

## THE EFFECT OF INCLUDING ADDITIVES IN FEEDS FOR PIGS ON THE APPEARANCE OF RESISTANCE IN *E. COLI*

VESELINA PUPAVAC,\* VERICA JURIĆ\*, M. RISTIĆ\*\* and S. FILIPOVIĆ\*\*

\*Faculty of Agriculture, Livestock Research Institute, Trg Dositeja Obradovića Novi Sad Yugoslavia

\*\*Faculty of Technology, Bul. Kralja Petra 4, Novi Sad.

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*The intestines of slaughter pigs which had been fed with diets containing additional copper flavomycin and salocin as growth stimulants were examined for the presence of E. coli. The test was done on six groups of pigs (I-control, II-200 ppm of copper, III 20 ppm of flavomycin, IV 200 ppm of copper and 60 ppm of salocin). Isolated strain of E. coli were tested for their susceptibility to some antibiotics and sulfapreparations.*

*The additives were found to affect the number of E. coli in the intestines of pigs. Thus E. coli from pigs in groups II and IV had less expressed hemolysis. Isolated strains of E. coli from all the experimental groups were penicillin, amoxycillin and lincomycin resistant. E. coli obtained from the groups given growth stimulants did not show changes in susceptibility to tetracycline.*

*Key words: Feeds, additives, resistance, E. coli*

### INTRODUCTION

The use of antibiotics and other stimulants in animal nutrition, has simultaneously created a real revolution in domestic animal breeding and two problems in health protection namely, resistance and residues. (Vissek, 1978; Brahma et al. 1982; Hedges and Linton 1988; Kaukas et al., 1988.). The use of biostimulators as ingredients in pig feed is based on the aspiration of science and practice to achieve better pig meat production. Some stimulants have bacteriostatic and bacteriocidal characteristics, which should be known because of their influence on bacteria in intestines and food. The effects of a number of combinations of antibiotics with other nutrient or growth promoters on animal performance have been investigated (Moser et al., 1988, Boucque et al. 1988.). When considering the protective and stimulative effects of additives in pig nutrition through a longer period or permanently, we must not forget the possibility of bacteria becoming resistant (Brahma et al., 1982; Hedges and Linton 1988; Semjen et al. 1991.)

In the literature we meet various opinions about the use of antibiotics and heavy metals as stimulators. The discovery of some scientists about resistance in "in vitro" conditions has interested researchers throughout the world (Popović et al. 1969; Mago et al. 1982; Brahma et al. 1982; Singh et al. 1985). A number of authors has concluded that stimulators can be used if they do not influence the activity of gram negative bacteria.

#### MATERIALS AND METHODS

Considering the stimulative effect of additives in pig nutrition, we have examined the presence of *E. coli* in intestines of slaughtered pigs, which had been fed diets enriched with copper and antibiotics as stimulators.

##### *Experiment*

The experiment was set up with six groups of 60 pigs, 10 pigs in each group (I control, II with the addition of 200 ppm of copper in feed, III with 20 ppm of flavomycin, IV with 200 ppm of copper and 20 ppm of flavomycin, V with 60 ppm of salocin and group VI with 200 ppm of copper and 60 ppm of salocin). The susceptibility of isolated *E. coli* strains to some antibiotics and sulfapreparations was tested.

##### *Isolation of bacteria*

For isolation and identification of intestinal microflora, 60 samples of intestine contents were sown up to ten hours after the pigs have been slaughtered, on appropriate nutritive selective and differential media. The total *E. coli* counts in intestine contents were determined in decimal dilutions of contents in sterile 1% pepton water, on McConey and Endo agar plates, which were incubated at 37 °C for 48 h. We made the identification of *E. coli* on the basis of their cultural and biochemical characteristics.

##### *Test of susceptibility*

We chose representative strains of *E. coli* for susceptibility tests, using the disk diffusion method. The susceptibility to penicillin, streptomycin, amoxycillin, gentamycin, lincomycin, trimethosul, cefalosporin and trofurantoin was examined.

