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Control on Dehydrated Lucerne as Component of Animal Diets Related to Nutrient Content and Hygienic Quality

[1]P.E. Zoiopoulos and [2]P.I. Natskoulis
[1]Laboratory of Animal Production, School of Management of Natural Resources and Enterprises, University of Ioannina, 2 Seferi Str., Agrinio, GR-30 100, Greece
[2]National Agricultural Research Foundation, Institute of Technology of Agricultural Products, 1 S. Venizelou str., Lycovrissi, GR-141 23, Greece

Abstract: Nineteen variables were studied in samples of pelleted lucerne taken from 4 dehydration establishments in Western Greece, to assess its quality as feed material. The analyses, based on European Union (EU) methods, included determination of major nutrients, ash insoluble in HCl, trace elements, heavy metals, aflatoxin B_1 and microbial load. Samples taken from three out of 4 drying factories were of excellent nutrient composition, whereas one was of lower quality, based on high crude fibre 282 and ash insoluble in HCl (18.2 g kg^{-1}) content, implying delayed cutting and excessive soil contamination of lucerne crop. Presence of Pb, Cd and aflatoxin B_1 was not detected in any of the samples, while enumeration of Total Bacteria Counts (TBC) and yeasts/moulds ranged from 4.3-5.5 and 2.3-3.0 log_{10} cfu g^{-1}, respectively, but remained below permitted levels. The low microbial load observed is apparently due to the noticeably low moisture content of the lucerne pellets (67-93 g kg^{-1}). Information from this study aims at contributing to measures taken by State authorities to cope with the EU legislation on food safety, developing traceability procedures and establishing HACCP systems as well as GMP programme.

Key words: Feed quality, lucerne pellets, nutrients, heavy metals, aflatoxin B_1, microbial load

INTRODUCTION

Lucerne, alfalfa in US, (*Medicago sativa*) is an important 4-year legume crop, which is grown worldwide under both tropical and temperate conditions and is used as ingredient in animal diets (McDonald *et al*., 2002). Lucerne owes its fame as top forage to high nutritional value and yields. It can be repeatedly harvested (up to 8 successive cuttings in the same year), depending on possibilities of irrigation and conditions of sunlight and temperature in a certain area (Frame *et al*., 1998; Posperi *et al*., 2001). In addition, lucerne is an important crop in the EU, where a common organisation in market of dried forages exists, based on quality traits and aid is given to dried forages meeting minimum requirements of quality (EU, 2005a). Among these forages, dehydrated (artificially heat dried) lucerne is the predominant product. The significance of fodder dehydration sector has been documented (Olle Marrugat, 2001).

Feedstuffs are not only a source of energy and nutrients (Coleman and Moore, 2003) but also can influence the quality of food in a variety of ways through the presence of undesirable substances that they may contain. Safety of foods of animal origin and feed safety are closely linked and interdependent (Flachowski and Danicke, 2005). Following the food crises of the second half of the 90's, the EU developed Regulation 178/2002 known as General Food Law (EU, 2002) which raised animal feed, in terms of safety, to the same level with that of food. This Regulation, among others, introduced the element of traceability in the food chain. More recently, EU Regulation on feed hygiene was adopted, stating that feed manufacturers should plan, apply and maintain permanent procedures based on the principle of HACCP (Hazard Analysis and Critical Control Points) (EU, 2005b). Because of initial claims against low performance of animals fed rations containing lucerne pellets as one of their main ingredients in Western Greece, lucerne pellet samples were taken from all four drying establishments operating in the area, to be analysed according to Community methods for major nutrients, trace elements, ash insoluble in Hcl, heavy metals, aflatoxin B_1 and microbial load, in order to investigate its quality. These data will serve as source of information to traceability concept in the lucerne dehydration chain (Laffi and Pasini, 2001) contribute to GMP programme and as prerequisite to the establishment of HACCP system for the feed and food sector.

Corresponding Author: Pantelis E. Zoiopoulos, Laboratory of Animal Production, School of Management of Natural Resources and Enterprises, University of Ioannina, 2 Seferi Str.,Agrinio, GR-30 100, Greece

MATERIALS AND METHODS

Samples: Samples of lucerne pellets were taken from 4 dehydration establishments in the area of Aetolia, Western Greece. Artificial drying is effected by means of a rotating drum with a heat source at one edge. Drying is achieved by passing an air draft through the mass of cut lucerne for 7 min. Prior to drying, lucerne is wilted to approximately 50% of its initial moisture content. Following drying, lucerne is ground and steam-pelleted. Conditions of irrigation, sunlight and temperatures in the Aetolian valley favour up to 8 successive cuttings of lucerne crop per year. Cuttings take place at the "bud" or beginning of bloom stage. Representative samples of lucerne pellets from the four drying establishments were obtained according to official Community methods for sampling animal feeds (EEC, 1976a).

