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ARTICLE

Fiber Reduction and Lipid Enrichment in Carotenoid-Enriched Distillers Dried Grain with Solubles Produced by Secondary Fermentation of *Phaffia rhodozyma* and *Sporobolomyces roseus*

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Carotenoid-enriched distillers dried grain with solubles (DDGS) developed as a value-added animal feed to provide carotenoids from mono and mixed culture (Mx) fermentation of red yeasts *Phaffia rhodozyma* (PR) and *Sporobolomyces roseus* (SR) were evaluated for their nutritional composition and compared to the control (C) DDGS. Apart from providing carotenoids, all three fermentation treatments reduced fiber with best reduction of 77% in PR, enhanced crude fat with highest of 81% in Mx, and reduced protein, amino acids and nitrogen by 50% in PR. DDGS fiber reduction by 77% was achieved by *P. rhodozyma* in the absence of any pretreatment. Qualitative and quantitative differences in fatty acid profiles were seen among the treatments. Vaccenic acid, a monounsaturated fatty acid produced in SR and Mx fermentation, was absent in C and PR. All these nutritional modifications are highly desirable in different DDGS-based animal feeds and can be explored to obtain tailor-made feeds/feed blends for specific animal diets.

KEYWORDS: Animal feed; astaxanthin; aquaculture; β-carotene; biofuel; coproduct; vaccenic acid; value addition

INTRODUCTION

Distillers dried grain with solubles (DDGS), a coproduct of corn ethanol, is primarily used as an animal feed. DDGS is an excellent source of protein and energy for beef cattle, and is used at 40 to 50% of ration dry matter (*27*). The recommended DDGS use varies from 20 to 40% dry matter in lactating cows, 10% in swine, 10 to 15% in poultry and 10 to 82% in aquaculture depending on the species (*27*). But the practical inclusion rates may be much lower than the recommended dosage. High fiber in DDGS is an impediment in using higher inclusion rates of DDGS in nonruminant and poultry feed. The poor digestibility of dietary fiber in swine (43% apparent total tract digestibility of dietary fiber) is the primary reason for reduced digestibility of dry matter and subsequently reduced digestibility of energy (*25*). Accordingly, based on the specific animal dietary needs, DDGS is supplemented with soybean meal or other agricultural products to overcome any nutrient limitation (*27*).

Recently, in an effort to sustain the biofuel industry and develop value added animal feed, we developed carotenoid-enriched DDGS by red yeast fermentation (*1*). By subjecting the whole stillage to carotenoid enrichment by monoculture and mixed culture fermentations of *Phaffia rhodozyma* and *Sporobolomyces roseus*, we not only were able to provide natural, inexpensive carotenoids within or twice the recommended daily dietary dosage of carotenoids in animal feed but also avoided the expensive process of extracting the carotenoids. Characteristic of yeasts, *P. rhodozyma* is a good source of proteins, lipids, vitamins and minerals (*18*). Also, carotenogenic yeasts are particularly rich in fatty acids, especially polyunsaturated fatty acids (PUFA) (*9*, *13*). It is therefore likely that the carotenoid-enriched DDGS produced by Ananda and Vadlani (*1*) is also enriched in nutrients like proteins, lipids and vitamins. Another interesting characteristic of *P. rhodozyma* is its ability to degrade corn fiber in the absence of any pretreatment (*12*). This ability is indeed valuable since corn fiber (composition of glucan 21.2%, xylan 17.2%, arabinan 12.9%, galactan 4.1% and starch 17.5% (*16*)) is a complex cross-linked structure not easily degraded by commercial enzymes. Hayman et al. (*10*) were able to produce astaxanthin from *P. rhodozyma* on six coproducts of corn wet-milling rich in corn fiber. Since corn whole stillage or DDGS is rich in percent crude fiber (5.3 to 13.5 (*3*, *7*, *11*, *20*, *22*)) and *P. rhodozyma* has the ability to degrade fiber, it is important to evaluate the carotenoid-enriched DDGS animal feed for reduced fiber.

