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Evaluation of Starch, Soluble and Insoluble Non-starch Polysaccharides and Metabolizable Energy of 15 Cultivars of Iranian Wheat

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ABSTRACT

Dry matter, crude protein, dry matter, metabolizable energy, starch and soluble and insoluble non-starch polysaccharides (NSP) of 15 wheat cultivars grown extensively in Iran were determined. The metabolizable energy content of the samples, were evaluated by using 270 Ross-308 broiler chicks, with two replicates per cultivar and 9 chicks per replicate. The regression coefficients of the apparent metabolizable energy (AMEn) with starch, soluble NSP and protein contents were determined. There were significant differences amongst cultivars in metabolizable energy content ($P < 0.01$). The Marvdasht cultivar had the highest (3062 Kcal/g) and the Kavir cultivar had the lowest (1893 Kcal/g) metabolizable energy, but the reverse was true for the soluble and total NSP contents. The AMEn was not significantly correlated with starch content (R2 = 0.12) and the degree of variation in starch content was not responsible for the wide range of AMEn values obtained. There were significant differences in crude protein, total NSP, insoluble NSP and soluble NSP contents. Crude protein content ranged from 10.2 to 14.0% and the ash content ranged from 1.52 to 1.82%.

Key Words: Wheat; Soluble NSP; Insoluble NSP; AME; Starch

INTRODUCTION

Wheat is the most widely cultivated crop in the world and has an important economical role in agriculture and industry. Although wheat takes second place to maize on a worldwide basis as a feed grain for livestock, its importance as a raw material in compound animal feeds varies markedly according to geographical location. For example 75% of wheat used for animal nutrition in the world, is consumed in Europe. European countries produce more than 120 million tons of wheat annually, of which more than 40% is used in poultry nutrition. In some countries, especially in Eastern Europe, wheat is the only grain in broiler ration (Pirgozliev *et al.*, 2003). In Denmark, wheat may constitute as mach as 70 - 80% of the finisher diet for broiler chickens (Steenfeldet *et al.*, 1998). In Iran, imported corn grain is the most widely used energy source in broiler rations; however, whenever the quality is not satisfactory or the price is high, wheat or wheat middlings are used. On the other hand, a large amount of wheat is produced that is not suitable for bread making and is fed to animals, especially the poultry. Under these conditions, wheat inclusion, in finisher ration of broilers, could be up to 70%. Wheat grain typically contains 67% starch, 13% protein and about 8% arabinoxylan (1.8% soluble fraction), 0.8% β-glucan (0.4% soluble fraction) and 2% absolutely insoluble cellulose (Englyst, 1989; Kim *et al.*, 2003). Although traditionally regarded as having a moderately uniform nutritional value, evidence is accumulating that there is considerable variability in the apparent metabolizable energy (AME) values, particularly with young birds (Austin, 1999). Non-starch polysaccharides especially soluble ones have anti-nutritional effects on young broilers and adversely affect the nutritive value of wheat. The negative effects can particularly be attributed to the water-soluble arabinoxylans in the endosperm, which increase the digesta viscosity and reduce the absorption of nutrients in the digestive tract (Choct *et al.*, 1995; Steenfeldet *et al.*, 1998). There is considerable variation among wheat cultivars in starch and NSP contents (Ordaz-Ortiz & Saulnier, 2005). For example in 21 cultivars of Australian wheat, starch content ranged from 59 - 72% (dry matter basis; Austin *et al.*, 1999) and total NSP content of Australian wheat (n = 81) ranged from 8 to 15% (Choct *et al.*, 1999). This wide variation in the chemical composition of wheat results in variation in the amount of available nutrients for monogastric animals (Anderson & Bell, 1983; Choct *et al.*, 1999; Mollah *et al.*, 1983), which affects production efficiency, however, the published data for broiler chickens are not consistent. For example the feed conversion in broilers between 1988 - 1989 in U.K was 2.06 and 2.11, but this small difference in FC increased the feed cost by about £12 million annually (Wiseman & Inbor, 1995; Smits & Annison, 1996). The difference has been attributed to the chemical composition, the level of inclusion in the diet and the ability to digest, absorb and utilize the feed components (Wiseman & Inbor, 1995). Although it is accepted that presence of NSP especially soluble NSP reduces starch, lipids and proteins digestibility (Smits *et al.*, 1998; Wiseman *et al*, 2000), research on the negative effects of NSP in young chicks, has recently received more attention (Wiseman *et al.*, 2000). It is known that the cultivar (Anderson & Bell, 1983; McNab, 1996), growing region (Dusel *et al.,* 1997), season (Choct *et al.*, 1999) and post-harvest storage conditions (Jood *et al.*, 1993; Kim *et al.*, 2003) influence the chemical composition of wheat and hence the utilizable energy content of wheat (Austin *et al.*, 1999; Kim *et al.*, 2003). The purpose of the present investigation was to determine the starch, soluble and insoluble non-starch polysaccharides and protein content of 15 Iranian wheat cultivars and the relationship between these components and the AMEn in young broilers.