Chemical and microbiological analyses: Existing Community methods of analysis were employed for the determination of chemical constituents of pelleted lucerne. Moisture content was determined by heating samples at 105°C to constant weight, crude protein by the Kjeldahl method (N×6.25), fat by the Soxhlet technique and the concentration of P in solution was determined spectrophotometrically as the coloured complex formed with molybdovanadate reagent (EEC, 1971a). Ash was determined by ignition at 550°C. To measure ash insoluble in HCl, the sample was ashed, the ash was boiled with HCl, the solution filtered and the residue ashed and weighed, whereas Ca was determined volumetrically (EEC, 1971b). For crude fibre determination, the sample was treated successively with boiling weak solutions of H_2SO_4 and KOH, whereas Mg and trace elements were measured by atomic absorption technique (EEC, 1978). Alfatoxin B_1 was determined by Thin Layer Chromatography technique (EEC, 1976b). Heavy metals Pb and Cd were measured by atomic absorption method (ADAS, 1986) using a Perkin Elmer AAS Analyst 700 instrument. Enumeration of total bacteria and yeast and moulds was carried out by the pour plate method (Busta *et al*., 1984) with the following culture media: total bacterial count in plate count agar (Oxoid) and yeast and moulds in malt extract agar (Oxoid), incubated at 30 and 25°C for 3 and 5 days, respectively. Analyses for each constituent and sample were carried out in triplicate.

RESULTS AND DISCUSSION

Results of chemical analyses for nutrient content of lucerne pellets are given in Table 1. In general, the results are consistent with already published values (Allen, 1989; McDonald *et al*., 2002; Morrison, 1957). However, crude protein content in samples taken from 3 out of the 4 drying establishments, appeared to be exceptionally high, combined with low values of crude fibre. This implies that lucerne for these pellets was of excellent quality, cut at very early bud stage (Borreani *et al*., 2000), with a wide ratio of leaves to stems. In contrast, lucerne pellets from one of the factories were of noticeably marginal quality, based on the low crude protein and high crude fibre values observed. Low crude protein implies a narrow leaf to stem ratio, which is enhanced by the lower Mg content for this particular feed material, since, Mg participates in the formation of the chlorophyll molecule of the green parts of plants. High crude fibre content implies that lucerne was cut at a late stage of maturity, in addition to loss of leafy fraction during handling, transportation and storage before being dehydrated and pelleted. It should be mentioned at this point that such lucerne pellets are still eligible of receiving a Community premium as dried fodder, since, EU standards for moisture and crude protein set at a maximum of 12% and a minimum of 15% on dry matter basis, respectively. These requirements were satisfied in our case, i.e., 6.7 and 16.5%, respectively.

Table 1: Values for major nutrients, trace elements and ash insoluble in HCl content in pellets of dehydrated lucerne (on dry matter basis)

	Drying establishment					
Nutrients	A	B	C	D	Mean	SD
Major (g kg^{-1})						
Dry matter	907	922	902	933	916	14.2
Crude protein	266	252	266	165	237	48.6
Crude fibre	209	217	228	282	234	33.8
Fat	21.6	28.6	23.7	27.4	25.3	3.24
Ash	118	111	94.3	103	107	10.2
Ash insoluble in HCl*	6.5	4.8	3.4	18.2	8.23	6.77
Ca	18.7	15.7	13.3	16.1	16.0	2.21
P	3.5	3.3	6.2	2.8	3.95	1.53
Mg	16.4	17.4	24.5	11.9	17.6	5.21
Trace elements (mg kg^{-1})						
Fe	1803	1815	1772	3361	2188	782
Zn	131	120	208	89	137	51
Mn	206	178	207	228	205	20.5
Cu	66	55	109	73	75.8	23.4
Co$^+$	ND‡	ND	ND	ND	-	-

*Ash insoluble in HCl is not a nutrient; its presence implies soil contamination of the crop or fraud. $^+$Detection limit for Co: 0.03 mg kg^{-1}, ‡ ND: Not Detected

Table 2: Microbial load in pellets of dehydrated lucerne ($\log_{10}$ cfu g^{-1})‡