Based on the above reports, we hypothesized that, apart from carotenoids, (1) carotenoid-enriched DDGS produced by red yeast *P. rhodozyma* would be enriched in proteins, lipids and PUFA along with reduced fiber; and (2) *S. roseus*, also red yeast, would produce nutritional changes in carotenoid-enriched DDGS similar to that of *P. rhodozyma*. Specifically, the objective of this study was to produce the carotenoid-enriched DDGS as outlined in Ananda and Vadlani (*1*) in a 2 L benchtop fermenter and evaluate and compare the nutritional composition of carotenoid-enriched DDGS between monoculture and mixed culture fermentation and control DDGS.

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Table 1. Nutrition Profile of DDGS and Carotenoid-Enriched DDGS

components[a]	control	mixed culture	*P. rhodozyma*	*S. roseus*
% crude protein[b]	27.77 ± 0.24	17.16 ± 0.11 (↓38%)	12.95 ± 0.064 (↓**53**%)	17.75 ± 0.05 (↓36%)
% crude fat[c]	14.59 ± 0.1	26.35 ± 0.1 (↑**81**%)	17.07 ± 0.035 (↑17%)	24.25 ± 0.028 (↑66%)
% crude fiber	5.31 ± 0.24	1.99 ± 0.007 (↓63%)	1.20 ± 0.021 (↓**77**%)	1.81 ± 0.023 (↓66%)
% NDF	22.25 ± 0.024	9.68 ± 1.71 (↓57%)	5.49 ± 0.67 (↓**75**%)	8.42 ± 0.61 (↓62%)
% ADF	7.00 ± 0.464	4.61 ± 0.26 (↓34%)	1.97 ± 0.01 (↓**72**%)	3.66 ± 0.74 (↓48%)
% N	4.44 ± 0.23	2.74 ± 0.1(↓38%)	2.07 ± 0.06 (↓**53**%)	2.84 ± 0.05 (↓36%)
% P	0.81 ± 0.038	0.85 ± 0.04	0.81 ± 0.033	0.67 ± 0.029
% K	1.00 ± 0.046	0.97 ± 0.11	1.01 ± 0.043	0.86 ± 0.03
% S	0.70 ± 0.021	0.67	0.59 ± 0.006	0.66 ± 0.012
ash	3.25 ± 0.35	0.08 ± 0.028	0.2	0.12 ± 0.07
astaxanthin (μg/g)	0.00	2.73	**50.91**	
β-carotene (μg/g)	0.00	**240.00**	79.86	119.99

[a] Means and standard deviation are provided; numbers in parentheses indicate the % increase (↑) or decrease (↓) compared to the control; maximum increase or decrease is boldfaced. [b] Kjeldahl. [c] Acid hydrolysis.

MATERIALS AND METHODS

Microbial Cultures and Inoculum Generation. Lyophilized cultures of *Phaffia rhodozyma* M.W. Miller, Yoneyama & Soneda 1976 (ATCC 24202) and *Sporobolomyces roseus* Kluyver & van Niel 1924 (ATCC 28988) were obtained form American Type Culture Collection (ATCC, Manassas, VA). The *P. rhodozyma* (ATCC 24202) strain is a known carotenoid producer along with xylose metabolizing ability (*16*, *28*). Both astaxanthin and β-carotene are produced by *P. rhodozyma*, whereas *S. roseus* produces only β-carotene. Culture maintenance and inoculum generation of *P. rhodozyma* and *Sporobolomyces roseus* are outlined in Ananda and Vadlani (*1*). A 10% (v/v) inoculum was used for monoculture fermentation, while 5% of each strain was used in mixed culture fermentation.

