British Journal of Nutrition (1995), **74**, 671–679

Screening tests of the protein quality of grain legumes for poultry production

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(*Received 25 November 1994 – Accepted 20 February 1995*)

Three screening tests for protein quality, modified limiting amino acid score (MLAAS), net weight gain (NWG) and net protein ratio (NPR), were compared. Two experiments using young broiler chickens were conducted in a temperature-controlled room at 28·5 ± 0·5° with no adaptation to cages and diets, or at 31 ± 0·5° with 2 d adaptation to cages and diets. Nine isoenergetic diets containing nominally 100 g crude protein/kg supplied by legume meals and one isoenergetic N-free diet were randomly allocated to chicks in single cages in each side of a four-tier battery brooder. Each dietary treatment had eight replicates. The chickens had access *ad lib.* to diet and drinking water throughout a 14 d observation period. Body weight and feed were measured at the start on day 7 and at the end on day 21. The results indicated that keeping the chickens at 31 ± 0·5° and giving them a 2 d adaptation period decreased the variability of chickens' responses to each treatment. MLAAS, NWG and NPR methods could distinguish legume proteins of high, medium and low feed values. MLAAS correlated well with NWG (*r* 0·90; *P* < 0·001) and NPR (*r* 0·78; *P* < 0·01) in evaluating the protein quality of grain legumes used as sole sources of protein for meat chickens. However, MLAAS did not predict the exact order of NWG and NPR. Growth was limited because dietary methionine, the first limiting amino acid, provided only 27·6–55·2 % of the recommended proportion in the protein. Although the results should be interpreted cautiously since a small sample size was used, it was concluded that the MLAAS calculation could be used as a reasonable estimate of the relative protein quality of most grain legumes, but that NWG and NPR were better methods as they detected limiting factors other than limiting amino acids in raw and processed legumes.

Protein quality: Amino acid score: Legumes: Broiler chickens

Conventional sources of protein for animals, such as fish-meals and soyabean meals, are often in short supply and expensive. Other grain legumes offer an alternative to fat-extracted soyabean meal (SBM) because they have a similar amino acid profile (Ravindran & Blair, 1992) and are often cheaper. Although grain legumes in Australia are grown seasonally for human consumption, spreading production throughout the year (Davies, 1989; Food and Agriculture Organization, 1989) would result in increased amounts of legumes becoming available for stockfeed. At the present time, however, the utilization of grain legumes as sources of protein for poultry is limited due to uncertainty about their nutritional quality. The variation in quality of grain legumes appears to be a combination of variable protein quality and variable amounts of antinutritional factors (ANF). The availability of a rapid protein-quality test would give feed manufacturers a greater ability to select and use grain legumes and hence build confidence in the market.

Johnson & Eason (1990) showed that the inclusion of 80, 140 and 200 g/kg of field pea (*Pisum sativum*), lupin (*Lupinus angustifolius*) or chickpea (*Cicer arietinum*) in a sorghum- and wheat-based diet did not affect the performance of broiler chickens but the same levels of narbon beans (*Vicia narbonensis*) significantly depressed growth. The discrepancy in results between the legumes may have been due to variability of activity of ANF such as protease inhibitors, lectins or tannins, and/or to the presence of non-digestible carbohydrate in some species or cultivars, leading to reduced digestibility, possibly because of low accessibility of the legume protein to digestive enzymes (Gatel, 1994; Liener, 1994). Although heat treatment can reduce the activity of ANF in grain legumes (van der Poel, 1990; Anderson-Hafermann *et al.* 1992; Singh *et al.* 1993), such treatments will probably increase costs for the feed miller, and excessive heating could reduce the availability of amino acids (Rani & Hira, 1993; van Barneveld *et al.* 1993). Because of the variation of both raw and processed material, each batch of grain legume probably should be tested for its nutritional quality.

Dingle (1972) identified sixty-one *in vivo* and thirty-three *in vitro* methods for the evaluation of protein quality. For the purpose of identifying a screening test which could be used to rank the protein quality of various sources of protein, limiting amino acid score (LAAS; Bender, 1958), growth rate or net weight gain (NWG), and net protein ratio (NPR; Bender & Doell, 1957), were investigated because of their simplicity and short test time. In protein-quality tests, diets containing 100 g protein/kg are usually applied, since more consistent and discriminating results have been obtained (Bressani, 1977).

