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Citrullus colocynthis Seeds as a Potential Source of Protein for Food and Feed

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Seeds of *Citrullus colocynthis* were investigated as a possible source of feed or food formulation. The seed contained 13.5% protein (N × 6.25), 26.6% oil, 2.1% ash, 52.9% crude fiber, and 4.9% nitrogen-free extract (NFE). The seeds contained substantial amounts of potassium, phosphorus, and iron. The essential amino acid contents of the seed protein were adequate (>95%) (FAO/WHO, 1973). Lysine was the limiting essential amino acid with a chemical score of 65. The seeds in particular were rich in methionine and cystine (5.5 g/100 g of protein). The in vitro protein digestibility (IVPD) and calculated protein efficiency ratio (C-PER) of the seed protein were 75.9% and 1.85, respectively, on the basis of Animal Nutrition Research Council casein values of 95.0% (IVPD) and 2.50 (C-PER). Feeding experiments with chicks showed normal growth with up to 15% whole seeds in the diet. Using the unprocessed meal however at the same level (15%) depresses growth and feed efficiency.

INTRODUCTION

Citrullus colocynthis, a member of the Cucurbitaceae family, grows as a wild perennial in desert regions of the world including Saudi Arabia (Khan and Gul, 1975; Sawaya et al., 1983). The fruit of this plant, called gourd, contains 200–300 seeds/gourd. The seeds have been investigated as a possible source of edible oil. Khan and Gul (1975) reported 13–19% oil in seeds collected from Pakistan, while Singh and Yadava (1978) reported 30–36% oil in seeds obtained from India. Sawaya et al. (1983) showed, from feeding experiment with 1-day-old chicks, that the oil extracted from *C. colocynthis* seeds was potentially suitable for human and for animal consumption. Apart from their potential as a source of oil, cucurbit seeds, in general, are reported to contain approximately 35% protein by weight of decorticated seeds that have a nutritionally adequate amino acid profile (Jacks et al., 1972). Since large-scale isolation of purified oilseed protein is readily accomplished by current technology (Hensarling et al., 1973), a study of the nutritional properties of *C. colocynthis* seed proteins was desirable particularly when reports in the literature are scarce on the protein quality of these seeds.

The present study was, therefore, initiated to investigate the nutritional characteristics of *C. colocynthis* seed proteins growing wild in the kingdom of Saudi Arabia.

MATERIALS AND METHODS

Wild gourds of *C. colocynthis* were collected from desert parts in the vicinity of Riyadh, Saudi Arabia. The dried gourds were crushed and the seeds separated. The seeds were ground in a laboratory micromill (Technilab Instruments, Pequannock, NJ) to pass through a 1-mm sieve.

Moisture, crude fat, ash, and crude fiber were determined in the ground sample by the official methods of the AOAC (1980). Crude protein (N × 6.25) was determined by an automatic protein analyzer (Kjel-foss Automatic 16210) by the procedure of the AOAC (1980).

For mineral analysis, a 1-2-g sample was ashed (AOAC, 1980) and the ash was dissolved in 5 mL of 20% HCl. Necessary dilutions were made with deionized water. For Ca and Mg, the final diluted solution contained 1% lanthanum to overcome interferences. Na, K, Ca, Mg, Fe, Cu, Zn, and Mn were determined with a Perkin-Elmer Model 603 atomic absorption spectrophotometer. P was determined via its molybdo blue reaction by the procedure of Watanabe and Olsen (1965).

For amino acid analysis, samples containing 5 mg of protein were hydrolyzed with 6 N HCl at 110 °C for 24 h (Moore and Stein, 1963). Cystine was determined as cysteic acid (Moore, 1963). Tryptophan was determined in a separate alkaline (NaOH) hydrolysate (Hugli and Moore, 1972). All the hydrolysates were analyzed on a Beckman Model 119CL automatic amino acid analyzer. Duplicate hydrolysates were prepared for the amino acid analysis.

Chemical Score. The chemical score of the protein was calculated by dividing the contents of the essential amino acids by the contents of the same amino acids in an FAO/WHO (1973) reference protein.

In Vitro Protein Digestibility (IVPD) and Calculated Protein Efficiency Ratio (C-PER). The IVPD of the protein was determined by the multienzyme technique reported by Satterlee et al. (1979), using the four enzymes trypsin, chymotrypsin, peptidase, and protease. The C-PER was calculated from the essential amino acid data and IVPD as described by Satterlee et al. (1979).