Media Preparation. Optimized medium as outlined in Ananda and Vadlani (*1*) was used. A liter of the fermentation medium contained 15% whole stillage, 1.5% corn steep liquor, 7.7% glycerol and mineral salts (0.6 g of KH_2PO_4, 0.3 g of $MgSO_4$, 0.3 g of $MnSO_4$ and 0.7 g of $ZnSO_4$). Corn whole stillage was procured from Abengoa Bioenergy (Colwich, KS, USA). Media pH was about 6.0 before sterilization and was not adjusted any further.

Fermentation. Fermentation was carried out using a 2 L BBraun Biostat-B fermenter. About 1.5 L of the fermentation medium was sterilized in the fermenter at 121 °C for 30 min. Batch fermentation was carried out for seven days at 20 °C, 500 rpm and 1 (v/v) sterile air. Dissolved oxygen and pH were monitored for every 2 h. Three fermenter runs, one each for *P. rhodozyma* and *S. roseus* monocultures, and mixed culture fermentation were carried out. The entire fermentation broth was harvested on day 7, aliquoted into five bottles and freeze-dried for five days. After freeze-drying, samples were pooled and blended using a coffee blender. Samples were stored at −20 °C until further analyses. The control sample contained all the media ingredients except glycerol. Two representative samples from each treatment were subjected to nutritional profiling. It is well-known that whole stillage and/or DDGS samples from different batches from the same ethanol plant can have variations in their nutritional composition. This can potentially affect the carotenoid production. Accordingly, multiple fermenter runs were not possible due to limited whole stillage sample. The whole stillage sample used in Ananda and Vadlani (*1*) was used in this study as well.

Carotenoid Extraction and Estimation. Extraction and estimation of carotenoids are outlined in Ananda and Vadlani (*1*). Briefly, carotenoids were extracted in dichloromethane solvent by grinding a known amount of freeze-dried sample with 0.2 g of acid washed sand (40−100 mesh size).

High performance liquid chromatography (HPLC) was used for quantification of carotenoids. Astaxanthin and β-carotene standards were obtained from Sigma Aldrich (St. Louis, MO, USA). A Shimadzu HPLC equipped with LC-20AB pump, SIL-20AC auto sampler, SPD-M20A PDA detector and CTO-20A column oven was used. Phenomenex Prodigy C_{18} column (150 mm length and 4.6 mm internal diameter) along with a C_{18} guard column was used for the separation of carotenoids. An acetonitrile and methanol (80:20) mobile phase was used. The carotenoid yield was expressed as μg/g of freeze-dried whole stillage sample instead of yield per gram of yeast dry weight as it was impossible to separate yeast cells from the whole stillage solids.

Nutrition Profiling. Nutrition composition ana1yses of the samples were conducted to include total amino acid profile, total fatty acid profile, crude fat and protein, crude fiber, % NDF and % ADF and % P, S and K. About 10 g of each representative sample from each treatment was analyzed at Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) for total amino acid profile (AOAC Official Method 982.30 E (a, b, c), chapter 45.3.05 (*17*)), total fatty acid profile (AOAC Official Method 996.06, AOCS Official Method Ce 2-66, AOAC Official Method 965.49, AOAC Official Method 969.33 (*17*)), crude fat (acid hydrolysis, AOAC Official Method 954.02 (*17*)) and protein (Kjeldahl method, AOAC Official Method 984.13 (A−D) (*17*)). Estimation of % P, K, S and crude fiber, % NDF and % ADF was conducted at Analytical Laboratory, Department of Animal Science and Industry, Kansas State University (Manhattan, KS).

RESULTS

The crude composition of DDGS and the secondary fermented products are presented in **Table 1**. Compared to the control, *P. rhodozyma*, *S. roseus* and mixed culture fermentation resulted in lesser protein, amino acids, N and fiber, and enhanced fat. Maximum reduction in percent protein, fiber and N was seen in *P. rhodozyma*, and the best fat enhancement was seen in mixed culture fermentation. Fiber reduction by yeast fermentations ranged from 63 to 77%. Percent P, K and S were not reduced drastically compared to the control. However, *S. roseus* reduced % P and % K by 17% and 14% respectively, and *P. rhodozyma* reduced % S by 15%.