MATERIALS AND METHODS

Samples from 15 wheat cultivars grown widely in Iran (Tables I & II) were obtained from wheat fields of 'The Seed and Plant Institute, Karaj, Iran. Total starch content was determined using the Megazyme Total Starch Assay Kit (Megazyme International Ireland Ltd., Bray Business Park, Bray Co., Wicklow, Ireland). This method has been adopted by AOAC (method 996.11) and AACC (method 76.13). This method allows the measurement of total starch in most cereal products (natural or processed). Starch hydrolysis proceeds in two phases; in phase I, starch is partially hydrolyzed and totally solubilized and in phase II, dextrins are hydrolyzed to glucose by amyloglucosidase. After hydrolysis of starch, the soluble materials are treated with glucose oxidase, peroxidase and 4-aminoantipyrine and the absorbance is read at 510 nm in a spectrophotometer. Soluble and insoluble NSPs were determined by using a modified method based on AOAC method 991.43 "total, soluble and insoluble dietary fiber in foods" and AACC method 32 - 07 "determination of soluble, insoluble and total dietary fiber in foods and food products". This method is the simplified modification of the AACC soluble/insoluble dietary fiber method, 32 - 21. Briefly, 1 g of dried wheat sample (in duplicate) is subjected to sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase. Then insoluble NSPs (INSPs) are filtered and the residue is washed with warm distilled water. Combined solution of the filtrate and water washings are precipitated with 4 volumes of 95% ethanol for soluble NSPs (SNSPs) determination. The precipitate is then filtered and dried. Both SNSPs and INSPs residues are corrected for protein, ash and blank for the final calculation of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) values. Dry matter, crude protein (N × 5.83) and ash contents were measured according to AOAC (1990). The wheat samples were finely ground and mixed with 0.5% Titanium oxide, which was used as a marker for determination of the apparent metabolizable energy. Twenty-four days old Ross broiler chicks (18 per cultivar & 9 per cage) were housed in metal cages. Each cage was provided with an individual feeder, drinker and plastic covered tray for collection of feces. Birds were fed with the experimental wheat for 4 days and the feces were collected twice daily at 12-h intervals. The fecal samples from each replicate were mixed and dried in an oven at 65°C until reaching a constant weight. The gross energy values of the wheat and feces were measured using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Loughborough, UK). The concentration of Titanium oxide in the feces and wheat samples was measured (Short *et al.*, 1996) and the apparent metabolizable energy calculated (Choct *et al.*, 1995) as follows:

$$AME/g\ feed = GEfd - \{(Tofd/Tofc) \times GEfc\}$$
$$AMEn/g\ feed = AME/g\ feed - 8.73\{Nfd - (Tofd/Tofc) \times Nfc\}$$

Which

Tofd = Titanium oxide/g feed; Tofc = Titanium oxide/g feces; GEfd = Gross energy/g feed; GEfc = Gross energy/g feces; Nfd = Nitrogen/g feed; Nfc = Nitrogen/g feces.

Statistical analysis. Statistical analysis was performed using the Proc GLM of the SAS (1996) according to the following general model:

$$Yi = \mu + \acute{a}i + \epsilon i$$

Where

Yi is the observed dependent variable; μ the overall mean; *ái* the effect of wheat cultivar and *єi* the random error. The means were compared by 'Tukey Studentized Range Test' and the correlation coefficients of AMEn with starch, crude protein and SNSPs were calculated.

RESULTS AND DISCUSSION

The chemical composition of the wheat cultivars is presented in Tables I and II. There were significant differences in the mean ash content of the wheat cultivars ($P < 0.01$), ranging from 1.52 to 1.82%. It is un-likely that this variation reflected the actual mineral content, but it may be due to differences in the soil content of the samples. Starch content of wheat cultivars tended to differ ($P = 0.15$). Marvdasht cultivar (73.4%) had the highest but Sardari and MV17 had the lowest (70.5%) starch content. Starch is the major energy–yielding component of wheat and it is likely that any variation in the nutritive value is associated with the differences in starch content and its digestibility. There was a significant effect of the cultivar on AME and AMEn contents (Table II). The relationship between AMEn and starch content is presented in equation [1]:

AMEn = -4504.97 + 99.08 × starch% (R2 = 0.12; P = 0.06; CV = 11.62)…………………………….…. [1]

Starch content explained a very small portion of the total variation in AMEn contents. The results are in agreement with those of Kim *et al*. (2003), Wiseman *et al*.

