CHAPTER 5 – EXPERIMENT 2

THE EFFECT OF NON STARCH POLYSACCHARIDES AND XYLANASE SUPPLEMENTATION ON THE NET ENERGY VALUE OF BROILER DIETS

5.1 Introduction

One of the most important factors that impact on the quality of wheat is its content of soluble, viscous non-starch polysaccharides (NSPs) (Choct and Annison, 1990; Annison, 1991; Bedford, 1996). Many other factors are also involved, including quantity and quality of protein and starch. There is an enormous literature (Annison *et al.*, 1992; Choct *et al.*, 1992; Bedford, 1996; Annison *et al.*, 1997) on the feeding value of wheat in poultry rations and while generally considered to be high, the AME values of wheats assayed in broiler chickens have proved (Annison, 1991, 1992; Choct *et al.*, 1992) to be extremely variable. Sibbald and Slinger (1962) found that the AME of Canadian wheats ranged from 12.32 to 16.57 MJ/kg while Schumaier and McGinnis (1967) showed a range of 12.05 to 13.47 MJ/kg AME for United States wheats. Wiseman and Inborr (1990) documented a range of 13.01 to 15.24 MJ/kg AME for the United Kingdom wheats whereas Mollah *et al.* (1983) and Rogel *et al.* (1987) found that Australian wheats ranged from 10.5 to 15.9 MJ/kg AME.

Recent studies (Choct and Annison, 1990; Annison, 1991; Choct *et al.*, 1995) have shown that a strong negative correlation exists between the AME values of wheat and the levels of water-soluble NSPs, which are predominantly arabinoxylans (Annison, 1991). When isolated and incorporated into broiler diets, wheat arabinoxylans cause a depression in AME and chick growth (Choct and Annison, 1990). Furthermore, it has been demonstrated that enzyme supplementation of wheat-based broiler diets is beneficial, indicating that wheat NSPs are deleterious to broiler chicken performance. The mechanisms by which NSPs depress bird performance include the ability of these polysaccharides to increase digesta viscosity (Choct and Annison, 1992b), their

interaction with the gut microflora (Choct and Annison, 1992b) and their effect on endogenous energy losses (Classen and Campbell, 1990). The details of these proposed mechanisms have been discussed in Chapter 2. It is, however, worth reiterating that one of the most obvious manifestations of the anti-nutritive effect of soluble NSPs is poor energy metabolism by the bird. This has in the past been measured by the AME bioassay, which is, however, unable to estimate the amounts of energy utilised by the bird. It is hypothesised that the effect of soluble NSPs on the NE value of feedstuffs for poultry may be more pronounced than that on the AME value because of: (1) the digestive system of the bird would have to work harder than usual to cope with a highly viscous gut environment, and (2) the proliferation of fermentative organisms in the small intestine of chickens is energy-inefficient and detrimental to the bird (Choct *et al.*, 1996). Thus, the current study was conducted to examine whether (a) consumption of diets with an elevated quantity of soluble NSPs would increase energy loss as heat production and VFAs in the excreta, and (b) enzymatic supplementation would enhance depolymerisation of the NSPs *in vivo,* and thus alleviate these losses.

5.2 Materials and Methods

Section 3.2 provided a general description of the materials and methods used throughout this project. The results were calculated and analysed for statistical significance according to procedures described under Section 3.7.

5.2.1.Experimental length

Experiment 2 ran for 14 days. The first 3-day period was allowed for the birds to adapt to the experimental diet as well as to adjust to the closed circuit calorimeter environment, in which they were confined for 20-22 h/d. About 2-4 h each day were allowed to replenish the feed and water containers and to readjust the system for the next run. On days 4 and 5, the birds were deprived of feed to establish their basal metabolism. A period of 4 days was then allowed for the birds to readjust to the diet. The following days (10 to 14) involved quantitative excreta collection. On the 14th day,

all the birds were killed by cervical dislocation. Intestinal contents were collected for digesta viscosity and VFA analysis.

5.2.2 Experimental diets and design

Wheat-based diets which included 4% NSPs were formulated with or without a commercial xylanase product (Biofeed Wheat, Novo Nordisk Bioindustrial Pty Ltd, Australia) as shown in Table 5.1. A soluble wheat NSP product was prepared from a wheat-milling by-product as described by Choct and Annison (1992a). It contained 78.5% soluble NSPs with two pentosan-rich fractions (water-extractable, WEP and alkali- extractable, AEP) with some residual starch, protein and minerals. The diets were cold-pelleted and were offered *ad libitum*. The birds had free access to clean water. Two replicates of two birds each were used for each diet. Feed intake and excreta output were recorded separately on each of days 10 to 14 in order to determine AME and HP on a daily basis.

Table 5.1 Diet composition

Ingredient (g/kg)	**Wheat + NSPs (- Enzyme)**	**Wheat + NSPs (+ Enzyme)**
Wheat(12% CP)	760	760
Dicalcium phosphate	20	20
Limestone	11	11
Salt	5	5
Choline chloride (50%)	2	2
Premix*	5	5
NSP product	40	40
Casein (Dried)	150	150
DL Methionine	7	7
Xylanase	NO	YES

*The active ingredients contained in each kg of the vitamin-mineral premix were as follows: retinol, 3.03 mg; cholecaliferol, 0.09 mg; all-*rac*-a-tocopherol acetate, 20 mg; menadione, 6.3 mg; riboflavin, 8 mg;

pyridoxine hydrochloride, 5 mg; biotin, 0.01 mg; niacin, 30 mg; Fe, 20 mg; Cu, 5 mg; I, 1 mg; Co, 0.3 mg; Se, 0.5 mg; Mo, 0.16 mg; cyanocobalamin, 0.15 mg.

5.3 Results

All performance data are shown in Table 5.2. The enzyme supplement did not have a significant effect on the feed intake, weight gain or FCR of the birds. Although there was a 13.6% decrease in feed intake, a 14.3% increase in weight gain, a 23.4% increase in body weight and a 22.7% decrease in FCR in birds fed the enzyme supplement.

Table 5.2 The effect of soluble NSPs and xylanase supplementation on bird performance

	WHEAT + NSPs (- enzyme)		WHEAT + NSPs (+ enzyme)		
Measure	**Mean**	**SE**	**Mean**	**SE**	**P-value**
Feed intake (g/kg b wt/d)	150.9	8.5	117.6	6.0	0.086
Feed intake (g/bird/d)	122.5	6.7	105.9	2.3	0.097
Gain (g/bird/d)	55.8	3.3	63.8	1.8	0.240
Overall mean bird wt (g)	777	24	959	50	0.243
FCR	2.2	0.08	1.7	0.03	0.131

The energy-related measurements are presented in Table 5.3. The respiratory quotient (RQ) was not affected by enzyme supplementation. Daily heat production per kg body weight was also not affected although there was a numerical decrease of 11.0% associated with the enzyme supplementation. Heat increment, on the basis of MJ/kg body wt/d was markedly ($P<0.01$) reduced by enzyme supplementation, whereas differences in HI on the basis of MJ/kg feed were insignificant ($P>0.05$). AME and NE increased significantly ($P<0.01$) with enzyme supplementation.

Table 5.3 The effect of soluble NSPs and xylanase supplementation on energy utilisation

	WHEAT + NSPs (- enzyme)		WHEAT + NSPs (+ enzyme)		
Measure	**Mean**	**SE**	**Mean**	**SE**	**P-value**
RQ	0.995	0 014	1.006	0.02	0.772
HP (MJ/kg b wt/d)	0.91	0 013	0.81	0.02	0.103
HI (HP-FH*) (MJ/kg b wt/d)	0.20	0.01	0.13	0.01	0.004
HI (MJ/kg feed)	1.28	0.11	1.17	0.09	0.402
AME (MJ/kg feed)	10.5	0.06	14.8	0.07	0.003
AME-HP (NE: MJ/kg feed)	4.7	0.23	7.5	0.16	0.008

*FH-Fasting heat

Table 5.4 summarises the effect of enzyme supplementation on digesta viscosity along the intestinal tract of broilers. The enzyme reduced digesta viscosity (mPa.s) from 61.5 to 2.5 in the duodenum ($P<0.05$), from 189.2 to 4.7 in the jejunum ($P<0.01$) and from 360.5 to 7.8 in the ileum ($P<0.01$).

