

Full Length Research Paper

## Effect of mung-bean fibre on acidification in culture broths using selected intestinal microflora

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The effect of mung-bean dietary fibre on the acidification in culture broths by using six intestinal bacterial strains was investigated. The strains were cultured anaerobically individually in broths containing 2 kinds of mung-bean fibre, mung-bean hull and cellulose at 37°C. The results showed that *Bifidobacterium bifidum* and *Bifidobacterium longum* in the broths containing each kind of mung-bean fibre had the highest acidification ability than the broths containing mung-bean hull and cellulose. *Lactobacillus acidophilus*, *Enterococcus faecalis* and *Escherichia coli*, and *Bacteroides fragilis* also showed acidification ability in the broths containing each kind of fibres, but the acidification ability of these intestinal bacterial strains was significantly different. However, the mung-bean fibre played an important role in acidification of broths cultured with intestinal microflora.

**Key words:** Acidification, fibre, intestinal, microflora, mung-bean.

### INTRODUCTION

Mung-bean or green gram is a kind of cheap and popular food material in Taiwan. The hulls of mung-bean are commonly used as stuff for pillow. It was reported that mung-bean was one of the good sources of proteins as well as abounding in dietary fibres. The dietary fibres of mung-beans are located in the hulls (Lee et al., 1988). Mung beans are similar in composition to other members of the legume family, with 24% protein, 1% fat, 63% carbohydrate and 16% dietary fibre (US Department of Agriculture, 2001). It was reported that the dietary fibres from mung-beans, such as pectin, could delay increase of blood sugar level. They found that the more the dietary fibres the slower the level of blood sugars went up. So they suggested that mung-beans may possibly delay the emptying process of stomach (Jenkins et al., 1975; Jenkins et al., 1999). Mung-beans are considered as low-GI (glycaemic index) foods observed in non-diabetic and diabetic participants (Trinidad et al., 2010). Moreover, all

fibres, both soluble and insoluble can entrap bile acid and prevent its re-absorption in the liver, thus inhibiting cholesterol synthesis (Mallillin et al., 2008).

It is well known that dietary fibres are needed by intestinal microorganism, and organic acids are generated during fermentation; these lead to lower pH in intestines, along with effective control on generations of harmful or carcinogenic substances caused by the activities of spoilage microorganism or bile acid decomposition (Vince et al., 1973). The effect of fermentation on the protein value, antioxidant activity and anti-nutritional factors of mung meals by *Lactobacillus* strains was reported (Khalil, 2006). The physiological effects of dietary fibre differ depending on its particle size (Heller et al., 1980). Heller et al. (1980) reported that finely milled wheat bran tends to be more readily fermented than coarse wheat bran. However, there is no definitive study on the effect of particle size of mung-bean

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fibre on intestinal physiology (Jenkins et al., 1978).

This study exhibited the effect of mung-bean fibre size on the intestinal microorganism growth by using a number of intestinal microorganisms in an *in vitro* experiment to mimic temperature and anaerobic condition in human intestinal system. The results indicated that mung-bean fibre showed effective function in stimulating acidification ability of the intestinal bacteria, and different size of mung-bean fibre had various effects depending on the bacterial strains.

## MATERIALS AND METHODS

### Preparation of mung-bean fibres

Mung-bean hulls were purchased from Flavour Full Foods Inc (Taipei) and stored at 4°C for use. For preparing mung-bean fibres, a part of mung-bean hulls was mixed with ether at the ratio of 1:4 (w/v) to free it from fat 3 times. The defatted mixture was collected and 10 volume of distilled water was added, and then the mixed solution was homogenized for 2 min. The homogenized solution was filtered to obtain the solid substances from the solution. The solid substances were washed with distilled water 3 times, followed by 85% ethanol 2 times and distilled water 2 times, respectively. The solid substances were dried at 50°C and ground to powders. The powders were sieved through a Japanese industrial standard sieve of mesh size 40 for providing two particle powder products (diameter being not less than or equal to 420 µm) and stored at -20°C for use. The cellulose was purchased from Sigma (MO, USA).

### Chemical analyses

The chemical compositions of mung-bean hull, two kinds of mung-bean fibre and cellulose were determined. Moisture content was measured by the weight difference before and after oven drying at 105°C for 16 h. Crude lipid content was measured by drying the sample in a 105°C oven for 6 h and then extracting the lipid with ether in a Soxhlet extractor for 4 h. Crude protein content was measured by the Kjeldahl method (AOAC, 1995). The soluble and insoluble dietary fibre measurements were determined according to the method of Prosky et al. (1998).

### Preparation of culture medium

The basic culture medium, PYF broth, which composed of peptone (10 g), yeast extract (5 g), salt solution (40 ml) that was prepared by dissolving 0.2 g anhydrous CaCl<sub>2</sub>, 0.2 g MgSO<sub>4</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>, 1 g KH<sub>2</sub>PO<sub>4</sub>, 10 g NaHCO<sub>3</sub>, and 2 g NaCl in 1,000 ml pure water, Field solution (40 ml), L-cysteine HCl.H<sub>2</sub>O (0.5 g) and water (920 ml) was prepared (Lee et al., 1997; Hodeman et al., 1977). The stock solutions of 10% of two kinds of mung-bean fibre, mung-bean hull and cellulose were also prepared with sterile water and then sterilized under UV lamp for 18 h. These solutions were diluted with PYF broth to form culture medium containing 0.5% (w/w) different glucide. The culture medium was put into a number of test tubes, with 8 ml culture medium/per tube.

### Experiment

To mimic human intestinal system, anaerobic condition was used in *in vitro* experiment and each of the 6 intestinal bacterial strains was added in the test tubes. These strains included *Bifidobacterium*

*bifidum* (BCRC11844), *Bifidobacterium longum* (BCRC11847), *Lactobacillus acidophilus* (BCRC10695), *Enterococcus faecalis* (BCRC10066), *Escherichia coli* (BCRC11509) and *Bacteroides fragilis* (BCRC10619) and supported by Food Industry Research and Development Institute (Hsinchu, Taiwan). Each strain was activated in the tubes with PYF broth. The volume of 0.1 ml (10<sup>5</sup> CFU/ml) bacteria solution was mixed well with every culture medium. The culture was conducted at 37°C in anaerobic condition, and pH value was recorded by using a pH meter (Seven Easy, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) to measure the culture medium after culturing for 0, 4, 8, 12, 24, 48, 32 and 48 h at 37°C anaerobic condition.

### Statistical analysis

All data were analyzed by one-way ANOVA analysis (Puri and Mullen, 1980). Duncan's new multiple range test was used to resolve the difference among treatment means. A value of P < 0.05 was used to indicate significant difference.

## RESULTS

### Proximate composition of mung-bean hull and fibre

Approximate compositions of mung-bean hull, 2 kinds of mung-bean fibre and cellulose used in this study are shown in Table 1. Two kinds of mung-bean fibre contained higher amount of crude carbohydrate and lower amount of moisture, crude protein, crude fat and ash than those of mung-bean hull. The percentage of dietary fibre in 2 kinds of mung-bean fibre was almost the same (about 70%), but higher than that (49%) of mung-bean hull. Among dietary fibre, the level of soluble one was small, about 8% in mung-bean fibre and 6% in mung-bean hull. The tested cellulose contained the main insoluble fibre (88.6%).

