

# Physicochemical Properties of Rice Grain and Starch from Lines Differing in Amylose Content and Gelatinization Temperature

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The amylose content of the grain of two crops of selected IR841 lines (IR262-43-8 X Khao Dawk Mali 3-2-105) correlated positively with the residual protein content of purified starch and negatively with the water content of steeped brown rice. A low value for water-soluble amylose at 100°C in high amylose (30%) lines was associated with a very high value of amylograph setback. Gelatinization

temperature was negatively correlated with alkali digestibility values of milled rice and the extent of acid corrosion of starch granules in both crops. Grain characteristics, such as hardness, protein, and amylose content, and gelatinization temperature, and sedimentation constant of amylopectin differed in the two crops in many of the lines.

Starch is the major constituent of milled rice. The amylose content and gelatinization temperature of rice starch differ among varieties (Juliano, 1967, 1970). Waxy rice has almost no amylose. Nonwaxy rices may contain 8 to 37% amylose (Raghavendra Rao and Juliano, 1970; Reyes *et al.*, 1965). Amylose content may be classified as low (<20%), intermediate (20–25%), or high (>25%). Final gelatinization temperatures range from 55 to 79°C (Juliano, 1970). Final gelatinization temperature may be low (<70°C), intermediate (70–74°C), or high (>74°C). To minimize complicating genetic and environmental factors, we have used the same crops of lines from the same cross, differing in either amylose content (International Rice Research Institute, 1971; Juliano, 1970; Vidal and Juliano, 1967) or gelatinization temperature (Juliano *et al.*, 1969). The identification, in the IRRI breeding program, of lines from the same cross that have different combinations of amylose content and gelatinization temperature permitted us to study the relation of these two starch properties to other grain properties in two crops.

## MATERIALS AND METHODS

Samples of IR841 lines and the two parents, IR262-43-8 (Peta/3 × Taichung Native 1) and Khao Dawk Mali 4-2-105, were obtained from the IRRI Varietal Improvement department in the 1970 dry and wet seasons. The two crops were grown under identical cultural management, except that 120 kg/ha of N was applied in the dry season crop and 90 kg/ha of N was applied in the wet season crop. Nine lines were represented in the dry season crop and only eight lines in the wet season crop. Three months after harvest the samples of rough rice were dehulled and milled.

**Brown Rice.** The weight of 100 grains was determined in duplicate and the length and width of 10 grains were measured. Moisture content was determined in duplicate in 40-mesh flour from the weight lost during 1 hr drying at 130°C (American Association of Cereal Chemists, 1962). Grain hardness was estimated from 20 individual grains with a Kiyatype hardness tester with a tapered plunger, and from the percentage by weight of flour coarser than 80-mesh after grinding duplicate 10 grains for 20 sec in a Wig-L-Bug amalgamator (Raghavendra Rao and Juliano, 1970). To determine the

water content of steeped grain, duplicate 15-g grain samples were soaked for 24 hr in distilled water in 50-ml beakers after vacuum infiltration for 10 min to remove air spaces in the grains, blotted with a moist chamois, weighed, and dried for 16 hr at 100°C. Water content was determined from the weight lost after drying.

Rough rice of selected samples was moistened and germinated in Petri dishes in darkness for 4 days at 25–28°C. The endosperm protein was separated by hand, dehulled, freeze-dried, and ground to 100-mesh flour with a Wig-L-Bug amalgamator. Free sugars were extracted from a portion of the flour with hot 80% methanol and determined by the anthrone method (McCready *et al.*, 1950).

**Milled Rice.** Moisture content, alkali spreading and clearing values, gelatinization or birefringence end point temperature, crude protein, elongation ratio, and amylograph characteristics were determined by previously described methods (Juliano *et al.*, 1969). Amylose content was assayed by a simplification of the method of Williams *et al.* (1958) adapted to an AutoAnalyzer (Juliano, 1971). Calibration was done with rice samples of predetermined amylose content by the method of Williams *et al.* (1958). The starch-iodine blue test at 100°C for water-soluble amylose was conducted in duplicate on 100-mesh rice flour according to Juliano *et al.* (1968a) with the iodine reaction of the aqueous extract adapted to an AutoAnalyzer.