Table I. Dry matter, ash, starch, SNSP, INSP and TNSP contents of 15 Iranian wheat cultivars

Wheat cultivars	Dry matter%	Ash%	Starch%	Soluble NSP%	Insoluble NSP%	Total NSP%
Alvand	91/3	1.80 [a]	73	1.45 [def]	11 [bcd]	12.4[cd]
Atrak	91/7	1.57 [bc]	72.5	1.90 [bc]	9.4 [ef]	11.3[d]
Chenab	91/9	1.78 [a]	72.5	1.75 [bcd]	11 [bcd]	12.8 [bcd]
$Darab_2$	91/9	1.58 [bc]	72.3	1.95 [bc]	12.3[ab]	14.3 [a]
Dez	91/9	1.71 [ab]	71.4	2.05 [ab]	12.1 [abc]	14.1 [ab]
Hirmand	91/7	1.60 [bc]	71	1.70 [cde]	10.7 [cde]	12.4[cd]
Kavir	92	1.52 [c]	71.4	2.25 [a]	12.4 [ab]	14.6 [a]
Mahdavi	92/1	1.59 [bc]	72	1.40 [ef]	9.9 [de]	11.3 [d]
Marvdasht	91/5	1.53 [c]	73.4	1.30 [f]	8.3 [f]	9.6 [e]
MV_{17}	92/1	1.57 [bc]	70.6	1.70 [cde]	13.2 [a]	14.9 [a]
Pishtaz	91/7	1.62 [bc]	71	1.60 [cdef]	10.5 [de]	12.1 [d]
Roshan	91.8	1.82[a]	72.6	1.65 [cde]	9.7 [de]	11.4 [d]
Sardari	91/5	1.79 [a]	70.4	1.85 [bc]	12 [abc]	13.9 [abc]
Shahryar	92/6	1.70 [ab]	71.6	1.70 [cde]	12.3 [ab]	14[ab]
Toos	92/3	1.81 [a]	72.1	1.70 [cde]	12 [abc]	13.7 [abc]
P-value	ns	0.0001	ns	0.005	0.0001	0.0001
CV	0.57	0.85	1.31	8.10	5.41	4.97
MSE	0.52	0.02	0.95	0.14	0.60	0.64

Column data with different letters are statistically different (p<0.05, Duncan test).

Table II. Crude protein, AME and AMEn contents of 15 Iranian wheat cultivars AMEn Cultivar Crude protein percent AME (kcal/g) (kcal/g)

Wheat cultivars	Crude protein%	AME(Kcal/g)	AME_n(Kcal/g)
Alvand	10.9 [bc]	2822 [abc]	2812 [abc]
Atrak	14[a]	2411 [efg]	2407 [efg]
Chenab	10.8 [bc]	2641 [cde]	2635 [cde]
$Darab_2$	9.5 [c]	2394 [def]	2388 [def]
Dez	10.5 [bc]	2336 [fg]	2330 [fg]
Hirmand	13.6 [a]	2662 [cde]	2654 [cde]
Kavir	10.4 [bc]	1896 [h]	1893
Mahdavi	13.3 [a]	2946 [ab]	2937 [ab]
Marvdasht	13.8 [a]	3072 [a]	3062 [a]
MV_{17}	11.2 [bc]	2682 [bcde]	2671 [bcd]
Pishtaz	13.7 [a]	2908 [abc]	2900 [abc]
Roshan	13.6 [a]	2729 [bcd]	2722 [bcd]
Sardari	12 [ab]	2245 [g]	2237 [g]
Shahryar	10.6 [bc]	2677 [bcde]	2667 [bcde]
Toos	10.2 [bc]	2908 [abc]	2904 [abc]
P-value	0.0003	0.0001	0.0001
CV	6.99	4.46	4.4
MSE	0.83	117	116

Column data with different letters are statistically different (p<0.05, Duncan test).

(2000) and Austin *et al.* (1999). Variation in starch digestibility may be a factor contributing to this failure to predict AMEn (Wiseman & Inborr, 1995; Wiseman, 2006). Wheat starch digestibility has been studied extensively in poultry (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Wiseman *et al.*, 2000; Kim *et al.*, 2003; Kim *et al.*, 2005), with the coefficient of digestibility ranging from 0.699 to 0.999. It is not possible at this stage to state whether this poor correlation is due to the intrinsic properties of the starch itself, or it is a consequence of another component such as anti-nutritive factors present in wheat. The average NSP content of all wheat samples was 12.8% (1.7% soluble & 11.1% insoluble) and there were significant differences in total NSP, insoluble NSP and soluble NSP (Table I) of the wheat cultivars. Increased content of NSPs may have been the cause of the low AME and AMEn values. The Kavir cultivar had the highest SNSPs and lowest AME and AMEn (2.25%, 1896 & 1893, respectively) and Marvdasht had the lowest SNSPs and the highest AME and AMEn (1.30, 3072 & 3062, respectively). Equations [2], [3] and [4] show the relationship of AMEn with soluble (SNP), insoluble (INSP) and total NSPs (TNSP).

