

# Microbial load on cowpea (*vigna unguiculata*) husks stored under different conditions for use as supplementary feed for small ruminants.

ABBEY, Charlotte and NAANDAM, Jakper.

University for Development Studies, Faculty of Agriculture, Animal Science Department, Box TL 1882, Tamale, Ghana.

Corresponding author email: jaknaan@yahoo.com

#### Abstract

A ninety- two (92) day experiment was carried out in Nyankpala to determine the microbial load on cowpea husks when stored in an enclosed room (T1) and in an airy or open shed (T2). There were 3 replicates for swabs taken on both treatments at three (3) different stages; i). at harvest time in the field, ii). during storage after transporting husks home and iii). at feeding time). The swabs were taken to the laboratory and serial dilution, inoculation of diluents onto a sterile blood agar for incubation and catalase test and gram staining were conducted. The results indicated that there was heavy, very little and no fungal growth in both treatments at the three stages of swab preparations respectively. Bacterial counts (log/cfu) at the field and during storage were slightly higher in T2 than in T1 (4.08 as against 3.76 and 5.07 as against 4.65 respectively) but there was no significant difference (P>0.01). At feeding however, bacterial counts in T1 was higher and significantly different (P<0.01) from that of T2 (6.23 as against 5.23). There was no fungal growth on the husks after storage on both treatments. When the cowpea husks were ready to be used, bacterial counts on the husks stored in the room were higher than that of the open shed.

Keywords: Bacterial count, cowpea husk, fungal growth, microbial load, Open shed storage

#### **INTRODUCTION**

The colonisation of plants by microorganisms starts almost as soon as leaves are exposed to the air. Bacteria usually colonise first, rapidly followed by yeasts, and then by pathogenic and saprophytic fungi. Filamentous fungi usually continue to develop at all stages of plant growth, including seed ripening (Flannigan, 1987; Lacey & Magan, 1991; Magan *et al.*, 2003). Fungi present on plants before harvest are traditionally termed 'field fungi'. Typically, these include species of *Cladosporium, Alternaria, Epicoccum* and *Fusarium* (Flannigan, 1987; Lacey & Magan, 1991; Magan & Lacey, 1984). Some severe toxicity from the consumption of mouldy hay sometimes occurs, more commonly in monogastric animals such as horses as a result of the presence of thermophilic fungi (Lacey, 1991). Most well authenticated toxicoses are attributable to *Aspergillus*  species especially *Aspergillus fumigates* (Yamazaki *et al.*, 1971; Cole *et al.*, 1977 and Lacey, 1991).

Ruminants eating Penicillium roqueforti infested feed displayed symptoms such as lack of appetite, ketosis, paralysis and spontaneous abortions (Häggblom, 1990). Aspergillus spp. are characteristic colonisers of stored products, different species vary considerably in their growth requirements; thus, the dominance of certain species may be indicative of previous storage conditions (Lacey, 1989). The dominant bacterial flora on plant surfaces are Gram-negatives -Erwinia, Pseudomonas and Xanthomonas (Flannigan, 1987) – with a smaller number of Gram-positive bacteria, such as Lactobacillus and Leuconostoc (Adams & Moss, 2000; Kaspersson et al., 1988). Presence of Enterobacteriaceae is indicative of the general hygiene status of a feed. High cfu values imply that further studies of potentially harmful organisms are required.

The population of microorganisms found in the rumen is diet specific and takes days to build up to cope with a new diet (MacMillan, 1996). Cowpea (*Vigna unguiculata*) husks are one of the cheapest and easiest materials to acquire and use as feed supplement. Generally all the plant parts of cowpea used for food are nutritious (Quin, 1997). Some farmers store the hull in enclosed places whiles others store them in open sheds. Information on changes in the population of microbes as a result of location or manner of storage is lacking. This study therefore sought to determine any changes in the type and population of microbes present on the cowpea husks from time of harvest to feeding time depending on the mode of storage.