**Starch.** Starch was prepared from milled rice by extracting the protein with sodium dodecyl benzene sulfonate according to Reyes *et al.* (1965). The starch was air-dried at 35°C. Moisture content and protein were determined in the same way as for the rice samples. The equilibrium moisture of triplicate 2-g starch samples was measured in a glove box at 96% relative humidity (saturated Na<sub>2</sub>SO<sub>4</sub> solution) according to Juliano (1964). Granule size distribution, blue value at 680 nm, and lintnerization loss after 4 days at 36.0 ± 0.5°C in 2.2 N HCl were determined by the method of Reyes *et al.* (1965).

Starch samples were dispersed in dimethyl sulfoxide at 100°C as a 0.6% solution and the sedimentation constants of amylose and amylopectin were determined in a Beckman Model E ultracentrifuge at 20°C at a bar angle of 60°C according to Adkins *et al.* (1970). A An-D rotor with a single-sector, 12-mm aluminum cell was rotated at 15,000 rpm for amylopectin and then at 39,460 rpm for amylose. The sedimentation constants were recalculated to standard conditions ( $S_{20,w}$ ). Within the variation in amylose and amylopectin contents of the 0.6% starch solutions, the  $S_{20,w}$  of amylose

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Table I. Range and Mean of Physicochemical Properties of Grain of Selected IR841 Lines and Their Parents, 1970 Dry and Wet Seasons

Property	Dry season crop				Wet season crop				LSD, 5 % <sup>a</sup>
	Nine lines		Khao Dawk Mali	IR262	Eight lines		Khao Dawk Mali	IR262	
	Range	Mean	Range		Mean				
Milled rice amylose, % dry basis	11.6-29.4	20.6	16.0	28.8	9.0-30.0	17.7	13.8	28.5	0.6**
Final gelatinization temperature, °C	66.5-78.5	73	67.5	73	64-77	71	66.5	59.5	1.5**
Brown rice									
100-grain wt, g	1.83-2.29	2.05	2.12	2.30	1.65-2.02	1.91	1.82	2.22	0.09**
Grain hardness									
Kiya test, kg/mm <sup>2</sup>	8.7-9.8	9.3	9.8	7.4	9.4-11.7	10.4	10.8	8.2	1.0**
Grinding, % retained by 80-mesh screen	50.7-61.3	56.2	55.7	41.3	52.7-62.8	57.5	57.8	47.3	2.0**
Water content of steeped grain, % wet basis	28.4-32.7	30.7	31.2	30.4	29.1-33.8	31.6	32.0	31.0	0.3**
Milled rice									
Elongation ratio	1.49-1.74	1.62	1.78	1.55	1.49-1.84	1.70	1.85	1.89	0.13**
Alkali spreading	2.7-7.0	4.8	7.0	5.2	2.7-7.0	5.0	7.0	6.6	0.6**
Alkali clearing	1.7-7.0	3.8	6.4	4.8	2.0-7.0	4.4	7.0	6.3	0.6**
Protein, % dry basis	6.6-7.9	7.2	6.7	7.4	10.6-13.0	11.7	9.9	10.4	0.3**
Water-soluble amylose at 100°C, %	2.44-14.0	8.82	7.16	11.8	3.47-13.3	7.64	6.56	10.3	0.43**

<sup>a</sup> For (crop × line) mean, including the two parents.

was not affected by differences in amylose concentration but the  $S_{20,w}$  of amylopectin tended to be negatively correlated with amylopectin concentration and may account for as much as 20 Svedberg units.

## RESULTS

**Rice Grain.** The rice lines that were tested consisted of five low amylose lines that had low, intermediate, or high gelatinization temperatures; one line that had low gelatinization temperature and intermediate or low amylose content; and three lines that had low or intermediate gelatinization temperatures with high amylose content. Thus, from a cross between a high and a low amylose parent, most of the IR841 lines had either low or high amylose contents.

The IR841 lines from a cross between an intermediate- and a low-gelatinization temperature parent (Table I) had low, intermediate, and high gelatinization temperatures. However, only low amylose lines had high gelatinization temperatures as Beachell (1967) observed in lines from crosses between *indica* and *japonica* varieties. The lines and Khao Dawk Mali tended to have lower amylose content and gelatinization temperatures in the wet season crop than in the dry season crop. Previous work in our laboratory showed a more consistent effect of crop season on amylose content than on gelatinization temperature (Juliano *et al.*, 1964, 1969).