The objectives of the present study were to develop and evaluate the above protein-quality test methods for their rapidity and repeatability in estimating the protein quality of some grain legumes for poultry production.

MATERIALS AND METHODS

Two experiments were conducted to compare three methods for the evaluation of protein quality of grain legumes for poultry production.

Proximate analysis

The proximate compositions of black gram (*Phaseolus mungo*), chickpeas (cv. Desi and cv. Kaniva), faba bean (*Vicia faba*), field pea, green gram (*Phaseolus aureus*), lupin, pigeon pea (*Cajanus cajan*), and solvent-extracted SBM were determined according to standard procedures (Association of Official Analytical Chemists (AOAC), 1984). Heat stable α-amylase (*EC* 3.2.1.1; AOAC approval number A3306; Sigma Chemical Co., St. Louis, MO, USA) was used in the crude fibre, acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) determinations.

Experiment 1

Diets. The SBM and all raw legumes were obtained from local (Queensland, Australia) suppliers. They were hand-cleaned to remove wastes, then ground to pass a 1 mm screen. As the sole source of dietary protein, each of the legume meals contributed 100 g dietary protein/kg calculated from published values. Maize starch, dicalcium phosphate, limestone, and vitamin and mineral premixes were added to each diet according to least-cost formulation (User Friendly Feed Formulation, 1986). All diets were made isoenergetic (13 MJ metabolizable energy (ME)/kg) by adding sunflower-seed oil and were made equal in fibre content by adding rice-hull meal (Tables 1 and 2). Ingredients for each diet were mixed in a single batch for approximately 25 min.

Chickens and experimental procedures. Eighty unsexed commercial broiler chickens were used in the assay. During days 1–6 post hatching the chickens were kept in a group and fed on a commercial starter diet, and on day 7 they were allocated to the dietary treatments (Table 1). The chickens were housed individually in a four-tier double-sided battery brooder. Each treatment had eight replicates and two replicates of each treatment were distributed randomly in each tier. The room temperature was maintained between 28 and

Table 1. *Expt 1. The compositions of dietary treatments (g/kg)*

Diet...	N-free	Soyabean meal	Black gram	Chickpea cv. Desi	Chickpea cv. Kaniva	Faba bean	Field pea	Green gram	Lupin	Pigeon pea
Soyabean meal		222·20								
Black gram (*Phaseolus mungo*)			427·4							
Chickpea (*Cicer arietinum* cv. Desi)				487·1						
Chickpea (cv. Kaniva)					511·5					
Faba bean (*Vicia faba*)						446·4				
Field pea (*Pisum sativum*)							448·4			
Green gram (*Phaseolus aureus*)								418·4		
Lupin (*Lupinus angustifolius*)									346·0	
Pigeon pea (*Cajanus cajan*)										500·0
Maize starch	851·2	676·0	486·9	400·6	418·6	463·2	498·6	498·0	588·0	372·4
Sunflower-seed oil	—	—	15·0	48·8	5·0	15·0	—	26·2	25·3	88·0
Limestone	16·5	20·0	19·9	16·0	16·0	19·9	17·9	22·6	18·3	19·9
Dicalcium phosphate	26·1	18·2	17·6	22·9	22·9	19·2	19·1	12·9	20·3	17·8
Rice-hull meal	100·0	61·5	31·2	20·5	22·1	34·1	13·9	20·0	—	—
Vitamin and mineral premix*	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Total	1000·0	1000·0	1000·0	1000·0	1000·0	1000·0	1000·0	1000·0	1000·0	1000·0

* Per tonne of diet the vitamin and mineral premix contained: retinol 2·4 g, cholecalciferol 75 mg, α-tocopherol 5 g, menadione 300 g, calcium pantothenate 3 g, riboflavin 3 g, nicotinic acid 15 g, cyanocobalamin 10 mg, biotin 5 mg, choline 100 g, Co 200 mg, I 500 mg, Cu 5 g, Fe 20 g, Mn 80 g, Zn 50 g, ethoxyquin 20 g, Se 100 mg, Mo 200 mg.

