EVALUATION OF PIGMENT EXTRACTED ANNATTO SEED (*BIXA ORELLANA*) BY CHEMICAL, *IN-VITRO* AND *IN-SACCO* TECHNIQUES IN BUFFALOES

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ABSTRACT

Annatto *(Bixa orellana)* seed after extraction of dye and colour was evaluated by chemical, *in vitro* and *in sacco* techniques. DM, OM, CP, EE, and NFE contents were 86.81, 94.77, 14.35, 1.95, and 52.87 %, respectively. NDF, ADF, hemicellulose, cellulose and lignin contents were 58.5, 24.5, 34.0, 14.4, and 10.86 %, respectively. The Ca, P, Mg, K and S contents were 0.04, 0.7, 0.2, 1.1 and 0.35 %, and the Fe, Mn, Co, Zn and Mo contents were 142, 2125, 2.07, 132 and 203 ppm, respectively. The IVDMD was 61.4 %, when incubated with buffalo rumen liquor. *In sacco* studies with fistulated rumen buffaloes revealed that the effective degradable DM and protein of pigment extracted annatto seed was 62.4 and 23.2 %, respectively. It was concluded from the present study that annatto seed can be used as substitute for cereal grains in the rations of buffaloes as a source of energy and by-pass protein.

Key words: Annatto seed, Chemical composition, IVDMD, EDDM, ERDP, Buffalo

INTRODUCTION

The annatto (*Bixa orellana*) plant is a native of tropical America but is now distributed in most tropical countries in both wild and cultivated forms (Anon, 1988).The main producers are Bolivia, Brazil, Colombia, the Dominican Republic, Ecuador, India, Jamaica, Mexico, Peru and Srilanka (Anand, 1983). In India, it is found mainly in Orrisa, Andhra Pradesh and Maharashtra and to some extent in Kerala, Karnataka and Tamil Nadu (Patnaik, 1971). The quantity of annatto seeds handled in markets during the harvesting season is roughly estimated to be about 150-200 metric tons. The yield of annatto seed is approximately 270 kg per tree. The seeds have a thin coating of highly coloured resin, which after purification and processing provides the basic pigment used in colouring preparation. After this process the pigment extracted annatto seed, constituting approximately 94.5-95.5% on weight basis becomes waste. Hence, in the present study on attempt was made to estimate nutritional quality and suitability of pigment extracted annatto seed for feeding of ruminant livestock as an alternative to conventional feed ingredients.

MATERIALS AND METHODS

The annatto seed meal used in this experiment was procured after pigment extraction from the Pramoneel Agro-industries, Hyderabad, and analyzed for proximate constituents (AOAC, 1997) and cell wall constituents (Van Soest *et al.*, 1991) after grinding in a Wiley mill using a 2 mm mesh screen. Minerals were estimated by AAS (Perkins Elmer model-2380). A two- stage *in vitro* fermentation technique (Tilley and Terry, 1963) was used keeping the period of incubation during the first stage at 48h for the determination of *in vitro* DM digestibility (IVDMD) using rumen liquor

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from a permanently cannulated graded Murrah buffalo steer maintained on a ration with a 50:50 roughage to concentrate ratio.

Dry matter disappearance and protein degradability were determined using a nylon bag technique (Kempton, 1980). Nylon bags of 25-28 μ mesh size with a bag area of 70 cm^2/g DM were used to incubate the samples. Nylon bags with a sample size of 5 g were incubated in duplicate simultaneously in the rumen of four fistulated graded Murrah buffalo steers (279 ± 2 kg) for 12, 24, 36, 48 and 72 h for DM disappearance and 3, 6, 9, 15 and 24 h for protein disappearance. The Murrah buffalo steers were adapted to a ration comprising 4.5 kg each of sorghum straw and concentrate mixture containing pigment extracted annatto seed. After each incubation interval, the bags were retrieved, washed under tap water, and dried at 70°C till constant weight. From the percent DM and protein disappearance data, constants i.e., a (readily soluble), b (insoluble but degradable with time) and c (rate constant) were derived (McDonald, 1981). The effective degradable dry matter (EDDM) and effective rumen degradable proteins (ERDP) were determined at an out flow rate of 0.05/h (Orskov and McDonald, 1979).

RESULTS AND DISCUSSION

Proximate composition and fibre fractions of pigment extracted annatto seed are presented in Table 1. The crude protein (CP) content was 14.35%, and this was similar to the reports of Wurts *et al.* (1983). Bressani *et al.* (1983) reported that the protein content of annatto seed fluctuated between 13 – 16%. However, a low protein value of 10.6% was reported by Glew *et al.* (1997). The crude fibre content in annatto seed was 25.60%, which was higher than the value reported by Bressani *et al.* (1983). The variation in chemical composition may be due to variation in soil and climatic conditions of the country where the crop is grown. The DM, OM, NFE, TA and fibre fractions such as NDF, ADF, hemicellulose, cellulose, lignin, silica values were similar to those reported by Jayawikrama (2006). The fibre fractions indicate the fibrous nature of annatto seed, and this makes it more suitable for feeding ruminants than monogastric animals.