Animal Experiments. Feeding trials were done with broiler-type 1-day-old straight-run chicks. Four experiments were conducted, each lasting up to 3 weeks. The composition of diets used in these experiments is shown in Table I. Chicks were reared in a thermostatically controlled, six-deck battery brooder, and feed and water were provided ad libitum. Chick weights and feed consumed were recorded weekly and at the end of the experiment. All diets in experiments 1–4 were equalized in protein, energy, fat, and crude fiber.

Experiment 1. Three pens of six chicks each were assigned to each treatment in a completely randomized design. The *C. colocynthis* seed meal was used in this experiment. (The meal was prepared by extracting the seeds with petroleum ether at 60–80 °C and then drying under a hood for 2 days.) The semipurified diets (Table I) patterned after Scott et al. (1982) were used in this experiment. The *C. colocynthis* meal was used at 15% of the diet replacing the cellulose and part of the glucose and soybean meal of the control diet. All other ingredients were kept constant.

Experiment 2. Methods used for this experiment were identical with those of experiment 1. The *C. colocynthis*

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Table I. Composition of Diets for Chick Experiments

ingredient, %	diets 1 (control)	2	3	4	5
	Experiments 1 and 2				
soybean meal[a]	43.00	39.00	39.00		
glucose (dextrose)	37.87	34.87	34.87		
C. colocynthis meal		15.00[b]	15.00[b]		
cellulose	8.00				
DL-methionine	0.30	0.30	0.30		
corn oil	4.00	4.00	4.00		
vitamin premix[c]	1.20	1.20	1.20		
mineral premix[c]	5.63	5.63	5.63		
	Experiment 3				
yellow corn	51.80	51.80	51.80	51.80	
soybean meal[a]	27.50	26.10	24.74	23.20	
super concentrate[d]	10.00	10.00	10.00	10.00	
C. colocynthis		5.00	10.00	15.00	
soybean oil	3.08	2.05	1.03		
cellulose	7.62	5.05	2.43		
	Experiment 4				
yellow corn	48.20	48.20	48.20	48.20	48.20
soybean meal[a]	27.50	26.10	24.70	23.30	21.80
super concentrate[d]	10.00	10.00	10.00	10.00	10.00
C. colocynthis		5.00	10.00	15.00	20.00
soybean oil	4.30	3.08	2.05	1.07	
cellulose	10.00	7.62	5.05	2.43	

[a] Protein content, 49%. [b] Meal was untreated in diet 2 and water soaked in diet 3. [c] Supplied the same level of nutrients as recommended by Scott et al. (1982) for semipurified chick diets. [d] Composition of super concentrate: protein, 50%; ME, 2200 kcal/kg; Ca, 8.7%; available P, 3.7%; salt, 3.2%; lysine, 3.6%; methionine, 1.07%; methionine + cystine, 2.6%.

meal was divided into equal halves. Half was soaked in water for 24 h, decanted through No. 10 and 20 mesh sieves, and then resoaked for 10 consecutive days. The meal was then dried at 40 °C for 15 h. The *C. colocynthis* meal was again used at 15% of the diet replacing cellulose and part of the glucose and soybean meal.

Experiment 3. In this experiment, three pens of eight chicks each were assigned to each treatment. The experimental period was from 1 day old to 19 days of age. Citrullus seeds were crushed and used as is in the diets at levels of 0, 5, 10, and 15% of the diets. Practical type diets were used in this experiment. The seeds were added in the rations to replace part of the soybean meal, soybean oil, and alphacel, and all other procedures followed were similar to those followed in experiment 1.

Experiment 4. Three pens of eight chicks each were assigned to each treatment. The experimental period was from 1 day old to 21 days of age. *C. colocynthis* seeds were used in this experiment at levels of 0, 5, 10, 15, and 20% of the diets. Practical type diets shown in Table I were used in this experiment. The seeds were added to replace part of the soybean meal, soybean oil, and alphacel. The procedures followed were similar to those followed in experiment 1.