The amino acid profiles of all the treatments are presented in **Table 2**. Both monoculture and mixed culture fermentation resulted in lesser amino acids compared to the control. *P. rhodozyma* reduced the amino acid content by more than 50%, followed by about 40% reduction in mixed culture and *S. roseus*. Lysine, threonine, tryptophan and methionine are important limiting amino acids in DDGS depending on the animal diet (*27*), and levels of these amino acids did not improve with yeast fermentation.

The fatty acid profiles of all the treatments are presented in **Table 3**. Both monoculture and mixed culture fermentation resulted in higher fatty acids compared to the control. Based on the abundance of different fatty acids (accounting for more than 2% of total fats), both, control and *P. rhodozyma* fermentation contained linoleic acid, oleic acid, palmitic acid and stearic acid. Linoleic acid in the control accounted for 52.7% whereas it accounted for only 34.6% in *P. rhodozyma*. Oleic acid, palmitic acid and stearic acid in *P. rhodozyma* fermented DDGS were higher than that in the control (**Table 3**). Both, *S. roseus* and mixed culture showed similar fatty acid profiles with vaccenic acid, linoleic acid, palmitic acid and stearic acid being the most

Table 2. Amino Acid Profile of DDGS and Carotenoid-Enriched DDGS

	w/w %[a]			
amino acids	control	mixed culture	*P. rhodozyma*	*S. roseus*
taurine	0.04	0.04 ± 0.007	0.03 ± 0.007	0.04
hydroxyproline	0.00	0.00	0.00	0.00
aspartic acid	1.78 ± 0.014	1.32 ± 0.028	0.81 ± 0.021	1.36
threonine	1.02 ± 0.021	0.69 ± 0.014	0.64 ± 0.021	0.75 ± 0.014
serine	1.15 ± 0.021	0.75 ± 0.021	0.65 ± 0.042	0.80 ± 0.028
glutamic acid	3.81 ± 0.177	1.94 ± 0.014	0.98 ± 0.021	1.87 ± 0.028
proline	2.02 ± 0.035	0.98 ± 0.021	0.76 ± 0.057	1.02 ± 0.085
lanthionine	0.00	0.00	0.00	0.00
glycine	1.18 ± 0.007	0.98 ± 0.021	0.62 ± 0.007	1.01 ± 0.007
alanine	1.95 ± 0.021	1.00 ± 0.021	0.71 ± 0.014	1.04 ± 0.007
cysteine	0.57	0.44 ± 0.021	0.23 ± 0.007	0.45
valine	1.43 ± 0.021	0.86 ± 0.007	0.79 ± 0.007	0.91
methionine	0.58 ± 0.007	0.27 ± 0.007	0.20 ± 0.007	0.29 ± 0.007
isoleucine	1.04 ± 0.007	0.62 ± 0.014	0.63 ± 0.007	0.65 ± 0.014
leucine	2.99 ± 0.021	1.17 ± 0.028	1.12 ± 0.007	1.24 ± 0.028
tyrosine	0.94 ± 0.028	0.52 ± 0.007	0.38 ± 0.057	0.51 ± 0.014
phenylalanine	1.19	0.61 ± 0.014	0.48 ± 0.007	0.61 ± 0.014
hydroxylysine	0.00	0.00	0.00	0.00
ornithine	0.04	0.04	0.01	0.04 ± 0.007
lysine	1.12 ± 0.007	0.93 ± 0.021	0.74 ± 0.057	0.94 ± 0.014
histidine	0.82	0.47	0.45	0.49
arginine	1.39	0.84 ± 0.007	0.61 ± 0.007	0.88 ± 0.007
tryptophan	0.22 ± 0.007	0.16	0.13 ± 0.007	0.18
total	25.22 ± 0.24	14.58 ± 0.28 (↓42%)	10.91 ± 0.064 (↓**57**%)	15.06 ± 0.19 (↓40%)

[a] Means and standard deviation are provided; numbers in parentheses indicate the % decrease (↓) compared to the control; the maximum decrease is boldfaced.

abundant in that order. Vaccenic acid was not seen in both the control and *P. rhodozyma* fermented DDGS, whereas oleic acid was absent in mixed culture and *S. roseus* fermentation (**Table 3**).