Table 2. *Calculated and proximate nutrient contents of the diets (g/kg air-dry weight)*

Diet...	N-free	Soyabean meal	Black gram	Chickpea cv. Desi	Chickpea cv. Kaniva	Faba bean	Field pea	Green gram	Lupin	Pigeon pea
Calculated values										
ME (MJ/kg)	13	13	13	13	13	13	13	13	13	13
Crude protein	—	100	100	100	100	100	100	100	100	100
Crude fat	—	8·6	29·9	34·6	34·6	23·9	39·3	29·9	43·6	96·8
Crude fibre	50·0	50·0	50·0	50·0	50·0	50·0	50·0	50·0	50·0	50·0
Ca	12·0	12·0	12·0	12·0	12·0	12·0	12·0	12·0	12·0	12·0
P	4·7	4·7	4·7	4·7	4·7	4·7	4·7	4·7	4·7	4·7
Amino acids										
Arginine	—	7·1	6·6	—	9·6	6·0	8·9	6·6	10·3	5·9
Histidine	—	2·6	2·1	—	2·6	3·8	2·2	2·9	2·6	3·8
Isoleucine	—	4·4	4·5	—	4·2	5·8	4·6	4·1	3·6	3·8
Leucine	—	7·4	7·4	—	7·4	7·2	8·2	7·4	6·4	7·0
Lysine	—	6·0	7·5	—	6·8	6·8	7·1	7·2	4·6	6·8
Methionine	—	1·2	1·1	—	1·0	0·7	1·1	1·1	0·5	0·9
Phenylalanine	—	4·9	5·9	—	5·6	5·4	4·9	5·9	3·4	4·4
Threonine	—	3·6	3·4	—	3·3	4·0	3·8	3·5	3·3	3·2
Tryptophan	—	1·6	1·0	—	1·8	1·0	0·8	1·8	0·9	0·8
Valine	—	5·2	5·0	—	3·9	4·7	4·9	4·8	3·4	4·3
Analysed values										
Dry matter	893·0	894·1	892·7	900·6	894·7	894·7	890·4	890·9	898·2	898·2
Crude protein	4·4	99·8	107·1	95·4	95·7	113·6	100·7	92·0	117·4	97·0
Crude fat	—	3·4	19·8	70·6	34·5	20·5	5·4	32·1	42·7	97·5
Crude fibre	34	33	29	55	26	51	31	25	51	37

ME, metabolizable energy; —, values not available.

29° and light was provided continuously. The chickens had access *ad lib.* to feed and water for 14 d. They were weighed at 7 d and 21 d after an overnight fast. Feed allowance, feed refusal and feed spills were measured.

The chickens' response to dietary protein was assessed in terms of NWG and NPR. NPR was calculated as (weight gain of the chickens fed on the test diet + weight loss of the chickens fed on the protein-free diet) divided by protein intake (Bender & Doell, 1957). The modified limiting amino acid score (MLAAS) used in these trials was based on the lowest ratios of mg amino acid/g protein in the legume to mg of the same amino acid/g dietary protein allowance recommended by the National Research Council (1994), as proposed by Dingle & Wiryawan (1994). The protein quality of grain legumes was ranked according to the descending value of their MLAAS, NWG and NPR scores. The distribution of the scores was further ranked into high, medium and low.

Experiment 2

This was a repeat of the first experiment with the following modifications: (a) the analysed protein content of each legume was used to calculate dietary protein (Table 3); (b) room temperature was increased to $31 \pm 0{\cdot}5°$; (c) the chickens were placed in the cages and fed on a mixture of commercial and SBM diet (3:1, w/w) for 2 d before the start of the feeding trial, and (d) body weights were measured at day 14 also.

Statistical analysis

Statistically significant differences between the mean NWG, protein intake and NPR value of chickens given each dietary treatment, and for each experiment, were calculated by using the Statistical Analysis Systems procedure General Linear Model (SAS Institute, Inc. 1990).

RESULTS AND DISCUSSION

The proximate compositions of the legumes tested showed that their protein contents were between 180 and 460 g/kg (Table 3). These were within the range of published values (Evans, 1985; Ravindran & Blair, 1992). Apart from SBM, lupin contained the most and pigeon pea the least protein (Table 4). Because approximately equal amounts of protein were included in the test diets, the extent to which the amino acid composition of each protein coincided with the chickens' requirements determined the protein quality score using the MLAAS calculation.