Energy loss as VFAs in the excreta was calculated by measuring the VFA concentrations in the samples and using a heat of combustion value for each type of VFA as described in Chapter 3. On a 4-day average basis, the energy loss as VFAs was 101.5KJ/chamber/d for birds fed the NSP-enriched diet compared with 34.3KJ/chamber/d for those fed the same diet supplemented with enzyme (Table 5.5). However, while the difference was not statistically significant, there was a numerical difference of 66.2% in favour of the enzyme supplemented diet.

Table 5.4 The effect of Wheat + NSPs (-Enzyme) or Wheat + NSPs (+Enzyme) on Gut Viscosity of the broiler chickens

	Mean viscosity (mPa.s)		
Diet	**Duodenum**	**Jejunum**	**Ileum**
Wheat + NSPs (-Enzyme)	61.5 ± 7.2	189.2 ± 9.8	360.5 ± 16.0
Wheat + NSPs (+Enzyme)	2.5 ± 1.1	4.7 ± 0.4	7.8 ± 2.1
P-Value	0.015	0.003	0.002

Table 5.5 The effect of Wheat + NSPs (-Enzyme) or Wheat + NSPs (+Enzyme) on Volatile Fatty Acid (VFA) Energy in the excreta of broiler chickens

	DIET	
Mean total VFA energy (KJ/chamber/d)	**Wheat + NSPs (-Enzyme)**	**Wheat + NSPs (+Enzyme)**
Day 11	65.2	34.9
Day 12	121.0	42.9
Day 13	152.2	37.5
Day 14	124.4	32.6
4-day mean	101.5	34.3
SE	17.4	2.4
P-value	0.1603	

5.4 Discussion

The previous study (Chapter 4) indicated that broiler chickens may lose substantial amounts of energy as volatile fatty acids in the excreta. This was the first time to the author's knowledge that the VFA concentrations in the excreta of poultry had been measured and their energy values calculated. The inference was that NSPs were the major factors responsible for the energy loss due to excreta VFAs. The current experiment was therefore conducted to examine whether (a) addition of isolated soluble

NSPs in a wheat-based diet would increase energy loss as heat increment and as VFAs in the excreta, and (b) enzymatic supplementation would enhance depolymerisation of the NSPs *in vivo,* and thus alleviate these losses.

Exogenous enzymes appropriately targeting the substrates have been shown to increase the efficiency of energy utilisation by birds, perhaps by eliminating the adverse effect of viscous NSPs on nutrient digestion and absorption in the gut (Classen and Bedford, 1991; Bedford, 1997; Choct, 1997; Marquardt, 1997). It is generally conceded (Choct and Annison, 1992a, b; Choct et al., 1996) that the anti-nutritive activities of NSPs are related largely to: (i) their ability to increase the thickness of the digesta which in turn hinders effective mixing of digestive enzymes and their substrates, (ii) increasing endogenous secretion of protein, lipids, electrolytes and water (Johnson and Gee, 1981), and (iii) changing the balance of the gut microflora (Choct *et al.*, 1996). The viscous nature of NSPs depends on their molecular size and characteristics (Classen and Bedford, 1991). Cleaving NSPs both *in vitro* and *in vivo* with enzymes can largely eliminate their anti-nutritive effects on nutrient digestion and absorption in broiler chickens (Choct *et al.*, 1994, 1996). The current study showed that the heat increment was markedly ($P<0.01$) reduced by enzyme supplementation, and that the NE value of the diet was significantly ($P<0.01$) improved. However, as with the experiment reported previously, any conclusions were drawn on only 2 replicates of 2 broiler birds and these may not be real differences.

The current AME system, for the evaluation of the energy content of poultry feed is not capable of measuring energy loss as gases, ammonia and methane, and VFAs, since the method used in the current experiment involved drying the excreta under force-draught. Preliminary data in Chapter 4 indicated that chickens might lose considerable amounts of VFAs in the excreta and that the extent of that loss appeared to depend on the type of cereal grain used in the diet. In the present study, depolymerisation of the NSPs by an exogenous enzyme appeared to reduce energy loss as VFAs in the excreta. Although the energy lost in VFAs was reduced from 101.5KJ in birds fed the NSP-enriched diet to 34.3KJ in those supplemented with xylanase, the difference was statistically not significant. This was due to the extreme variability in the VFA data over the four collection days and the small number birds used in the experiment. For instance, the

energy lost as VFAs on the first day on the diet without xylanase was only about half that on each of the subsequent days. This could indicate that the three-day adaptation before the excreta collection was not long enough for the gut microflora of the birds to become fully adapted after the introduction of the high-NSP diet, although this is unlikely.

The effectiveness of the enzyme in reducing the viscosity of the digesta in the duodenum, jejunum and ileum was clearly demonstrated, and it is presumed that this was due to the depolymerising effect of xylanase on the NSPs (Bird, 1997; Marquardt, 1997). However, because of the impure nature of several enzyme products, the effect could have been as a result of other enzyme contaminants, such as lipases and proteases, acting on other dietary functions. Work by Martin (1996) showed a range of impurities in, supposedly single enzyme products.

The current study demonstrated that energy loss via heat increment and as VFAs in the excreta may be minimised by enzyme supplementation, but undoubtedly, the data reported here are preliminary and more extensive work is needed to elucidate the significance of this finding.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The AME is the default system of estimation of energy in the poultry industry today. However, the system is not capable of accounting for the utilisation of ME within the birds. The system is also limited because it does not account for losses of chemical energy in the solid, liquid and gaseous excreta or as heat. Such energy losses may be substantial depending on the composition of the diet. Thus, the basis for the current study was to determine the heat production and the loss of energy in the excreta as VFAs in broiler chickens. The data reported at this exploratory stage were obtained using small-scale experiments because facilities were limited and thus, there is an obvious need for full-scale trials. If the results from such trials tallied with those of the current experiments, then this would be a sound basis for a fundamental change in the feed evaluation system.

Two experiments were conducted for the current project. Experiment 1 indicated that the amount of energy lost via HI may not be influenced significantly by the type of cereal grains, i.e., maize or barley, included in the diet of broiler chickens. However, the energy loss as VFAs in the excreta was significantly higher in birds fed a barley-based diet than with birds fed a maize-based diet. These results may indicate the significance of gut microflora and their influence on energy losses in chickens.

Experiment 2 showed that elevated levels of NSPs in the diets of broiler chickens increased energy loss as HI by 35% and VFAs in the excreta by 66%. Enzyme supplementation significantly reduced duodenal, jejunal and ileal digesta viscosities. It also alleviated energy loss via HI and as VFAs in the excreta. HI on the basis of MJ/kg body weight per day was markedly decreased by enzyme-supplementation, but the variations in HI on the basis of MJ/kg feed were not significant. This demonstrated that soluble NSPs can change the gut dynamics of the chicken, which was manifested by elevated digesta viscosity, and

increased energy loss via HI and as VFAs in the excreta. Another important consideration is the likelihood that wide between-bird variation in the loss of VFAs in the excreta was partly responsible for the extreme variability seen in the AME value of viscous grains, such as wheat, barley, rye and triticale, assayed in broiler chickens. Energy is the most expensive part of poultry diets and it is therefore imperative to obtain accurate estimates of the energy in the feed ingredients used in least cost formulations.

In the future, the industry should consider the effect of NSPs on loss of energy as VFAs in a practical sense, perhaps by adopting a VFA-corrected AME system for high NSP ingredients. Another suggestion for future work is the investigation of the effect of different types of NSPs, i.e., β-glucans, pectic polysaccharides and arabinoxylans (the NSPs used in current work) on heat increment and VFA loss in conjunction with appropriate enzymes. Effects of excreta VFA loss on the individual variability in an AME assay system using birds of various ages may also yield useful information.