The Effect of Different Sources of Urease Enzyme on the Nutritive Value of Wheat Straw Treated with Urea as a Source of Ammonia

M. J. Khan*, J. R. Scaife[1] and F. D. Hovell
Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ABSTRACT : Wheat straw samples (3-4 cm) were sprayed with solutions of urea (U) alone or with a dry addition of garden soil (GS), midden soil (MS), soya bean meal (SM) or jack bean meal (JM) as crude urease sources and with a pure urease (UR) enzyme. