | Pos. | ONT | - | + | Pos. | ONT | - | + |
| 28 days | Pos. | ONT | - | + | Pos. | ONT | - | + |
| 28 days | Pos. | O8 | F5 | - | Pos. | O8 | F5 | - |
| 28 days | Pos. | O8 | F5 | - | Pos. | O8 | F5 | - |
| 28 days | Pos. | O139 | F4 | + | Pos. | O139 | F4 | + |
| Total | 5 | 5 | 3 | 3 | 5 | 5 | 3 | 3 |

No significant differences between the results in the control and experimental groups can be seen (Table 4). Out of 19 examined RSS from pigs at day 14 of age from the control group, 26.3% were positive for *E. coli*, similar to the OEO group, in which 21.05% were positive. *E. coli* of serogroup O8, O139 and O non-typing were isolated from 4 RSS in the experimental group and from 5 RSS from the control group in the first examination. Equal numbers positive samples (5 RSS) in both groups were found in the second examination at day 28 of age. *Salmonella* spp. was not isolated from neither of the 76 examined RSS taken on day 14 and day 28. During the clinical observation of pigs from both groups, diarrhea was recorded for one day in one piglet from the control group, from which *E. coli* O139:F4:Hem+ was isolated on the day 14. The lack of obvious clinical signs in piglets from which pathogenic *E. coli* was isolated could be explained by the fact that both sows and suckling pigs were treated with oregano in a different form. However, we need more trials with larger number of piglets to be able to prove such a causal relationship. For the entire study period, the condition of the piglets was rated as 1 - normal, well-formed, firm brown stools, in contrast to the previous trials conducted with suckling pigs (Ivanova et al., 2010; Ivanova & Kanev, 2014; Ivanova et al., 2016). This could be due to the well-cleaned and disinfected farrowing room of the sows and the arrival of the piglets in a stable microbiological environment. Information on the growing conditions should be taken into account for a more complete interpretation of the experimental data. Simitzis et al. (2010) pointed out that well-nourished and healthy animals do not respond to growth-promoting additives, for example OEO. In the same context, Gāliņa & Valdovska (2017) concluded that oregano essential oil exerts its effects only during pathological conditions. Pirgozliev et al. (2014) also found that the beneficial effects of essential oils were more pronounced in less hygienic housing conditions. In contrast to Hall et al. (2021), who demonstrated a relative reduction of *Enterobacteriaceae* in feces from pigs at day 14 and 28 of age originating from sows receiving OEO, in the present experiment we found no significant differences in the carriage of pathogenic *E. coli* in the control and experimental piglets groups. In a study of the effect of combining benzoic acid with an essential oil, Rodrigues et al. (2020) found no difference in indicators of diarrhea and microbial population, in the experimental and control groups of piglets over 21 days of age, but reported an improvement in the growth indicators of the piglets. We share the opinion of Canibe et al. (2022) that essential oils show potential to reduce the development of enteritis in piglets and there seems to be no doubt that some of them have essential benefits, especially their specific antimicrobial effect against ETEC. However, according to them, a major challenge

is the mixing of essential oils with organic acids, particularly benzoic acid, which complicates the assessment of the effects of essential oils on their own. Another major challenge in general evaluation of the results and providing recommendations is that none of the experiments are directly comparable because they vary widely in the type, amount, purity, and combinations of essential oils involved. The results of the studies depend on factors related to age, health and immune status of the pigs used in the experiment, the feed fed and the farming technology, the dose and concentration of the added product, the duration of the treatment and the level of stress and hygiene, as well as research design.

## CONCLUSIONS

The application of OEO emulsion of 1 ml per piglet per day orally and OEO powder of 1 g per kg of feed in suckling piglets paralleled with the application of OEO powder of 0.5 g per kg of feed in sows, showed a trend for an increased suckling piglet growth performance from day 7 to day 14. Application of OEO significantly increased sows' feed intake during the third and fifth weeks of lactation ($P<0.01$). In general, the use of oregano emulsion and powder had no effect on the growth performance and health status of suckling pigs. No significant difference was found in the results of bacteriological tests for the isolation of *E. coli* and *Salmonella* spp. of rectal swab samples between the experimental and control groups.

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