Most of the lines and Khao Dawk Mali had lower grain weights than the IR262 parent in both crops (Table I). The grain weights were lower in the wet season crop as previously reported for 16 varieties (Juliano *et al.*, 1964). Grain hardness values differed among the lines and overlapped the values of Khao Dawk Mali but they were higher than those of IR262, both by the two methods employed (Table I). The grain hardness values were higher for the wet season crop.

The range of water content values for steeped grain was wide and exceeded those of the parents (Table I). Water content was higher for the wet season crop.

The grain length and width of milled rice were within the values shown by the two parents and were not affected by season. In contrast, differences in values for alkali digesti-

bility among the lines were significant and the values were lower than those of both parents. IR262 showed higher alkali values in the wet season crop.

The elongation ratios of the lines tended to overlap those of the parents in the dry season crop but were lower in the wet season crop (Table I). Values tended to be higher in the wet season crop.

Protein content was higher in the wet season crop for all samples (Table I) because of an actual increase in protein per grain, and probably because the starch content was lower in the wet season, as reflected by the lower grain weight and lower amylose content. Similar seasonal effects were obtained previously for 16 varieties (Juliano *et al.*, 1964). A contributing factor was the lower solar radiation during grain development in the wet season, which resulted in lower grain yields.

The lines had a wider range of water-soluble amylose values than the parents and these values were higher in the dry season crop, reflecting differences in amylose content between the two crops (Table I).

**Starch.** The lines had a wider range of blue values of the starch than the two parents (Table II), reflecting the lines' wider range of amylose content (Table I). The lines also tended to have a wider range of mean starch-granule size than their parents. They had a smaller mean starch-granule size in the wet season crop than in the dry season crop. Khao Dawk Mali had the same starch granule size in both crops, but IR262 had a larger mean size in the wet season crop.

The equilibrium moisture content of starch at 96% relative humidity differed among the lines but was higher in the wet season crop (Table II). The moisture content showed the same trend as water content of steeped brown rice (Table I). Values previously reported for waxy rice starch granules ranged from 23.7 to 24.7% (Juliano *et al.*, 1969).

Residual protein differed significantly among the lines and between the two parents and between crops, but tended to increase with amylose content (Table II). These results agree with those of Horiuchi and Tani (1966). Reyes *et al.* (1965) found no correlation between residual protein and amylose

**Table II. Range and Mean of Physicochemical Properties of Starch of Selected IR841 Lines and Their Parents, 1970 Dry and Wet Seasons**

Property	Dry Season Crop					Wet Season Crop				
	Nine lines		Khao Dawk Mali	IR262		Eight lines		Khao Dawk Mali	IR262	LSD, 5% <sup>a</sup>
	Range	Mean				Range	Mean			
Final gel. temp., °C	66.5-78.5	73	67.5	73		64-77	71	66.5	69.5	1.5**
Blue value at 680 nm, A	0.127-0.336	0.231	0.180	0.286		0.126-0.286	0.196	0.168	0.306	0.014**
Mean granule size, μ	4.6-5.9	5.4	5.0	5.3		4.2-5.5	5.0	4.9	6.0	0.4**
Equilibrium moisture at 96% r.h., % wet basis	20.5-24.4	22.5	21.5	20.4		22.4-24.6	23.9	25.2	23.4	0.5**
Protein, % dry basis	0.09-0.51	0.27	0.10	0.41		0.14-0.63	0.28	0.16	0.61	0.04**
S <sub>20,w</sub> amylose, Svedbergs	4.9-6.6	5.8	6.2	5.4		4.9-6.4	5.5	5.7	5.4	0.8**
S <sub>20,w</sub> amylopectin, Svedbergs	73-229	128	112	85		76-237	139	109	86	21**
4-Day lintnerization loss, %	37.5-56.0	45.1	57.1	42.8		41.6-59.0	48.6	61.0	49.0	1.2**

<sup>a</sup> For (crop × line) mean, including the two parents.