Statistical Analysis. Data obtained from the animal experiments were analyzed statistically by the analysis of variance method (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Chemical Composition. Data on the proximate composition and mineral analysis are presented in Table II. The seeds contained 13.5% protein, 26.6% oil, 2.1% ash, and 52.9% crude fiber on a dry weight basis. The oil content was within the range reported for *C. colocynthis* seeds, which varied from 13% (Khan and Gul, 1975) to 36% (Singh and Yadava, 1978). On the other hand, the value for protein was lower than that of other cucurbit seeds (Jacks et al., 1972) such as buffalo gourd that contain 28.5–31.7% protein (Shahani et al., 1951; Khoury et al., 1982). It was also lower than the protein content of conventional oilseeds (Watt and Merrill, 1963; Bemis et al., 1975). The lower protein content in *C. colocynthis* seeds can be attributed to the high fiber content of the seed that accounts for more than half of the total seed weight. If the full potential of this oil seed is to be utilized in feed or food preparations, the seed or the defatted meal has to be processed further to remove or decrease the crude fiber, thus increasing protein content. With the current state of technological advancement, it probably can be readily accomplished. For example, cottonseed, which closely resembles *C. colocynthis* seed in protein and oil content, has been successfully processed into usable products despite a relatively higher amount of crude fiber relative to other oilseeds (Bemis et al., 1975). The *C. colocynthis* seed after decortication will then contain theoretically a higher protein (28.7%) and oil (56.5%) content in the kernels (Table II), due to the removal of the high crude fiber. Even the undecorticated oil-free residue contains higher amounts of all the nutrients (Table II), and further processing to decrease the crude fiber content would result in higher nutrient density. Data on mineral elements showed that the seeds, with the exception of sodium, contained relatively lower concentrations of mineral elements compared to other oilseeds including buffalo gourd (Watt and Merrill, 1963; Shahani et al., 1951). As mentioned earlier, the high fiber content (seed coat) has a dilution effect on the mineral elements also. However, on dehulling, the mineral elements would be increased in the kernels to levels comparable to other oilseeds (Table II). Among the macroelements K followed by P and Mg would be predominant, while among the microelements Fe and Zn would be present in fairly good amounts.

Table II. Proximate Composition[a] and Mineral Element Contents of *C. colocynthis* Seed, Defatted Meal, and Seed Kernel

nutrient	whole seed	defatted seed meal	seed[b] kernel
protein (N × 6.25), %	13.5	18.4	28.7
fat, %	26.6	<1.0	56.5
ash, %	2.1	2.9	4.5
crude fiber, %	52.9	72.0	
nitrogen-free extract (NFE), %	4.9	6.7	10.3
Na, mg/100 g	79	108	168
K, mg/100 g	322	439	684
Ca, mg/100 g	13	18	28
Mg, mg/100 g	50	68	106
P, mg/100 g	119	162	253
Fe, mg/100 g	3.3	4.5	7.0
Zn, mg/100 g	1.4	1.9	3.0
Cu, mg/100 g	0.3	0.4	0.6
Mn, mg/100 g	trace	trace	trace

[a] Results are the average of two determinations (variation <10%) expressed on a dry weight basis. The moisture content of the seed was 4.6% and meal 5.7%. [b] Composition of the seed kernel by calculation on fiber-free basis.

Amino Acid Composition. Data on the amino acid composition are compared with other oilseeds in Table III. Like other oilseeds, aspartic acid, glutamic acid, and arginine were the most abundant amino acids in *C. colocynthis* seed protein. Its amino acid profile compared well with that of buffalo gourd except for a relatively lower concentration of cystine and tryptophan in the seed of buffalo gourd. With the exception of lysine, all the essential amino acids were present in amounts equal to or more than 95% of the FAO/WHO (1973) reference pro-

Table III. Comparison of Amino Acid Composition of *C. colocynthis* Protein with Other Oilseed Proteins (g of Amino Acid/100 g of Protein)