Fibre content, in terms of crude fibre, NDF or ADF, also varied between legumes. Lupin had the highest and chickpea (cv. Kaniva) the lowest. It is also worth noting that grain legumes are a source of dietary carbohydrate energy since they contain significant amounts of N-free extract (NFE), mainly sugars and starch (Reddy *et al.* 1984). Most of the raw legumes analysed did not contain appreciable amounts of lipid. Chickpeas and lupin however, contained 40–60 g lipid/kg (Table 3). The significance of legume lipid and NFE in providing energy for chickens has not been elucidated. It is possible that their ME value may vary inversely with their concentration of non-starch polysaccharides, as with cereals (Annison, 1991).

There were some differences between calculated and analysed values of dietary protein, fibre and fat for Expt. 1. These differences were small except in the case of crude fat in chickpea (cv. Desi) and field pea. In the case of protein content, chickpea was about 15% lower, while faba bean and lupin were about 15% higher than the calculated values. The analysed protein content of the non-protein diet may be accounted for by residual amounts of N in maize starch and the rice-hull meal.

NWG and protein intake of chickens given different legumes in Expt. 2 were significantly ($P < 0{\cdot}001$) higher than those in Expt. 1, indicating that the modified experimental

Table 3. *Proximate composition of grain legumes (g/kg air-dry weight)*

Legumes	Dry matter	Ether extract	Crude protein	Crude fibre	NDF	ADF	Ash	NFE
Soyabean meal	888·0	15·2	454·5	53·5	164·9	147·8	61·8	303·0
Black gram (*Phaseolus mungo*)	903·7	11·3	249·9	43·0	148·4	96·3	34·6	564·9
Chickpea (*Cicer arietinum*) cv. Desi	903·4	44·7	193·2	99·0	234·2	159·1	34·6	531·9
Chickpea cv. Kaniva	902·6	57·7	188·9	36·7	108·5	61·9	26·1	593·2
Faba bean (*Vicia faba*)	908·6	12·3	234·7	87·5	209·6	150·3	31·3	542·8
Field pea (*Pisum sativum*)	906·1	10·2	223·6	57·8	177·6	98·5	43·7	569·0
Green gram (*Phaseolus aureus*)	899·3	14·1	223·5	43·5	139·9	92·9	47·9	570·3
Lupin (*Lupinus angustifolius*)	916·4	50·4	313·5	147·0	239·4	203·7	34·0	371·5
Pigeon pea (*Cajanus cajan*)	885·1	19·0	181·4	74·7	195·7	140·2	37·9	572·1

NDF, neutral-detergent fibre; ADF, acid-detergent fibre; NFE, nitrogen-free extract.

Table 4. *The order of protein quality of grain legumes on the basis of modified limiting amino acid score (MLAAS), net weight gain (NWG) and net protein ratio (NPR) in broiler chickens*

			Protein intake (g)		NWG (g)		NPR		Order of protein quality						
										NWG		NPR		
Legume	MLAAS*	Order of protein content	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	MLAAS	Expt 1	Expt 2	Expt 1	Expt 2	Growth test category
Soyabean meal	55·2	1	19·06^{ab}	21·52^{ab}	8·65^{ab}	27·20^{a}	3·01^{a}	3·53^{a}	1	2	2	2	1	High
Chickpea (*Cicer arietinum* cv. Desi)	54·4	7	16·14^{bc}	21·89^{ab}	1·06^{bc}	27·62^{a}	3·09^{a}	3·49^{a}	2	4	2	2	1	High
Chickpea (cv. Kaniva)	54·4	8	20·03^{a}	24·33^{a}	20·26^{a}	37·06^{a}	3·45^{a}	3·53^{a}	2	1	1	1	1	High
Green gram (*Phaseolus aureus*)	47·0	6	16·28^{bc}	19·34^{b}	−0·97^{bc}	6·61^{b}	2·94^{ab}	2·86^{b}	4	5	5	4	4	Medium
Pigeon pea (*Cajanus cajan*)	46·0	9	19·74^{ab}	20·11^{b}	8·67^{ab}	9·12^{b}	2·91^{ab}	2·88^{b}	5	2	4	5	4	Medium
Black gram (*Phaseolus mungo*)	42·0	3	14·85^{c}	22·29^{ab}	−23·11^{d}	1·22^{bc}	1·73^{c}	2·24^{c}	6	8	7	8	8	Low
Faba bean (*Vicia faba*)	36·8	4	15·57^{bc}	14·74^{c}	−10·33^{c}	−7·54^{bc}	2·74^{bc}	2·80^{b}	7	7	8	7	7	Low
Field pea (*Pisum sativum*)	34·6	5	15·84^{bc}	19·37^{c}	−7·30^{c}	6·98^{b}	2·62^{ab}	2·88^{bc}	8	6	5	6	4	Medium
Lupin (*Lupinus angustifolius*)	27·6	2	14·72^{c}	15·00^{c}	−23·56^{d}	−15·54^{c}	1·71^{c}	2·22^{c}	9	9	9	9	9	Low
SEM	—	—	1·43	1·43	5·06	5·06	0·24	0·18						

