LAYING HEN FECES FOR RUMINANT FEEDS
MICROBIOLOGICAL ANALYSES[1]

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ABSTRACT - The objectives of the present study were: to evaluate the variation in total number of microorganisms in laying hen fecal samples and to identify the pathogenic microorganisms possibly present such as *Staphylococcus aureus, Salmonella* sp., *Clostridium botulinum, C. perfringens, C. chauvoei, Campylobacter* sp., *Escherichia coli,* and *Corynebacterium* sp. The experiment was conducted at the School of Agrarian and Veterinarian Sciences of Jaboticabal, - UNESP. Fecal samples were collected from the aviary. A fully randomized experimental design was used with 7 treatments and 4 replications, for a total of 28 samples. A decrease in total number of microorganisms was detected when treatment T_0 (zero days of storage) was compared with treatment T_6 (42 days of storage), with oscillations in numbers being observed during this period of time. Pathogenic bacteria such as *Clostridium noyvi, C. perfringens, C. sordelli, C. septicum* and *C. noyvi* type B were detected, as well as non-pathogenic bacteria such as *Bacillus* sp. and *Staphylococcus epidermidis.*

Index terms: microorganisms, storage.

FEZES DE GALINHAS POEDEIRAS NA ALIMENTAÇÃO DE RUMINANTES
ANÁLISES MICROBIOLÓGICAS

RESUMO - Os objetivos deste trabalho foram: avaliar a variação do número total de microrganismos em amostras de fezes de galinhas poedeiras e identificar os microrganismos patogênicos possivelmente presentes, tais como: *Staphylococcus aureus, Salmonella* sp., *Clostridium botulinum, C. perfringens, C. chauvoei, Campylobacter* sp., *Escherichia coli* e *Corynebacterium* sp. O experimento foi conduzido na Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, UNESP, onde foram coletadas amostras de fezes procedentes do aviário. O delineamento experimental foi inteiramente casualizado, com 7 tratamentos e 4 repetições, totalizando 28 amostras. A avaliação demonstrou que houve decréscimo no número total de microrganismos quando se comparou T_0 (zero dias de estocagem) com o tratamento T_6 (42 dias), havendo, no intervalo, oscilações neste número. Constatou-se a presença de bactérias patogênicas nas amostras de fezes, tais como: *Clostridium noyvi, C. perfringens, C. sordelli, C. septicum, C. noyvi* tipo B, havendo a presença de outras bactérias não patogênicas como *Bacillus* sp., *Staphylococcus epidermidis.*

Termos para indexação: microrganismos, estocagem.

INTRODUCTION

The use of avian dejecta as a supplementary source of nitrogen in ruminant feeds is being extensively studied. Blair (1974) emphasized the importance of subproducts for cattle feeding, and especially the use of poultry feces in rations as an effective process of nutrient recycling.

However, the use of untreated avian dejecta in cattle feeds is a source of concern due to the

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possible dissemination of *Salmonella* (Boots et al. 1952, Tucker 1967, Fanelli et al. 1970, Smyser et al. 1970.

Alexander et al. (1968) isolated the following bacteria from samples of poultry litter: *Salmonella, Mycobacterium, Clostridium, Corynebacterium, Actinobacillus, Staphylococcus, Streptococcus,* as well as *Escherichia coli* which normally causes diarrhea in calves. Smibert (1978) detected the presence of *Campylobacter jejuni* in sheep and cattle feces, and Park et al. (1981) detected the same species in chicken and turkey feces.

Shane & Montrose (1985) demonstrated the importance of the colonization of chicken intestine by *Campylobacter jejuni* in terms of contamination of both humans and animals.

Schocken-Iturrino (1986) demonstrated experimentally that avian residues such as chicken litter may carry *Clostridium botulinum* spores as well as botulin toxin. *Clostridium perfringens* and *C. chauvoei* are normally transmitted through the digestive tract, with the consequent possibility of infecting animals fed ration containing chicken feces or chicken litter highly contaminated with these microorganisms. The results reported by Alexander et al. (1968) have demonstrated that poultry litter feed is a significant vehicle for these bacteria.

In view of the importance of this material as a complement of animal feeds, and ruminant feeds in particular, because of its good nutritional value, a microbiological study of the feces of laying hens was undertaken in order to determine the total number of microorganisms existing in this material and to identify possible pathogens.

MATERIALS AND METHODS

A fully randomized design with 7 treatments and 4 replications was used for data analysis, for a total of 28 samples. Fecal samples were collected and stored in a place protected from rain and sunlight. Samples were collected on a weekly basis and the following treatments were established: T_0, T_1, T_2, T_3, T_4, T_5, and T_6, corresponding to 0, 7, 14, 21, 28, 35 and 42 days of feces storage, respectively.

An experimental mound was built with feces from three poultry rearing units in which the hens were kept in hanging cages. The feces were deposited on a cement floor.