**Table III. Simple Correlation Coefficients of Physicochemical Properties of Grain and Starch with Amylose Content and Final Gelatinization Temperature of IR841 Lines, 1970 Dry and Wet Seasons**

Property	Simple correlation coefficient <sup>a</sup> with			
	Amylose content		Final gelatinization temperature	
	Dry season	Wet season	Dry season	Wet season
Final gel. temp. of starch	-0.443	-0.316		
100-grain wt of brown rice	0.499	0.440	-0.731*	-0.612
Grain hardness, Kiya test	-0.318	-0.485	0.330	0.206
Grain hardness, grinding	-0.418	-0.567	+0.406	+0.097
Alkali spreading values, milled rice	0.576	0.373	-0.971**	-0.951**
Alkali clearing values, milled rice	0.439	0.249	-0.996**	-0.958**
4-Day lintnerization loss, starch	-0.095	-0.088	-0.836**	-0.885**
Water-soluble amylose at 100°C	0.955**	0.930**	-0.399	-0.175
Blue value of starch	0.980**	0.965**	-0.534	-0.312
Protein content of milled rice	-0.391	-0.025	0.541	-0.264
Protein content of starch	0.904**	0.966**	-0.233	-0.198
Water content of steeped brown rice	-0.975**	-0.949**	0.425	0.296
Moisture content of starch at 96% r.h.	-0.525	-0.968**	0.681*	0.116
Granule size of starch	0.295	0.030	0.016	-0.233
S <sub>20,w</sub> of amylopectin	-0.567	-0.628	0.834**	0.619
S <sub>20,w</sub> of amylose	0.487	-0.124	0.017	-0.382
Elongation ratio during cooking	0.726*	0.375	0.127	0.348

<sup>a</sup> Nine lines in the dry season and eight lines in the wet season.

content of starch from different varieties grown in different seasons and locations.

Molecular weights of amylose as indexed by S<sub>20,w</sub> values differed among the lines but each line had the same value in the two crops (Table II). Only three of eight lines differed in amylose S<sub>20,w</sub> values for the two crops. S<sub>20,w</sub> values for amylopectin showed a wider range and were higher than S<sub>20,w</sub> values of amylose. Four of the eight lines differed in amylopectin S<sub>20,w</sub> values between the two crops. These differences reflect true differences in molecular weights, since the two crops of each line had similar amylose content. The differences in amylopectin S<sub>20,w</sub> values among the lines also reflect true differences in molecular size since they are much greater than the observed concentration effect on S<sub>20,w</sub> of 20 Svedbergs. Reported S<sub>20,w</sub> values are 3.5 to 5.8 Svedbergs for amylose and 30 to 1400 Svedbergs for amylopectin (Juliano, 1967).

Weight loss after lintnerization for 4 days differed among the lines but showed a wider range of values than those of the parents (Table II). Lintnerization loss was higher in the wet season crop.

#### DISCUSSION

The amylose content and gelatinization temperatures of both the dry season and wet season crops of the IR841 lines were not significantly correlated (Table III). This verifies the reported general independence of these two starch properties (Beachell, 1967; Juliano *et al.*, 1969).

Grain weight and grain hardness were not correlated with amylose content and gelatinization temperature, except for a significant negative correlation between grain weight and final gelatinization temperature in one crop (Table III). Vidal and Juliano (1967) found similar hardness scores for three isogenic pairs of waxy and low amylose, nonwaxy rices. In contrast, conflicting correlations with gelatinization temperature were obtained with waxy and nonwaxy samples (International Rice Research Institute, 1966). Since Nagato and Kono (1963) found the best hardness distribution score for a variety that has high gelatinization temperature, Century Patna 231, in relation to other varieties with lower gelatinization temperature, grain hardness probably correlates with gelatinization temperature, a physical property of the starch. Presumably differences in gelatinization temperature of the starch are reflected in some properties of the whole endosperm, as shown by the use of the alkali test on milled rice to measure gelatinization temperature (Juliano *et al.*, 1969). In both crops, in all lines alkali spreading and clearing values were highly significantly correlated with gelatinization temperature but not with amylose content (Table III).