### Impact of mung-bean fibre on intestinal bacteria

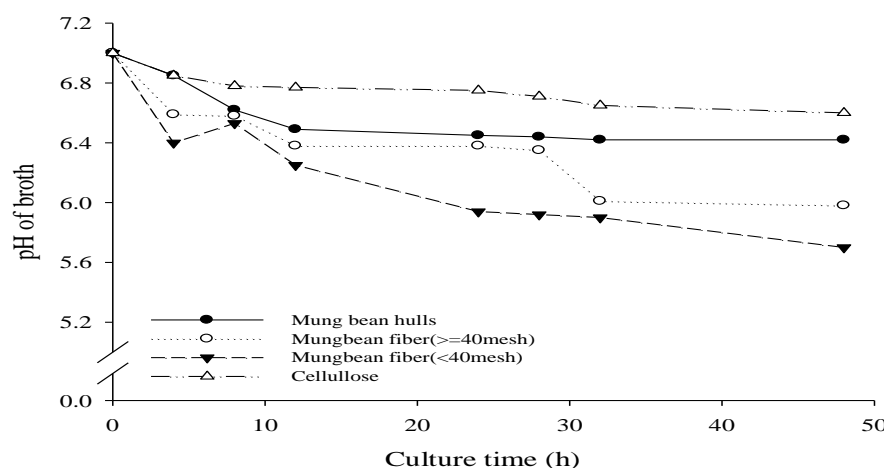
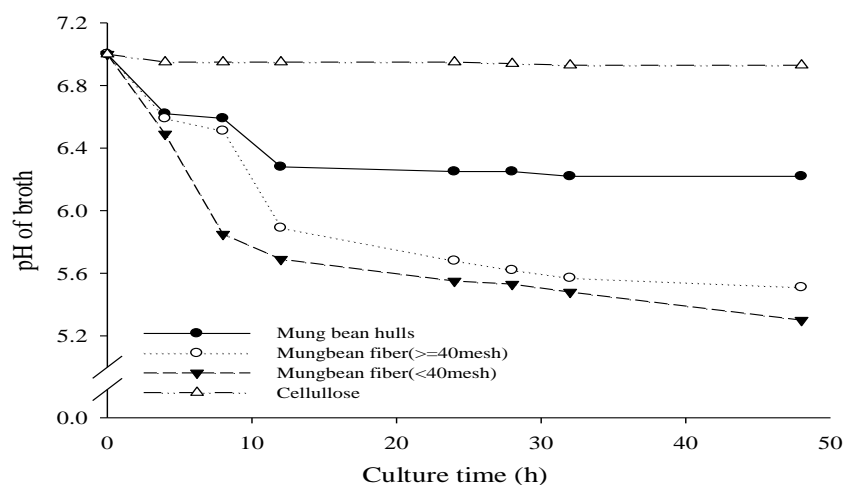
Dietary fibres are usually difficult to digest, and the utilization of dietary fibre depends on the kinds of material sources and intestinal bacteria. Figure 1 shows effects of different fibres in PYF broths on acidification ability of *B. bifidum*. The value of pH in culture medium containing each kind of fiber decreased with increase of culture time. Among them, the large particle (sieve < 40 mesh) of mung-bean fibre showed the least pH value in the culture medium, followed by small particle of mung-bean fibre, mung-bean hull and cellulose. Figure 2 shows effects of different fibres added in PYF broths on acidification ability of *B. longum*. Except for cellulose, 2 kinds of mung-bean fibre and mung-bean hull exhibited efficient acidification ability for *B. longum*. Among these three fibres, the large particle of mung-bean fibre also showed the least pH value in the culture medium, followed by the small particle of mung-bean fibre and mung-bean hull.

Figure 3 shows effects of different fibres in PYF broths

**Table 1.** Proximate composition and dietary fiber contents (%) of mung-bean hulls with different treatments.

Fiber source	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Dietary fiber		
						Soluble	Insoluble	Total
Mung-bean hull	11.3±0.8 <sup>a</sup>	17.1±0.7 <sup>a</sup>	4.5±0.3 <sup>a</sup>	3.7±0.2 <sup>a</sup>	63.5±0.8 <sup>c</sup>	2.7±0.2 <sup>b</sup>	46.3±2.0 <sup>c</sup>	49.0±2.3 <sup>c</sup>
Mung-bean fibre								
Size ≥ 40 mesh	6.9±0.8 <sup>b</sup>	12.6±0.9 <sup>b</sup>	0.8±0.1 <sup>b</sup>	1.9±0.3 <sup>b</sup>	77.8±1.2 <sup>b</sup>	5.2±0.3 <sup>a</sup>	63.6±2.6 <sup>b</sup>	68.8±3.0 <sup>b</sup>
Size < 40 mesh	7.0±0.6 <sup>b</sup>	12.5±0.1 <sup>b</sup>	0.8±0.2 <sup>b</sup>	2.0±0.2 <sup>b</sup>	77.7±1.3 <sup>b</sup>	5.1±0.2 <sup>a</sup>	64.1±1.8 <sup>b</sup>	69.2±2.1 <sup>b</sup>
Cellulose	5.8±0.2 <sup>c</sup>	0.1±0.1 <sup>c</sup>	0.1±0.1 <sup>c</sup>	0.1±0.1 <sup>c</sup>	93.9±0.3 <sup>a</sup>	0.2±0.1 <sup>c</sup>	88.6±1.3 <sup>a</sup>	88.8±1.5 <sup>a</sup>

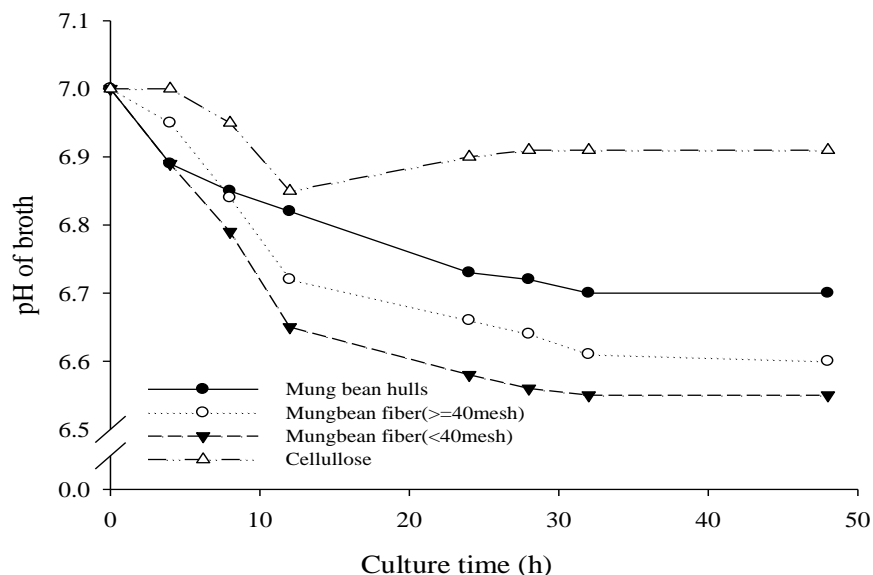
Data are mean ± standard deviation (n = 3). Mean values in the same column that are followed by the same superscript are not significantly different (P > 0.05).

**Figure 1.** Effect of mung-bean fibre in PYF broth on acidification ability of *Bifidobacterium bifidum*.**Figure 2.** Effect of mung-bean fibre in PYF broth on acidification ability of *Bifidobacterium longum*.

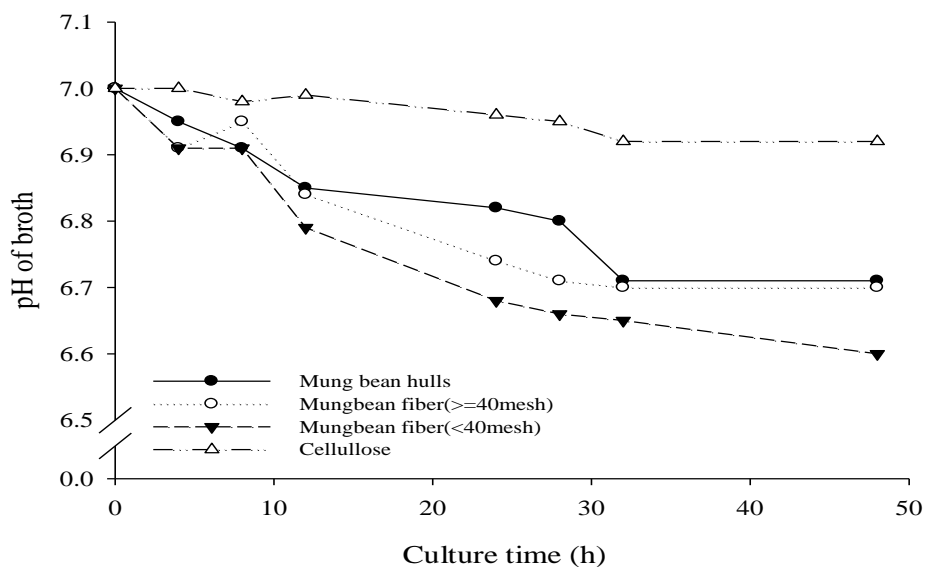
on acidification ability of *L. acidophilus*. Except for cellulose, the value of pH in culture medium containing each kind of fibre was decreasing with increase of culture time. Among them, the large particle (sieve < 40 mesh) of

mung-bean fibre showed the least pH value in the culture medium, followed by small particle of mung-bean fibre and mung-bean hull.