The microbiological analyses were carried out starting from dilutions in which 50 g of the samples were mixed with 450 ml of sterile peptone water. The mixture was placed in an Erlenmeyer flask and shaken; a 1-ml aliquot was then used for serial dilution up to 10^{-6} in peptone water with 3 replications per dilution. The dilutions were inoculated in agar for total counts and Saboraud dextrose agar for fungal growth and incubated at 37 and 25°C for 24, 48 and 96 hours, respectively. The initial mixture was used to inoculate blood-agar, EMB agar, MacConkey agar, clostrisel agar, *Staphylococcus* 110 agar, Skirrow agar, Tarozzi broth, and brain-heart infusion broth tubes. Plates were incubated at 37°C for 24 and 48 hours under conditions of aerobiosis and anaerobiosis in jars with the GAS-PAK system. Smears were obtained from typical colonies, stained with the Gram method and replated onto nutrient agar for later identification by a biochemical series as recommended by the Food and Drug Administration (1984) and American Public Health Association (1976).

RESULTS AND DISCUSSION

The mean microbiological composition (number of microorganisms per gram analyzed material) of laying hen feces is presented in Table 1.

Variation between treatments was observed and attributed to the unfavorable conditions for the microorganisms, since a decrease in humidity, greater release of ammonia and elevation in temperature occurred within the mound with storage time.

A larger number of microorganisms naturally inhabiting the gastrointestinal tract of hens was isolated at the beginning of storage. With increasing storage time and probably because of the changes in pH caused by the release of ammonia and of the high temperatures brought about by the decomposition of the piled material, followed by later fecal dehydration, the number of bacteria decreased and some species died and were replaced by others commonly found in soil and in the environ-

TABLE 1. Mean values of the number of microorganisms per gram of laying hen feces with storage time.

Treatments (Storage time)	Replications				Mean
	1	2	3	4	
T_0 - 0 days	125.900	150.730	136.500	163.600	144.180a*
T_1 - 7 days	3.720	4.330	4.300	3.180	4.040b
T_2 - 14 days	8.560	5.460	6.540	7.930	7.120b
T_3 - 21 days	7.990	6.970	5.200	2.300	5.620b
T_4 - 28 days	14.240	3.640	5.140	2.110	6.280b
T_5 - 35 days	7.570	5.180	13.130	8.030	8.480b
T_6 - 42 days	3.120	4.270	5.820	11.390	6.170b

* Differents letters on the column differ significatively ($P < 0.01$).

ment. This fact was also pointed out by Vilela Junior & Baliana (1982) who considered fecal drying and temperature to be the main causes of the drop in number of microorganisms initially present.

When the data were analyzed by the Tukey test, a statistically significant difference ($P < 0.01$) was detected between treatment T_0 and the remaining treatments, probably due to the causes listed above (Table 1).

Table 2 lists the microorganisms isolated from the fecal samples. It is interesting to point out the importance of some of the highly pathogenic genera and species isolated such as *Clostridium sordelli, C. chauvoei, C. septicum, C. noyvi* and *C. perfringens,* which may cause enterotoxemia in cattle and sheep (Beer 1981), blackleg in cattle (Guerreiro et al. 1984) and gas gangrene in both species (Sterne & Batty 1978). These microorganisms were found indistinctly in all treatments because of their characteristics of being sporulated and of resisting dehydration.

Campylobacter jejuni was isolated only from samples of the first treatment possibly because of its limited resistance and inability to survive outside the host for prolonged periods of time without protection against desiccation and sunlight (Carter 1988).

As to *Corynebacterium pyogenes,* little is known about the mechanism by which it induces diseases. Its toxin is relatively weak but frequently causes abscesses, suppurative pneumonia, arthritis, and infection of wounds and surgical incisions in cattle, sheep and swine (Carter 1988).

TABLE 2. Microorganisms isolated from the fecal samples during storage period.

Microorganisms isolated	Storage time (days)						
	0	7	14	21	28	35	42
Bacillus sp.	X	X	X	X	X	X	X
Campylobacter jejuni	X						
Clostridium chauvoei						X	
Clostridium noyvi tipo B	X	X			X		
Clostridium perfringens	X						
Clostridium saprofiticum					X		
Clostridium septicum		X				X	X
Clostridii sordelii	X						
Clostridium sp.	X	X	X	X	X	X	X
Corynebacterium pyogenes			X		X		
Enterobacter sp.				X	X	X	
Escherichia coli	X	X		X		X	
Levedura	X	X	X	X	X		X
Proteus mirabilis		X			X		
Proteus vulgaris		X					
Staphylococcus epidermidis		X	X	X	X	X	X

X - Presença

There are pathogenic serotypes of *Escherichia coli* associated with enteritis and diarrhea which are pathogenic only to a particular animal species and not to others. The *E. coli* strains isolated in the present study were not pathogenic to cattle.

One of the *Proteus* strains isolated was *mirabilis.* This bacterium has been reported to be responsible for a variety of sporadic infections in cattle, and urinary infections in particular (Guerreiro et al. 1984).

CONCLUSIONS

1. Considering the microbiological aspects, the use of laying hen feces for ruminant nutrition is recommended after the first month of storage since the number of microorganisms decreases during this period.

2. The origin of the material to be purchased (poultry feces and/or litter) should also be carefully checked since the history of these poultry farms is a very important factor in the partial determination of feed quality.

3. A wide variety of sporulated bacteria continued to be present in the material even after storage.

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