### **MATERIALS AND METHODS**

A total of eighteen swabs were taken; six each time (i.e. 2 swabs on each of 3 purposively sampled farmers who store and use cowpea as a supplementary feed) for three (3) consecutive times. The 2 swabs from each farmer, one for treatment 1(T1)and the other for treatment 2 (T2). All T1s were room stored while T2s were shed stored. Swabs were taken i). at harvest time in the field, ii). during storage after transporting husks home and iii). at feeding time). Laboratory procedure involved serial dilution, inoculation on a growth media and culturing in an incubator at a temperature of 37<sup>°</sup>C. Microbial growth in colonies was then identified by type and counts. Bacterial counts were subjected to ANOVA in Genstat version 3

#### **RESULTS AND DISCUSSION**

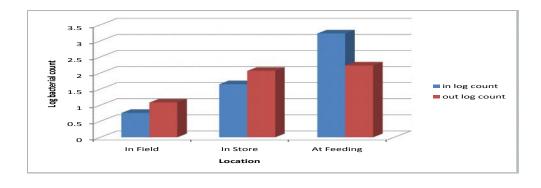
#### Presence of microbes

Microbes were present on both treatments (Room storage and Shed storage) which corroborates observations by Cameron and Collymore (1979) who reported that microorganisms are present in water, dust, soil, sewage, and even on the hands of man and as a result, their presence on feed supplements should be inevitable.

#### **Microbes identified**

The microbes identified on the cowpea husks for field and during storage samples for both treatments were bacterial and fungi. However, at the time of feeding i. e. after 48 hours of culturing the ready to feed material, there was no fungal growth on both treatments probably because both storage conditions did not favour fungal growth.

Bauchop (1979) reported that most fibrous and least- digestible diet support the greatest population of fungi, but Smith et al (1991) noted that cowpea husks has a high degradability ranging from 74% to 78%, consequently cowpea husks may not be supporting huge fungi growth due to its high digestibility. The severely reduced water activity, indeed probably lower than 0.7, may have also contributed to lack of fungal growth because Hui and Khachatourians (1995) noted that fungi and yeast can tolerate generally lower water activities than bacteria, they will grow at a<sub>w</sub> values around 0.8 and particularly xerophilic species grow at values as low as 0.7, since the period was very dry.



### **Bacterial Count.**

# Fig 1: Mean bacterial counts expressed in log<sub>10</sub>.

Figure 1 indicates that the mean log bacterial counts on the cowpea husk for both treatments increased from the field, before it was stored and after storage when it was ready to be used. This could also be due to changes in temperature and moisture which encouraged bacterial growth (Iherekonye and Ngoddy, 1985).

The mean log bacterial counts for cowpea husks under room storage increased considerably from the field to the time it was about to be stored. There was however a higher increase in log bacterial counts at the time the husk was about to be used for feeding as storage in an enclosed room could have contributed to high bacterial growth due the increased temperature (Fig. 1)(Adams and Moss, 1990). On the other hand, log bacterial counts for cowpea husks under shed storage also increased considerably from the field to the time it was to be stored. However there was a very little increase in log bacterial counts at the time the husk was about to be used as a supplementary feed. This is in line with what Hui and Khachatourian (1995) reported that some bacterial growth is facilitated by anaerobic conditions and since cowpea husks under shed storage was subjected to enough air, bacterial growth was not as high as that of those stored in the room.

From table 1, there was no significant difference in bacterial load between the two treatments (Room storage and Shed storage) at the threshing and storage time. This could be due to the fact that both treatments were subjected to the same conditions when the swabs were taken. There was however a significant difference in bacterial load between Room storage and Shed storage at the time of feeding. This could be due to the differences in storage and this corroborates with the findings of Adams and Moss (1990) that the conditions of the storage environment encourage, limit or prevent the growth microorganisms in foods.

**Table 1: Mean bacterial counts between treatments expressed in log**<sub>10</sub> (Means in a row with different superscripts are significantly different. P is probability).