a, b, c, d Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).
* Based on amino acid requirement of broiler chickens aged 0–3 weeks (Dingle & Wiryawan, 1994).

conditions improved responses of chickens to the dietary proteins. The small increase in room temperature may have been partly responsible for the difference. Many chickens in Expt. 1 showed signs of discomfort (chirping and shivering) throughout the trial, indicating that the temperature of $28{\cdot}5 \pm 0{\cdot}5°$ was not warm enough for chickens aged 1–3 weeks. The introduction of dietary treatments, at the same time as the chickens were placed in the single-cage environment, caused some chickens to stop eating for several days, resulting in a low total feed intake which caused a reduced weight gain. A 2 d period of adaptation to the cages and the mash diet was justifiable since the chickens accepted the experimental mash diets immediately. The limitation of using mash diets was a relatively larger amount of feed spill, especially with diets incorporating a large amount of starch, compared with a crumbed diet. Careful recovery of feed spill increased the precision of measuring feed intake.

There was no significant difference between blocks, indicating that, under the conditions of our studies, tier height and differences in light intensity in the room did not affect the chickens' performances.

Duncan's multiple range test showed that the NWG for chickens given chickpea (cv. Kaniva) in Expt. 1, or both chickpea diets in Expt. 2, were similar to those of chickens given SBM and significantly ($P < 0{\cdot}05$) higher than the NWG of chickens given the other legumes (Table 4). This indicated that the nutritive values of SBM and chickpeas were superior to those of other selected legumes.

The greater NWG of chicks given cv. Kaniva than those given cv. Desi could partly be explained by the lower fibre content of cv. Kaniva (2·6 *v.* 5·5%, Table 2). The MLAAS, NWG and NPR values of chickens fed on SBM and chickpea cv. Kaniva were not significantly different, indicating that raw cv. Kaniva chickpeas may not have contained ANF. It is possible, however, that there were ANF in both chickpeas and SBM. Other possible reasons for less than maximal growth of chickens fed on SBM are that overheating may reduce the availability of certain amino acids in SBM (Rani & Hira, 1993; van Barneveld *et al.* 1993), and chickens given SBM as the sole source of protein may not grow as well as chickens fed on a combination of SBM and another source of protein (Irish & Balnave, 1993).

The low NWG from feeding legumes other than chickpeas and SBM, and the severe weight losses of chickens given lupin, were apparently due to a combination of insufficient intake of sulphur-containing amino acids, due to their limiting amounts in the diets, and to low feed intake possibly associated with the sticky nature of the lupin diet. ANF may also have been responsible for some of the weight loss.

Fig. 1 indicates the amino acids that were well supplied and those that were in short supply for chickens' requirements from chickpea (cv. Kaniva) and lupin.

The amounts of most amino acids supplied in both experiments fulfilled the birds' requirements at 100 g protein/kg. However, methionine, the first limiting amino acid in all diets, supplied only 27·6–55·2% of the requirement. Lupin, especially, was not only very deficient in methionine but also contained insufficient lysine. Supplementation of lupin with both methionine and lysine improves its protein quality (Sarwar *et al.* 1978; Perez-Alba *et al.* 1990). Threonine is the next limiting amino acid for both of these legumes.