Figure 4 shows effects of different fibres in PYF broths



**Figure 3.** Effect of mung-bean fibre in PYF broth on acidification ability of *Lactobacillus acidophilus*.



**Figure 4.** Effect of mung-bean fibre in PYF broth on acidification ability of *Enterococcus faecalis*.

on acidification ability of *E. faecalis*. The value of pH in culture medium containing each kind of fibre was decreasing with increase of culture time. Among them, the large particle (sieve  $< 40$  mesh) of mung-bean fibre showed the least pH value in the culture medium, followed by small particle of mung-bean fibre, mung-bean hull and cellulose. Figure 5 shows effects of different fibres in PYF broths on acidification ability of *E. coli*. The value of pH in culture medium containing each kind of fibre was decreasing with increase of culture time. Among

them, the large particle (sieve  $< 40$  mesh) of mung-bean fibre showed the least pH value in the culture medium, followed by small particle of mung-bean fibre, mung-bean hull and cellulose after culturing for 12 h. However, the effect of mung-bean hull showed the least pH value in the culture medium before culturing for 12 h. Figure 6 shows effects of different fibres in PYF broths on acidification ability of *B. fragilis*. Although the value of pH in culture medium containing each kind of fibre was decreasing with increase of culture time, the lowest pH was about 6.9

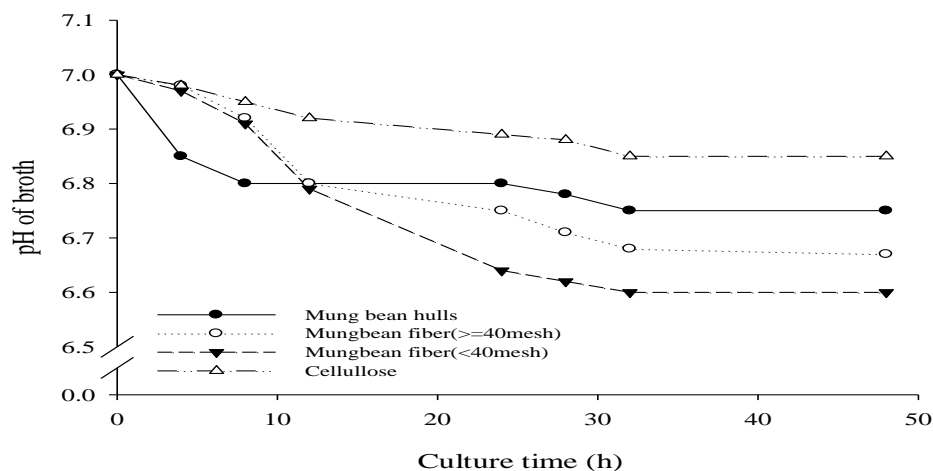


Figure 5. Effect of mung-bean fibre in PYF broth on acidification ability of *Escherichia coli*.

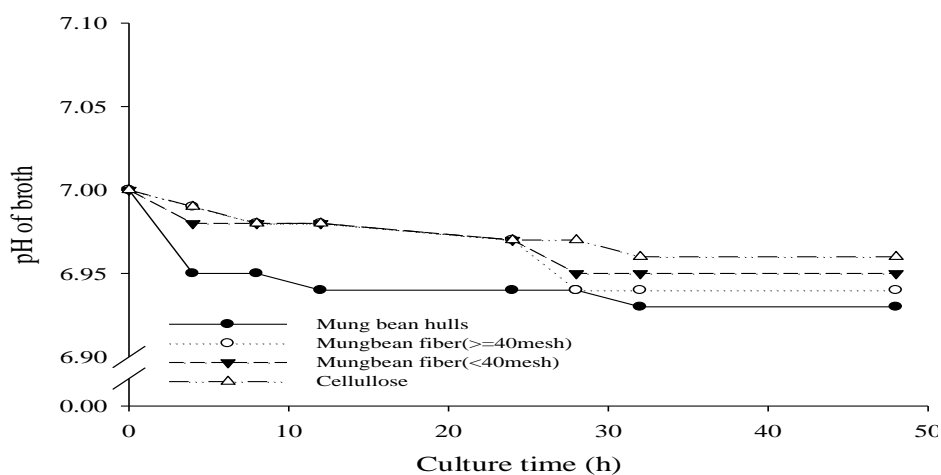


Figure 6. Effect of mung-bean fibre in PYF broth on acidification ability of *Bacteroides fragilis*.

even in the culture medium containing mung-bean hull. It indicated that the acidification ability of *B. fragilis* was the least.