	Drying establishment					
Microorganism	A	B	C	D	Mean	SD
TBC*,$^+$	5.3	4.3	5.3	5.5	5.1	0.5
Yeasts and moulds$^+$	3.0	3.0	2.3	2.8	2.8	0.3

*TBC: Total Bacterial Count (aerobic-mesophilic), $^+$EU maximum permitted levels do not exist; informal standards as working guidelines for this feed material in Greece are set at 6.47 and 4.6 $\log_{10}$ cfu g^{-1} for TBC and yeasts/moulds, respectively. ‡ cfu: colony forming units ($\log_{10}$ cfu g^{-1})

One interesting finding in this study was the 3-5 fold higher values for content of ash insoluble in HCl for lucerne obtained from the drying establishment producing the lower quality pellets reported above, compared to the material from the other 3 factories. Ash insoluble in HCl is not a nutrient and its presence implies detection of silica due to the addition of sand. This can be explained by the contamination of lucerne crop with soil during harvesting and handling, a fact which is enhanced by the approximately 2 fold increase of Fe content (3361 mg kg^{-1}) in this material, compared to the Fe content in the samples from the other three dehydration factories. However, the marketing of lucerne meal or pellets with higher content of ash insoluble in HCl is allowed, since according to Community legislation for feed materials, such feedingstuff can be marketed, but with the compulsory declaration of ash insoluble in HCl in the labelling, if its concentration exceeds the level of 3.5% (EU, 1996).

Only contaminants included in the EU legislation for undesirable substances (EU, 2002b) were dealt with. The issue of mycotoxin presence in foods and feeds has been extensively studied (Fink-Gremmels, 2006; Sangare-Tigori *et al*., 2006; Silva *et al*., 2007). The subject of risk of contamination of foods with toxic substances present in animal feed, including heavy metals and mycotoxins has been also reviewed very recently (Kan and Maijer, 2007). The presence of either Pb and Cd or aflatoxin B_1 was not detected in any of the samples, since all these contaminants were below the detection limits of the methods used. Detection limits of the quantitative assessment techniques employed in this study were 0.05, 0.01 and 0.01 mg kg^{-1} for Pb, Cd and aflatoxin B_1, respectively. In addition, Community legislation maximum permitted levels are 30, 1 and 0.02 mg kg^{-1} for Pb, Cd and aflatoxin B_1, respectively. It is apparent that the low moisture content of lucerne pellets does not favour growth of *Asperillus flavus* for the production of aflatoxin B_1. Vlachou *et al.* (2004) reported that, in general, aflatoxin B_1 does not seem to constitute a problem for animal feeds in Greece.

Results for TBC and yeasts/moulds counts are shown in Table 2. The effects on livestock of feed contaminated with bacteria and fungi were reviewed very recently (Maciovowski *et al*., 2007). Results of Table 2 were expressed in $\log_{10}$ numbers to enable us to include also standard deviations. All values for TBC and yeasts/moulds reported in this study, tended to be low. Apparently, this is due to the beneficial effect of hot (steam) pelleting that has taken place (McDonald *et al*., 2002), as well as to the noticeably low content of moisture of the lucerne pellets (67-93 g kg^{-1}). Although EU standards for TBC and yeasts/moulds counts do not exist, there are informal standards that are used as working guidelines for routine agricultural practice in Greece. According to these guidelines, TBC and yeast/moulds counts of dried forages should not exceed 3×10^6 and 4×10^4 cfu gr^{-1} or if one transforms to $\log_{10}$ numbers 6.47 and 4.60 cfu g^{-1}, respectively. TBC and yeast/moulds counts for all samples in the present study were below these values, but TBC counts for lucerne pellets taken from the drying establishment producing the material of lower quality was relatively higher.

CONCLUSION

The approach employed in this investigation to assess the quality of dehydrated lucerne, revealed that one out of the four drying establishments in the area produced, at the time of the study, a product of lower quality, based on high fibre and relatively low crude protein and Mg content, combined with higher ass insoluble in HCl and Fe content, implying soil contamination of the lucerne crop during handling. Presence of aflatoxin B_1 was not detected, enhancing the existing view that due to low feed moisture content, in general, aflatoxin B_1 does not seem to constitute a problem for animal feeds in Greece. Given the recent adoption of EU legislation on Feed Hygiene, the information from the present investigation will contribute to appropriate measures taken by the State to cope with EU General Food Law, to develop traceability procedures and to establish a HACCP system, as well as GMP programme.

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