The mineral analysis (Table 2) revealed that annatto seeds have a high P content and a low Ca content, and this is in accordance with the findings of Bressani *et al.* (1983) and Glew *et al.* (1997). But the Ca and P values of pigment extracted annatto seed were lower and higher than those reported by Glew *et al.* (1997), respectively. The Mg value for annatto seed observed in the present study was similar to the value reported (Glew *et al.*, 1997). Similarly, among the trace minerals, the Fe and Mo were lower and Zn and Mn were higher than the values of Glew *et al.* (1997). Further, the trace element Se was not present at detectable levels (<1μg/g). The fluctuations in mineral content may be due to the soil profile and the agro-climatic conditions in addition to the fertilizers used. However, Wurts *et al.* (1983) opined that the mineral content as well as the fibre fractions were very similar to those determined in cereals.

The IVDMD value of pigment extracted annatto seed was 61.4% and was similar to that of barley (58.8%) and soybean meal (63.6%) as reported by Mehta and Srivastava (1998). The DM disappearance of annatto seed meal increased linearly as its incubation period in the rumen increased. The average DM disappearance increased by 10.25, 16.85, 31.94 and 35.04 percentage units, by extending the incubation period in the rumen of buffalo from 12 to 24, 36, 48 and 72 h, respectively (Table 3). The instantly soluble DM fraction (a) and insoluble but degradable with time fraction (b) were almost equal in annatto seed meal with an effective degradable DM (EDDM) value of 62.40% (Table 3). The EDDM value of annatto seed was higher than that of green gram (*Vigna radiata*) husk (Radhakrishna *et al.*, 2002), salseed (*Shorea robusta*) meal, and mahua seed (*Madhuca indica*) cake (Barman and Rai, 2004). Wurts *et al.* (1983) reported a satisfactory dry matter degradation index for pigment extracted annatto seed in ruminants.

A linear increase in the protein disappearance of annatto seed meal was also observed with increase in the period of incubation in the rumen. Only 28.54 % of protein disappeared

by the end of 24 h incubation (Table 3) indicating its resistance to microbial action. The a and b fractions of annatto seed meal were 10.48 and 21.76 % (Table 3) and comparable with degradation characteristics of bajra (*Pennisetum americanum*) grain and salseed meal (Gangadhar *et al.,* 1992). The effective rumen degradable protein (ERDP) was 23.20 % and was much lower than the ERDPs of maize (*Zea mays*) and jowar (*Sorghum bicolor*) grain, wheat bran, and deoiled rice bran (Gangadhar *et al.,* 1992). Of the total CP present, nearly 76.8% (10.92 g) was undegradable in the rumen, indicating that annatto seed meal is a good source of by-pass protein for high yielding ruminants. Though the protein contents of annatto seed and other cereal grains were similar, differences exist in the protein degradability and this could be attributed to feed characteristics including protein structure and amino acid sequence (Leng and Nolan, 1984), type of binding between protein and other components mainly ADIN content of feeds (Orskov, 1982) and animal factors like rumen environment (type of microbes, ruminal pH), retention time and out flow rate (Van Soest and Mason, 1997). The RDP and UDP content of annatto seed meal were 23.2 and 76.8 g per 100g of protein and 34.3 and 109.2 g/kg DM, respectively; it falls thus under group D (<30% degradability) based on protein degradability in the rumen.

The results of the present study revealed that pigment extracted annatto seed meal is a potential alternate unconventional basal feed with high by –pass protein content and can replace cereal grains in the rations of buffaloes.

Table 1. Chemical composition (% DM) of pigment extracted annatto seed.

Parameter	%
Proximate composition	
Dry matter	86.81
Organic matter	94.77
Crude protein	14.35
Ether extract	1.95
Crude fibre	25.60
Total ash	5.23
Nitrogen free extract	52.87
Fibre fractions	
NDF	58.50
ADF	24.50
Hemicellulose	34.00
Cellulose	14.40
ADL	10.86
Silica	0.30

On DM basis except for DM

Table 2. Mineral profile of pigment extracted annatto seed.

Minerals	%
Macro minerals	
Ca	0.04
P	0.70
Mg	0.21
K	1.10
S	0.35
Trace minerals	**ppm**
Fe	141.60
Mn	57.86
Co	2.07
Zn	131.60
Mo	203.30

Table 3. *In sacco* DM and protein degradability (%) of pigment extracted annatto seed in Murrah buffalo steers**.**

Incubation period	DM disappearance	Incubation period	Protein disappearance
12	59.67	3	14.35
24	69.92	6	17.91
36	76.52	9	21.39
48	81.01	15	22.96
72	84.71	24	28.54
Degradation kinetics			
a	42.40		10.48
b	45.20		21.76
c	0.0397		0.097
a + b	87.60		32.24
ED (%)	62.40		23.20

Each value is the mean of four observations

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