## DISCUSSION

The normal profile of intestinal microflora can maintain the functions of intestinal system (Jawetz et al., 1989; Miysuoka, 1990). Among them, Bifidobacteria and lactic acid bacteria can help digestion and absorption of nutrients, as well as vitamins synthesis. The content of Bifidobacteria in intestinal system decreases gradually in aged people, while the spoilage microorganism like *E. coli* and harmful ones like Clostridia increase. It is well known that the increase of intestinal probiotics and decrease of spoilage and harmful microorganisms are

beneficial for human health and aging-process delay (Castell and Moore, 1971). The natural dietary fibre is needed by intestinal microorganisms, through which fermentation generates organic acids, and the pH value in intestinal system is lowered (Castell and Moore, 1971). In this way, the generation of harmful substances by spoilage microorganisms in intestinal system is suppressed.

Our results showed that the acidification abilities of intestinal bacteria in in vitro experiment (*B. bifidum* and *B. longum*) showed the best, followed by *L. acidophilus*, *E. faecalis* and *E. coli* (the middle) and *B. fragilis* (the least). Except for *E. coli* and *B. fragilis*, other 4 intestinal bacterial strains showed the highest acidification ability when the culture medium was added with the large particle of mung-bean fibre, followed by small particle mung-bean fibre, mung-bean hull and cellulose. It

indicated that different intestinal bacteria have different acidification ability. Although the digestion of dietary fibres provides carbon sources needed by growth of intestinal microorganism, a large quantity of acids will be generated during fermentation of intestinal microorganisms, and, in turn, the pH of intestines is lowered, which is beneficial for health. When the pH of human intestines is lowered, the enterokinesia is faster under the stimulation of acids, which lowers ammonia absorption by intestinal system. Therefore, lower pH decreases synthesis and absorption of ammonia (Castell and Moore, 1971). With this connection, Chau et al. (2005a) reported that a water-insoluble fibre-rich fraction (WIFF) was extracted from sweet orange peel and examined for its influence on gastrointestinal function and health-related parameters in hamsters, resulting in decrease of caecal pH. Their group (Chau et al., 2005b) further indicated that insoluble fibre fractions (IFF) of carambola and carrot significantly lowered caecal pH (6.50-6.64). Sembries et al. (2003) reported that apple fibre increased the weight of caecal contents and lowered luminal pH values in rats. Crittenden et al. (2002) reported that arabinosylian could be used in symbiotic combination with probiotic *B. longum* strains. Lee et al. (1997) indicated that apple dietary fibre significantly stimulated the growth of intestinal bacteria in *in vitro* experiment.

Due to cellulose having lower effect on acidification ability of intestinal bacteria, intestinal bacteria seem like to utilize the soluble dietary fibre. Furthermore, the large mung-bean fibre showed better effect on acidification ability of intestinal bacteria than the small mung-bean fibre. This result contrasts that of wheat bran (Heller et al., 1980). The experimental model is different from each other. Therefore, the real reason for different physiological effects of various kinds of fibre size needs further study.

In conclusion, in *in vitro* experiment, *B. bifidum* and *B. longum* showed the highest acidification ability in the culture broths when mung-bean fibre was added than mung-bean hull and cellulose. *L. acidophilus*, *E. faecalisand*, *E. coli*, and *B. fragilis* showed low acidification ability in the other broths. Although the variation of acidification in the broths induced by the intestinal bacterial strains was significant, the mung-bean fibre played an important role in acidification of broths cultured with intestinal microflora.

## ACKNOWLEDGMENT

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**Abbreviations:** **PYF**, Peptone yeast field; **WIFF**, water insoluble fibre fraction; **IFF**, insoluble fibre fractions.

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