AMEn/g = 4468-1083.4 × SNSP% (R2 = 0.82; P = 0.0001; CV = 5.1)……………………………..…… [2]

AMEn/g = 3765-103.6 × INSP% (R2 = 0.21; P = 0.01; CV = 11.0)……………………………………. [3]

AMEn/g = 4095-115.34 × TNSP% (R2 = 0.32; P = 0.001; CV = 10.2)………………….………… [4]

There is considerable evidence that some cell wall NSP play an important role in poultry nutrition (Steenfeldet *et al.*, 1998). The soluble NSPs in the endosperm cell wall of wheat have anti-nutritional effects in broiler chickens (Steenfeldet *et al.*, 1998). More than 50 g of total pentosans per kg in low AME Australian wheat had an anti-nutritive effect in poultry (Kim *et al.,* 2003). Soluble NSPs are capable of increasing the digesta viscosity and consequently reduce the digestibility of fat, starch and protein and reduce the absorption of nutrients (Choct *et al.*, 1995; Steenfeldet *et al.,* 1998; Smits & Annison, 1996). Annison (1993) and Choct *et al.* (1995) showed that the variable energy content of certain Australian wheat was directly attributed to variations in the concentration of NSPs. Annison (1991) found a strong negative correlation between wheat AMEn and soluble NSPs. Crude protein content (Table II) ranged from 9.5 (Darab cultivar) to 14.0% (Atrak cultivar) with a mean value of 11.9% and was significantly affected by the cultivar. Equation [5] shows the relationship between AMEn and the crude protein content (CP):

AMEn/g = 1865 + 62.9 × CP% (R2 = 0.11; P = 0.08; CV = 11.7)…………………………..……….. [5]

The very small coefficient of determination indicated that crude protein content did not have a considerable effect on wheat AMEn. Table III show the overall correlation coefficients between various chemical components of the wheat samples. There was a small and positive correlation between AMEn and the crude protein content (r = 0.34). The correlation coefficients of the starch content with INSP and TNSP were negative (r = -0.60; P < 0.01). The negative correlation between crude protein and total starch has also been reported by other investigators (Choct *et al.,* 1999; Kim *et al*., 2003; Wiseman & Inbor, 1995). The finding that the CP content was inversely correlated with total starch content might have implications in plant breeding programs aimed at animal nutrition. There were significant negative correlations (P < 0.01) between AMEn and soluble NSPs (-0.92), AMEn and insoluble NSPs (-0.46) and AMEn and

Table III. Overall Pearson's correlation coefficients between chemical composition of 15 wheat cultivars

	Starch	Protein	SNSP	INSP	TNSP	Ash	AME	AME_n
Starch	1							
P-value	0.0							
protein	-0.08	1						
P-value	0.67	0.0						
SNSP	-0.42	-0.34	1					
P-value	0.22	0.07	0.0					
INSP	-0.61	-0.7	0.53	1				
P-value	0.0004	0.0001	0.0025	0.0				
TNSP	-0.62	-0.68	0.65	0.99	1			
P-value	0.0003	0.0001	0.0001	0.0001	0.0			
ash	0.074	-0.27	-0.1	0.13	0.096	1		
P-value	0.6	0.15	0.6	0.51	0.61	0.0		
AME	0.34	0.32	-0.92	-0.46	-0.57	0.14	1	
P-value	0.064	0.08	0.001	0.011	0.0011	0.47	0.0	
AME_n	0.34	0.32	-0.92	-0.46	0.57	0.14	.9997	1
P-value	0.63	0.08	0.0001	0.011	0.0011	0.47	0.0001	0.0

total NSPs (-0.57). Arabinoxylans (AX) are the principal endosperm and aleurone cell wall component (Annison, 1991) in wheat (50 - 80 g/kg DM). In the wheat endosperm cell wall, AX constitute as much as 880 g/kg of NSP of which one-third is soluble in water, but this small amount of soluble NSP negatively affects the nutritive value of wheat. The present experiment highlighted the wide variations in the chemical composition of Iranian wheat cultivars grown in Karaj city. Variations in AMEn and crude protein contents are very important in feed formulation for poultry, especially for the young broilers. Since the cultivar, growing season, precipitation level and storage conditions affect the chemical composition of wheat, more extensive investigation with a larger number of cultivars are recommended. More experiments should be carried out for evaluation of *in vivo* and *in vitro* digestibility of starch and for determination of the relationship between AME, AMEn and digestible starch and the factors affecting starch digestibility.

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