DISCUSSION

The primary goal of the carotenoid-enriched DDGS was to provide carotenoids in DDGS based animal diets. However, as hypothesized, red yeast fermentation of DDGS also reduced fiber and enhanced fat and PUFA, modifications which are desirable depending on the specific needs of various animal diets. Red yeast fermentation did not enhance protein content as hypothesized. On the contrary, reduction in protein content, amino acid and % N was seen. Red yeast modifications of protein, fat and fiber content of DDGS are discussed from an animal feed standpoint as they can be exploited to develop tailor-made DDGS diets catering to the demands of different animal diets.

Effect of Red Yeast Fermentation on Fiber. High fiber in DDGS, though desirable for ruminants, is responsible for negative effects on poultry growth (*15*). Reduction in DDGS fiber can allow the expansion of the DDGS feed base, especially in nonruminant, poultry and aquaculture feeds. Srinivasan et al. (*23*) developed "elusieve", a process of sieving and elutriation to produce low fiber DDGS: sieving alone produced two fractions, one with low fiber and the other with increased fat and protein, and elutriation of these fractions further concentrated the fat and protein, and fiber, allowing the high fat and protein DDGS with low fiber for use in nonruminant feed. Additionally, Srinivasan et al. (*24*) showed that the sieving and elutriation process reduced the quantity of DDGS but increased the value of DDGS, as high fat (13%) and high protein (33%) DDGS attracts $5−$20 more per ton than DDGS with low fat (11%) and protein (28% (*3*)). Secondary fermentation of whole stillage by red yeasts to reduce fiber is likely to be more economical than mechanical methods simply because additional processing or equipment costs are not accrued (elusieve costs $1.4 million with a payback in 2.5 to 4.6 years (*24*)), and is an added bonus in the production of a premium product, namely, carotenoid-enriched DDGS. More than 75% reduction in DDGS fiber by yeast fermentation without any pretreatment or any procedural modifications is indeed remarkable. Fiber reduction in DDGS animal feeds by *Phaffia* yeast should be explored considering that the yeast is permitted as a salmonid feed additive by the U.S. Food and Drug Administration (FDA; 21 CFR Section 73.355 (*2*)).

Effect of Red Yeast Fermentation on Protein. The protein and amino acid levels and % N in DDGS were reduced substantially by red yeast fermentation. Ideally, protein enhancement in DDGS by 33% would attract a premium price (*3*). Contrary to expectations, protein and amino acid levels were reduced by red yeast fermentation. More than 50% reduction was seen in *P. rhodozyma* fermentation. Feed blends of carotenoid-enriched DDGS with DDGS or other protein rich sources like soybean products or fish meal can provide optimal protein and/or amino acid levels in DDGS-based animal diets.

Effect of Red Yeast Fermentation on Fat. Red yeast fermentation increased the crude fat and fatty acid content and altered the fatty acid composition of DDGS. This alone should be able to attract a higher price for DDGS. Soybean oil, oil seeds, vegetable oils, marine oils or animal fats are often used to supplement fat in animal feeds (*5*, *27*). Instead, carotenoid-enriched DDGS with enhanced fat can be used to supplement diets. Vaccenic acid, a monounsaturated fatty acid, was produced in *S. roseus* and mixed culture fermentation. Vaccenic acid is primarily found in bovine milk and meat, accounting for 70% of *trans* fatty acids in ruminant-derived lipids (*8*, *14*). It is a known precursor of conjugated linoleic acid (CLA), and the principal sources of CLA in human diets are dairy products and ruminant meat (*4*). CLA is known to confer many health benefits to animals and humans (*4*, *29*). Santora et al. (*19*) studied the effects of feeding specific fatty acids and their fate in mice and found that (i) elaidic acid and *trans*-vaccenic acid (TVA) from feed were incorporated similarly in mice, (ii) CLA found in mice fed with TVA was greater than that found in mice fed with fed with CLA, and (iii) the conversion of TVA to CLA was about 11% of TVA or 50% of stored TVA. Additionally, CLA in