#### RESULTS AND DISCUSSION

In the groups of pigs fed with the diets containing non-absorbable antibiotic, the number of *E. coli* was the  $1 \times 10^3/\text{g}$  to  $63 \times 10^3/\text{g}$ . Thus additives influenced the number of *E. coli* in the large intestines of the pigs in our test (table 1). The total *E. coli* counts in control group of pigs was higher than in the experimental groups.

Fagerberg et al. (1978), Jukes (1990) and Shurson et al. (1990) found changes of intestinal microflora after the addition of antibiotics to the feed. According to Semjen et al. (1991) enrofloxacin at a dose of 75 mg/kg feed reduced



Lactobacillus and Enterococcus, but *E. coli* showed an increase in the small intestine.

Table 1. Numbers of *E. coli* per gram of intestinal contents from slaughtered pigs

Group	I	II	III	IV	V	VI
Copper ppm	—	200	—	200	—	200
Flavomycin ppm	—	—	20	20	—	—
Salocin	—	—	—	—	60	60
<i>E. coli</i>	$240 \times 10^5$	$3 \times 10^5$	$8 \times 10^3$	$1 \times 10^3$	$4 \times 10^3$	$63 \times 10^3$

Table 2. Susceptibility of *E. coli* to therapeutic agents

Group	Penicillin	Streptomycin	Amoxycillin	Gentamycin	Trofuran-tain	Lincomycin	Cefalosporin	Trimethosul
I	0	+	0	+	+	0	+	+
II	0	+	0	+	+	0	+	0
III	0	+	0	+	+	0	+	+
IV	0	+	0	+	+	0	+	+
V	0	0	0	0	+	0	+	+
VI	0	0	0	0	+	0	0	0

0 = resistant

+ = susceptible

++ = less susceptible

We observed that *E. coli* strains isolated from various groups of pigs had some different characteristics. Thus, the strains which originated from groups I and V fermented glucose whereas the strains from groups I, II, III and V fermented sucrose. All the isolated strains were lactose positive. Strains from groups II and IV group had less expressed hemolysis.

Isolated strains of *E. coli* from all the experimental pig groups were penicillin, amoxicillin and lincomycin resistant (table 2.). Adding copper and salocin to the feed, influenced the appearance of resistance in the examined bacteria. In the group of pigs which were given only salocin bacteria also became streptomycin and gentamycin resistant. Susceptibility to trofurantoin was reduced only in group VI. Similar results were obtained by Limbuška (1990). She found that 200 ppm of added copper was favourable for the selection of *E. coli* resistant to antibiotics (tetracyclin, ampicillin, kanamycin). In our tests *E. coli* did not show susceptibility changes with this preparation. Trimethosul resistance was noticed in groups II and VI.

#### CONCLUSION

The results obviously show that the doses of antibiotics and copper influenced on the appearance and degree of resistance of *E. coli* to antibiotics (when permanently added to the feed).

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EFEKAT DODAVANJA ADITIVA OBROCI ZA ISHRANU SVINJA NA POJAVU REZISTENCIJE *E. COLI*

VESELINA PUPAVAC, VERICA JURIĆ, M. RISTIĆ I S. FILIPOVIĆ

## SADRŽAJ

U našim istraživanjima ispitali smo prisustvo *E. coli* u crevima zaklane prasadi koja je uzimala hranu sa dodatkom bakra, flavomycina i salocina kao stimulatora rasta. Ogled je bio izveden na šest grupa prasadi (I-kontrolna, II-sa 200 ppm bakra, III sa 20 ppm flavomycina, IV sa 200 ppm bakra i sa 20 ppm flavomycina, V sa 60 ppm salocina i VI 200 ppm bakra i 60 ppm salocina). Izolovane sojeve *E. coli* ispitali smo na osetljivost prema nekim antibioticima i sulfopreparatima.

U odnosu na kontrolnu grupu prasadi u našem ogledu aditivi su uticali na broj *E. coli* u crevima. *E. coli* iz II i IV grupe imali su slabije izraženu hemolizu. Izolovani sojevi od prasadi iz svih oglednih grupa bili su rezistentni na penicilin, amoksisicilin i linkomicin. *E. coli* iz svih grupa sa stimulatorom nije pokazala promene osetljivosti na trofurantoin.