amino acid	*C. colocynthis*[a]	buffalo gourd[b]	soybean[c]	peanuts[d]	FAO/WHO, 1973
aspartic acid	9.3	10.9	12.01	12.51	
threonine	4.0	3.5	4.31	2.76	4.0
serine	5.1	5.9	5.57	5.23	
glutamic acid	19.8	21.5	21.00	21.53	
proline	4.4	4.5	6.28	4.57	
glycine	7.2	4.8	4.52	6.05	
alanine	5.6	5.0	4.51	4.19	
valine	4.8	5.0	5.38	4.24	5.0
methionine (M)	3.5	2.5	1.56	1.23	
cystine (C)	2.0	0.7	1.58	1.53	
M + C	5.5	3.2	3.14[e]	2.76	3.5
isoleucine	3.8	4.1	5.10	3.61	4.0
leucine	7.2	7.8	7.72	7.01	7.0
tyrosine (T)	2.2	3.9	3.90	4.47	
phenylalanine(P)	5.5	5.9	5.01	5.33	
T + P	7.7	9.8	8.91	9.80	6.0
histidine	2.3	2.5	2.55	2.49	
lysine	3.6[e]	3.3[e]	6.86	3.53[e]	5.5
arginine	15.9	16.8	8.42	13.23	
tryptophan	1.4	0.6[e]	1.28	0.83	1.0
NH_3	2.1		2.05		
chemical score	65	60	90	64	100

[a] Results are the average of duplicate determinations. [b] Data from Hensarling et al. (1973). [c] Data from Smith and Circle, 1978 (p 72). [d] Data from Khalil and Chughtai, 1983. [e] Limiting essential amino acid.

tein. The concentration of sulfur amino acids (SAA) (methionine + cystine) was considerably higher in *C. colocynthis* seed protein compared to other oilseed proteins and exceeded the suggested level of the FAO/WHO (1973) reference protein. In this respect, it was similar to sesame seed protein that contained about 6 g of SAA/100 g of protein (Brito and Nunez, 1982). This is of significant nutritional importance since most seed proteins including oilseeds are generally low in SAA (Evans et al., 1978). The high SAA content of *C. colocynthis* protein indicated a good supplementary value with other seed proteins. Lysine, which is generally deficient in cereal proteins, was the only deficient essential amino acid in the protein of *C. colocynthis* seed with a chemical score of 65 for the protein. The chemical score of *C. colocynthis* was comparable to that of buffalo gourd (60) and peanut (64) protein (Hensarling et al., 1973; Khalil and Chughtai, 1983). These data point toward the good nutritional quality of *C. colocynthis* seed proteins.

IVPD and C-PER. The IVPD and C-PER values of *C. colocynthis* protein were 75.9% and 1.85%, respectively, in comparison to ANRC casein values of 90% and 2.50. The IVPD value was close to the average digestibility value of cotton seed (79.6%) but lower than the digestibility values of soybean (90.5%), peanut (86.6%), and sunflower seed (81.9%) reported by the FAO (1970). The C-PER value of *C. colocynthis* protein was higher than the C-PER values of many other protein sources such as cornmeal (1.1), wheat flour (0.7–0.8), and soy flour (1.3–1.4) reported by Satterlee et al. (1979). The IVPD and C-PER were determined with whole seed; hence, a reduction in the fiber content would be expected to increase the IVPD and consequently the C-PER value of the decorticated seed.

Animal Experiments. Results of the feeding experiments with 1-day-old chicks are shown in Table IV.

Experiment 1. Feeding *C. colocynthis* meal at 15% of the diet significantly depressed body weight and feed efficiency of chicks. This growth depression was not primarily due to depressed feed intake because differences in feed consumption were not found to be statistically significant. Since the two diets used in this experiment were equalized in all nutrients known to be required by the growing chicken, the growth depression caused by the experimental diet is most probably due to either the protein quality of the *Citrullus* meal or the presence of antinutritional factors in it. Feeding experiments with mice have also indicated high toxicity in *C. colocynthis* (Weber et al., 1977).

Table IV. Evaluation of *C. colocynthis* Meal and Seeds in Feeding Experiments with 1-Day-Old Chicks

treatment	body wt,[a] g/bird	feed consumed,[a] g/bird	feed/gain[a]
Experiment 1 (1 day old–20 days of age)			
soybean (control)	535 ± 12.5[a]	749 ± 15.2	1.40 ± 0.026[a]
C. colocynthis meal	433 ± 31.2[b]	725 ± 33.6	1.68 ± 0.050[b]
Experiment 2 (1 day old–20 days of age)			
soybean (control)	493 ± 12.8[a]	727 ± 9.3	1.47 ± 0.035[a]
C. colocynthis meal			
untreated	453 ± 17.8[b]	755 ± 7.2	1.67 ± 0.080[b]
water soaked	459 ± 10.7[b]	736 ± 10.7	1.60 ± 0.050[b]
Experiment 3 (1 day old–19 days of age)			
soybean (control)	474 ± 18.1	642 ± 35.1[a]	1.35 ± 0.021[a]
C. colocynthis seeds			
5%	498 ± 4.3	695 ± 10.4[a,b]	1.39 ± 0.01[a,b]
10%	517 ± 5.9	758 ± 6.0[b]	1.47 ± 0.010[b]
15%	502 ± 11.7	750 ± 23.8[b]	1.49 ± 0.014[b]
Experiment 4 (1 day old–21 days of age)			
soybean (control)	455 ± 9.6	674 ± 9.6	1.48 ± 0.026
C. colocynthis seeds			
5%	464 ± 3.8	693 ± 4.1	1.49 ± 0.017
10%	440 ± 14.3	689 ± 1.7	1.57 ± 0.052
15%	443 ± 19.5	691 ± 8.7	1.57 ± 0.053
20%	454 ± 13.6	661 ± 4.5	1.45 ± 0.035