Table 3. Fatty Acid Profile of DDGS and Carotenoid-Enriched DDGS

	% of total fat[a]			
fatty acid	control	mixed culture	*P. rhodozyma*	*S. roseus*
myristic (14:0)	0.06	0.45 ± 0.007	0.18	0.45
myristoleic (14:1)	0.00	0.00	0.00	0.00
(C15:0)	0.00	0.13	0.09 ± 0.007	0.14
palmitic (16:0)	14.12 ± 0.11	14.30 ± 0.021	17.59 ± 0.085	14.02 ± 0.007
palmitoleic (16:1)	0.22 ± 0.064	0.84	0.16	0.69
(17:0)	0.08	0.12	0.23 ± 0.007	0.12
(17:1)	0.05 ± 0.007	0.12	0.05	0.12
stearic (18:0)	2.53 ± 0.1	2.98 ± 0.007	10.10 ± 0.035	4.07 ± 0.007
elaidic (18:1*t9*)	0.06 ± 0.007	0.12 ± 0.007	0.07	0.12 ± 0.007
oleic (18:1*n9*)	26.98 ± 0.16	0.00	33.94	0.00
vaccenic (18:1*n7*)	0.00	61.66 ± 0.78	0.00	60.95 ± 0.071
linoleic (18:2)	52.70 ± 0.18	15.73 ± 0.12	34.64 ± 0.14	15.41 ± 0.049
linolenic (ω18:3)	1.49 ± 0.014	0.72	0.88 ± 0.007	0.74 ± 0.007
(ω18:4)	0.00	0.00	0.00	0.00
arachidic (20:0)	0.44 ± 0.007	0.30	0.85	0.34
(20:1*n9*)	0.25	0.62 ± 0.007	0.09	0.66 ± 0.007
(20:3 ω3)	0.00	0.00	0.00	0.00
arachidonic (20:4*n6*)	0.00	0.00	0.00	0.00
arachidonic (20:4 ω3)	0.00	0.00	0.00	0.00
(20:5 ω3; EPA)	0.00	0.00	0.00	0.00
docosanoic (22:0)	0.23 ± 0.042	0.45 ± 0.014	0.45	0.53
erucic (22:1*n9*)	0.00	0.06	0.00	0.07 ± 0.007
(22:5 ω3; DPA)	0.00	0.00	0.00	0.00
(22:6 ω3; DHA)	0.16 ± 0.007	0.09	0.03 ± 0.035	0.11
lignoceric (24:0)	0.34	0.79 ± 0.007	0.32 ± 0.007	0.93
nervonic (24:1*n9*)	0.00	0.03	0.00	0.03
% crude fat	14.59 ± 0.1	26.35 ± 0.1 (↑**81**%)	17.07 ± 0.035 (↑17%)	24.25 ± 0.028 (↑66%)

[a] Means and standard deviation are provided; numbers in parentheses indicate the % increase (↑) compared to the control; the maximum increase is boldfaced.

the carcass was found only when CLA or TVA was fed to the mice. Since vaccenic acid is abundant in *S. roseus* and mixed culture fermented DDGS, providing this to cattle may possibly increase the TVA and CLA levels in milk and meat especially since different types of forages and lipid supplementations are known to exert different effects on milk fat composition and synthesis in cow and goat (*5*, *6*). Depending on the fat requirement in specific animal feeds, *P. rhodozyma* fermented DDGS may be more suitable for low fat feed or feed blends and mixed culture fermented DDGS may be ideal for high fat animal diets.