There were negative correlations between the protein contents of the legumes and their MLAAS (r −0·80; $P < 0{\cdot}01$), NWG (r −0·87; $P < 0{\cdot}01$) and NPR (r −0·76; $P < 0{\cdot}01$) values. These indicate that the protein quality of unprocessed legumes with a high protein content is poorer than that of those with a lower protein content. Grain legumes with a high protein content have been found to be associated with relatively low concentrations of lysine, sulphur amino acids, tryptophan and threonine (Mosse, 1990). The legumes with moderate protein concentrations were sources of medium quality protein.

The MLAAS values were positively correlated with NWG values and NPR values. The

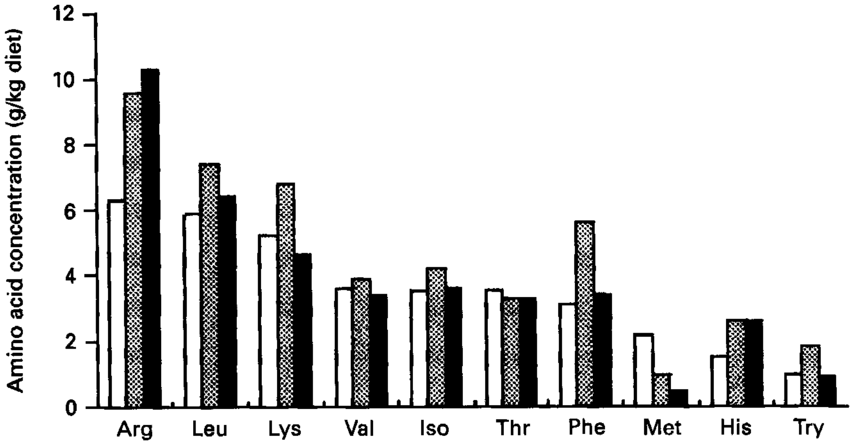

Fig. 1. Concentrations of amino acids (g/kg diet) required by chickens (□) and those provided by diets containing chickpea (*Cicer arietinum* cv. Kaniva; ▒) or lupin (*Lupinus angustifolius*; ■) at a level providing 100 g protein/kg diet.

correlation coefficients were 0·74 and 0·75 ($P < 0{\cdot}002$) respectively in Expt 1 and 0·90 ($P < 0{\cdot}001$) and 0·78 ($P < 0{\cdot}01$) respectively in Expt 2. These suggest that the protein quality of most grain legumes for broiler chickens could be reasonably estimated from their analysed amino acid content relative to the dietary amino acid requirement of the chickens.

Although the protein quality of some grain legumes, when based on NPR value, was in most cases of a similar order to their theoretical value (MLAAS), in other cases their order was higher or lower than expected (Table 4). The three protein quality indices distinguished three low, three medium and three high nutritional value legumes. The categories designated for black gram and field pea by MLAAS were reversed in the NWG and NPR scores (Table 4).

MLAAS and NPR methods showed consistent results for SBM, chickpeas, faba bean, green gram and lupin. However, the range of NPR values was much narrower than the range of MLAAS values (1·6:1 *v.* 2:1, the highest to the lowest) and therefore NPR may not be useful as MLAAS for grading feed proteins into high, medium and low values. However, these results should be interpreted with caution because only small sample sizes were used.

In conclusion, the rapid screening tests MLAAS, NWG and NPR could be used to grade grain legumes into high, medium and low quality. On the basis of NPR values the two chickpea varieties and SBM were grouped together into sources of high quality protein; green gram, pigeon pea and field pea were sources of medium quality protein and black gram, faba bean and lupin were sources of low quality protein. In categorizing the protein value of grain legumes into high, medium or low these rapid tests gave reasonably repeatable results in two experiments. Any of these is probably an adequate basis for purchasing and formulating decisions. The correlation between the three screening tests was high, therefore the use of the MLAAS, the simplest and quickest method, employing calculating and no growth test, was a reasonable estimate of protein quality for most grain legumes.

The research was supported by the Egg Industry Research and Development Council of Australia. Mr F. Gorbacz is thanked for his technical assistance.

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Printed in Great Britain