[a] Means ± SE. Means with different superscripts are significantly different ($p < 0.05$).

Experiment 2. Results of this experiment showed that water soaking of the meal did not significantly improve chick performance as measured either in terms of body weight or in feed efficiency. There was a slight improvement in feed efficiency, but this improvement was not statistically significant. Water soaking therefore was not found to be an efficient method of removing the antinutritional factors in *Citrullus* seeds.

Experiment 3. Results of experiment 3 indicate that feeding whole *Citrullus* seeds is not as growth depressing as feeding the meal. Feeding up to 15% seeds in the diet had no significant effect on growth. Birds consuming 10–15% *Citrullus* seeds consumed significantly more feed than the controls, and as a result feed efficiency was significantly reduced. In a previous report (Sawaya et al., 1983) it was shown that the oil extracted from *Citrullus* seeds was free from any antinutritional factor and in fact was equal in its nutrient value to corn oil when fed to growing chicks.

Experiment 4. Results of experiment 4 confirm the findings of experiment 3 and show no significant differences in growth, feed consumption, or feed efficiency of chicks consuming up to 20% *Citrullus* seeds in their diet. However, it is not recommended that *Citrullus* seeds be fed at such high levels in practical poultry formulations since this brings up the fiber level of the ration to over 10% and thus reduces feed utilization drastically. They can easily be incorporated in broiler rations at levels of 5–10% of the ration if all nutrients are kept constant. On the basis of data presented in this study, the undecorticated seed as well as the meal contain significant amounts of protein, oil, and ash but a lower amount of nitrogen-free extract. Although both the seed and meal contain substantial amounts of protein, the crude fiber content of both the seed and meal is high. Consequently, the seed would have to be decorticated prior to extraction or the meal processed further to make it more suitable as a stock feed. Purified protein can be isolated from such products for possible use in human food formulations. However, the meal contains certain growth-depressing factors for chicks, and more studies are needed to determine the detoxification procedure in order to exploit the full potential of *C. colocynthis* seed protein. These seeds are reported to be heavily consumed by humans in some African countries (Afolabi et al., 1985), and processing prior to consumption is regarded as the possible way for detoxification.

Registry No. Na, 7440-23-5; K, 7440-09-7; Ca, 7440-70-2; Mg, 7439-95-4; P, 7723-14-0; Fe, 7439-89-6; Zn, 7440-66-6; Cu, 7440-50-8; Mn, 7439-96-5.

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Isolation and Characterization of the Major Fraction (2S) of *Madhuca* (*Madhuca butyraceae*) Seed Proteins

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A method is described for isolating the major protein of *Madhuca* seeds in a homogeneous form. Its $S_{20,w}$ value was found to be 2S. It contains 4.1% carbohydrate and no phosphorus. The 2S protein consists of at least two subunits. Circular dichroism measurements indicate that this protein consists of 35% α helix, 38% β structure, and 27% random coil.

Madhuca (*Madhuca butyraceae*) (family Sapotaceae) is a minor oil seed of tree origin in India. The seed contains about 5–8% protein and 50–60% fat, which is being used in soap and cosmetic industries. the defatted flour, though a good source of protein, is not edible due to the presence of saponins (Wealth of India, 1952). In this laboratory, we have undertaken a systematic study on *Madhuca* seed proteins to obtain information on their chemical, physicochemical, nutritional, and functional properties.

Madhuca seed protein consists of three fractions with $S_{20,w}$ values of 2S, 10S and 15S (Shanmugasundaram and

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