Production of vaccenic acid in *S. roseus* is most likely due to the substrate, namely, DDGS. In the case of *S. roseus* grown in synthetic yeast extract dextrose (YED) broth, vaccenic acid production was not seen: the abundant fatty acids from yeast cells were linoleic acid (60−64% of total fats), followed by palmitic or stearic acid (16−20%) depending on aeration, with other fatty acids in trace amounts (< 5% (*9*)). However, fatty acid profiles of *P. rhodozyma* cells (Red Star Phaffia Yeast from Red Star Specialty Products, Milwaukee, WI, USA in ref *18*) were very similar to that seen in this study, with abundant fatty acids: linoleic acid (40%), oleic acid (32%) and palmitic acid (13%). Effect of culture media on fatty acid composition and relative abundance was seen in carotenogenic yeasts including *Sporobolomyces patagonicus* from Patagonia (*13*). The major fatty acids were linoleic acid (40%), oleic acid (34%), palmitic acid (13%) and α-linoleic acid (9%), and their relative abundance was influenced by the type of media.

Libkind et al. (*13*) hypothesized that carotenoids are lipid-based protection against oxidative stress and, as more carotenoids are produced by the carotenogenic yeasts, more fatty acids, especially PUFA, are produced. Similarly, Davoli et al. (*9*) noted that fatty acid (14.4 to 42.2 mg/g) and carotenoid levels (109 to 412 μg/g) increased relative to biomass in *S. roseus* with enhanced aeration on synthetic YED medium. However, this may not be true for all red yeasts. Carotenoid and lipid levels of *Rhodotorula gracilis* were inversely related depending on C/N ratio of the synthetic media (*21*). Similarly, *Rhodotorula glutinis* showed minimal increase in carotenoid levels (from 113 to 206 μg/g) upon aeration with unchanged levels of fatty acid at 19.6 mg/g in synthetic YED medium (*9*). Apart from the hypothesis of Libkind et al. (*13*), it is also likely that the higher fatty acid levels seen in some red yeasts and in the red yeast fermentation of DDGS are due to the antioxidant protection conferred by carotenoids that prevent lipid peroxidation.

It is probably convenient that the carotenoid-enriched DDGS is also enriched in fatty acids. Surai et al. (*26*) have reviewed the uptake of carotenoids and the intrinsic role of fatty acids in carotenoid transport and absorption. Micelles formed from dietary lipids transport and deliver carotenoids to the absorptive surfaces, implying the importance of the feed matrix. They also note that the amount and quality of dietary fat and fatty acids of varying chain length and saturation affect the transport and absorption of carotenoids.

Effect of Red Yeast Fermentation on Minerals. The red yeast fermentation of DDGS reduced the % N composition from 36 to 53%. This is probably useful in reducing nitrogen in animal wastes and fish farm effluents. The % P, K and S remained largely unchanged except for 17% and 14% reduction in % P and % K respectively by *S. roseus*, and 15% reduction of % S by *P. rhodozyma*. These reductions may not be significant and warrant further investigation.

In conclusion, secondary fermentation of corn whole stillage by red yeasts not only provides carotenoid-enriched DDGS but also brings about two valuable nutritional modifications: increase in fat and reduction in fiber. Additionally, there is reduction in protein and % N. Carotenoid-enriched DDGS is ideally suited for use in feed blends. The use of microbial modification of DDGS to obtain tailor-made DDGS catering to different animal diets is a definite possibility and should be explored to further the market base of DDGS to sustain the biofuel industry. The potential benefits of the carotenoid-enriched DDGS should be thoroughly evaluated in animal studies.

ABBREVIATIONS USED

ADF, acid detergent fiber; ATCC, American Type Culture Collection; CLA, conjugated linoleic acid; DDGS, distillers dried grain with solubles; NDF, neutral detergent fiber; N, P, K, S, nitrogen, phosphorus, potassium, sulfur (%); PUFA, polyunsaturated fatty acid; TVA, *trans*-vaccenic acid; YED, yeast extract dextrose.

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