	Treatments			
Levels	Room Storage(T1)	Shed Storage(T2)	SED	P. value
Threshing Time	3.76 <sup>a</sup>	4.08 <sup>a</sup>	0.39	P >0.01
Storage Time	4.65 <sup>a</sup>	5.07 <sup>a</sup>	0.39	P >0.01
Feeding Time	6.23 <sup>a</sup>	5.23 <sup>b</sup>	0.39	P <0.01

#### CONCLUSION

Bacteria (possibly *Enterobactereceae*) and fungi were detected on the cowpea husks. Storage of cowpea husks reduced fungal presence considerably to zero levels. Bacterial counts on the cowpea husks increased from the field through storage; however the increase tended to be relatively higher for cowpea husks stored in the room as against the open air storage under the shed outside.

#### RECOMMENDATIONS

To avoid toxicity from thermophilic fungi in mouldy feed cowpea husks should be stored for about 3 months before feeding to animals. Further studies should be conducted to isolate and ascertain the levels of *Enterobacteriaceae* if any, and other specific bacteria on cowpea husks in storage so as to decide on the risk of using such feed.

## REFERENCES

- Adams, M.R. and Moss, M.O, 1990 Food Microbiology. University of survey Guilford U.K. pp 1-219
- Adams, M.R. & Moss, M.O. 2000. Food Microbiology. 2nd ed. Cambridge, UK: The Royal Society of Chemistry.
- Bauchop, T. 1979. Rumen anaerobic fungi of cattle and sheep. Appl. Environ. Microbiol, 38: 148-158
- Cameron, A and Collymore, Y. 1979. The science of food and cooking (tropical edition). Edwards Arnold publishers Ltd 41 Bedford square London pp 152-154.
- Cole, R.J., J.W. Kirksey, J.W. Dorner, D.M. Wilson and J.C. Johnson Jr. 1977 Mycotoxins produced by Aspergillus fumigatus species isolated from moldy silage. J. Agric. Food Chem., 25: 826-830.
- Flannigan, B. 1987 *The microflora in barley and malt.* London: Elsevier.
- Häggblom, P. 1990 Isolation of Roquefortine C from feed grain. *Applied and Environmental Microbiology* 56, 2924-2926.
- Hui, Y. H. and Khachatourians, G. G. 1995 Food biotechnology: microorganisms published Wiley-VCH, Inc. pp 75-78.
- Ihekoronye, A. I. and Ngoddy, P. O. 1985 Integrated food science and technology for the tropic. University of Nigeria, Nsukka. Macmillan publishers Ltd. Pp 108-109, 326, 328-329,334-335.
- Kaspersson, A., Lindgren, S. & Ekström, N. 1988 Microbial dynamics in barley grain stored under controlled atmosphere. *Animal Feed Science and Technology* 19, 299-312.

- Lacey, J. 1989 Pre- and post-harvest ecology of fungi causing spoilage of foods and other stored products. *Journal of Applied Bacteriology* 67, 11-25.
- Lacey, J. 1991 Natural occurrence of mycotoxins in growing and conserved forage crops. *In:* Mycotoxins and Animal Foods, J. E. Smith and R. E. Henderson (Eds.). CRC Press, Boca Raton, Florida.
- Lacey, J. & Magan, N. 1991 Fungi in cereal grains: Their occurence and water and temperature relationships. In: Chelkowski, J. (Ed.) *Cereal grain. Mycotoxins, fungi and quality in drying and storage.* Amsterdam, The Netherlands: Elsevier Science Publishers. pp. 77-118.
- Macmillan 1996 The tropical agriculturalists, GOATS; 3:22-36.
- Magan, N. & Lacey, J. 1984 Effect of temperature and on pH on water relations of field and storage fungi. *Transactions of the British Mycological Society* 82, 71-81.
- Magan, N., Hope, R., Cairns, V. & Aldred, D. 2003 Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. *European Journal of Plant Pathology* 109, 723-730.
- Quin, F.M. 1997 Advances in Cowpea Research. Copublication of IITA and JIRAC.IITA, Ibadan Nigeria.
- Yamazaki, M., Maebayashi, Y., Miyaki, K. 1971 Biosynthesis of ochratoxin A. Tetrahedron Letters 25, 2301–2303.