The significant negative correlation between lintnerization loss of starch granules and gelatinization temperature (Table III) indicates that gelatinization temperature reflects accessibility or porosity of the starch granule to solvents and hydrolyzing agents, including alkali, acid, α-amylase, and hot water (Juliano *et al.*, 1969). After 4 days' germination in

Table IV. Properties of Milled Rice of Two Lines Differing in Water-Soluble Amylose at 100°C

Property	IR841-49-1		IR841-28-2	
	Dry season	Wet season	Dry season	Wet season
Amylose, % dry basis	29.4	29.6	28.4	30.0
Water-soluble amylose at 100°C, %	12.3	10.2	14.0	13.3
Final gel. temp, °C	66.5	64	74	74
Protein, % dry basis	6.7	12.5	6.7	11.1
Amylograph setback viscosity, B.U.	+440	...	+190	...

light, two lines of similar protein (10.6–10.7%) and amylose (15.0–15.4%) contents showed different increases in free sugars. The line that had low gelatinization temperature of 68°C showed an increase of 93 µg of glucose, while the line with high gelatinization temperature of 76.5°C increased only by 37 µg of glucose. Although the difference in the increase in free sugars was related to difference in gelatinization temperatures (Reyes *et al.*, 1965), another factor may be actual differences in amylolytic activity in the germinating grain.

The water-soluble amylose at 100°C of milled rice and the blue value of starch were correlated only with amylose content of milled rice (Table III). But, two of the three high amylose lines and IR262 gave at least 2% lower values than the other high amylose sample. This observation confirmed previous findings that above a critical amylose content of about 30%, the linear relationship between water-extractable amylose and total amylose content does not hold because of *in situ* retrogradation of amylose (Juliano *et al.*, 1968a). The amylograph characteristics of two such high amylose lines differing in soluble amylose values at 100°C revealed that the line with low-soluble amylose value was more resistant to decrease in viscosity during cooking and had a higher setback value when cooled to 50°C than the other sample (Table IV). A high amylose variety, such as IR8, shows this amylograph setback character of over 300 Brabender units (B.U.) although its amylose content may vary from 27 to 33%. In fact IR841-28-2 had 30.0% amylose in the wet season crop but it still retained its high soluble amylose value (Table IV). Hence, low values for water-soluble amylose at 100°C may be used for identifying rice varieties that have 27 to 33% amylose. Such varieties are ideal for noodle-making because their cooked grains readily harden and they resist disintegration if they are overcooked. Varieties with 25 to 27% amylose, which show maximum values for water-soluble amylose (Juliano *et al.*, 1968a), should find general acceptance in tropical Asia since their cooked grains are flaky but not very hard. We propose to call this fourth amylose type as intermediate-high, in addition to low (<20%), intermediate (20–25%), and high (>27%).

Protein content, amylose content, and gelatinization temperature were not significantly correlated (Table III). The absence of correlation between these three properties has been previously reported (Juliano *et al.*, 1965). However, the protein content of the purified starch was positively correlated with the amylose content but not with the gelatinization temperature of the starch. Because of the association of such protein as bound adenosine diphosphate glucose-starch glucosyltransferase with the nonwaxy starch granule and its relationship to amylose content (Baun *et al.*, 1970), this residual protein may include enzymes complexed or bound to amylose.

The water content of steeped brown rice was negatively correlated with amylose content, but it was not correlated with gelatinization temperature (Table III). Moisture content of starch at 96% relative humidity, however, was negatively correlated with amylose content only in the wet season crop.

Juliano (1964) reported that waxy rices have a higher moisture content than nonwaxy rices above 75% relative humidity. That may explain why waxy rice seeds lose their viability during storage faster than nonwaxy grains. Interestingly, Juliano *et al.* (1969) did not find a correlation between gelatinization temperature and moisture content of starch at 96% relative humidity for five waxy rice lines. The lower absolute density of waxy starch in comparison with nonwaxy starch (Reyes *et al.*, 1965) and the presence of micropores in the waxy rice starch granule (Watabe and Okamoto, 1960) may explain the greater capacity of low amylose rices to hold water.

The size of the starch granule was not correlated with its amylose content and gelatinization temperature. However, the  $S_{20,w}$  of amylopectin was positively correlated with gelatinization temperature in the dry season crop but it was not correlated with amylose content. The  $S_{20,w}$  of amylose was not correlated with either amylose content or gelatinization temperature. Our previous studies on lines differing in either amylose content or gelatinization temperature showed that an increase in molecular weight of amylopectin or a decrease in molecular weight of amylose, as indexed by intrinsic viscosity, was correlated with a lower gelatinization temperature or a higher amylose content (Juliano, 1970; Juliano *et al.*, 1969). Differences in amylose content were more consistently related to differences in amylose viscosity than to differences in amylopectin viscosity, whereas differences in gelatinization temperature were better related to differences in amylopectin viscosity. Starch fractions differing widely in intrinsic viscosity also differed in  $S_{20,w}$  values. Since the trend observed for molecular size of amylopectin in relation to gelatinization temperature in the lines differing in both amylose content and gelatinization temperature was the opposite of that found previously for lines differing only in gelatinization temperature, and since no trend was found for molecular size of amylose, no relationship between molecular size of starch fractions and differences in amylose content or gelatinization temperature may be expected among different varieties, as reported by Reyes *et al.* (1965).

Although the elongation ratio was not significantly correlated with gelatinization temperature, it was positively correlated with amylose content in the dry season crop. A contributing factor was the narrow range of elongation ratios exhibited by the lines.

Most physical properties of the grain (except water content of steeped rice) are probably better correlated with gelatinization temperature, a physical property of the starch, than with amylose content, a chemical property. Properties of the cooked rice, however, are better correlated with amylose content than with gelatinization temperature, since most of the physical structure of the raw starch granules changes during cooking. But, in correlation studies on rice properties, only the properties that are correlated in the same two crops of the samples should be considered valid because environment affects such properties as grain weight; grain hardness; water

content of steeped rice, and of starch at 96% relative humidity; amylose content, blue value, and starch-iodine blue values at 100°C; gelatinization temperature, alkali spreading, and clearing values and lintnerization loss of starch; protein content; and  $S_{20,w}$  of amylopectin (Tables I and II).

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## Distribution of Phytate and Nutritionally Important Elements among the Morphological Components of Cereal Grains

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Kernels of rice, wheat, and corn, a high lysine and a commercial hybrid variety, were dissected into the major components, including germ, endosperm, and pericarp. The whole kernels and fractions were analyzed for total P, phytate P, K, Mg, Ca, Fe, Cu, Zn, and Mn. Phytate phosphorus made up more than 80% of the total phosphorus and, in the case of corn, nearly 90% of phytate was in the germ. The concentration of phytate in rice and wheat germ was high but the major proportion in the total kernel

existed in the outer layers. In general the highest concentrations and proportions of the mineral elements of corn were in the germ. There were no significant differences between the high lysine and control corn samples except that the high lysine corn contained a higher concentration of potassium. Although the highest concentrations of some trace elements were found in germ fraction of rice and wheat, the highest proportions were present in the pericarp and aleurone layers.

Cereal grains constitute a major source of human food and animal feeds. Although they contribute most significantly as a source of carbohydrate and to a less extent as protein, their potential contribution of minor nutrients, including the trace elements, is frequently overlooked. The degree and type of milling used in preparation of human food has an important effect upon the concentration of the minor nutrients available to the consumer.

In evaluating the nutritional value of a foodstuff one must consider not only the concentration of a particular nutrient but also its biological availability to the animal that consumes it. Inositol hexaphosphate, phytate, strongly binds several mineral elements and from the nutritional point of view is deleterious in that it renders zinc unavailable (O'Dell, 1969). There is also evidence that it decreases the availability of iron (Sharpe *et al.*, 1950), magnesium (Roberts and Yudkin, 1960), and calcium (Harrison and Mellanby, 1939). Phytate is found almost exclusively in plants and primarily in seeds.

Cereal grains and oil seeds are particularly rich sources of phytate. For these reasons it is important to know the distribution of phytate and the nutritionally important mineral elements in plant seeds that serve as sources of human and animal food.

The purpose of this study was to determine the distribution of nutritionally significant substances among the dissectable components of the economically important cereal grains. Because of its potential significance in human nutrition, high lysine corn was compared to a commercially available hybrid variety of corn grown at the same location and during the same season.

## MATERIALS AND METHODS

**Source and Dissection of Seeds.** The corn samples were produced by the Pioneer Hi-Bred Corn Co. near Johnston, Iowa. The high lysine corn contained 10.2% crude protein and 0.44% lysine, air dry basis; the commercial hybrid, which served as the control, contained 8.1% protein and 0.30% lysine. The wheat was a soft (Arthur) variety and was produced locally by the Agricultural Experiment Station. The

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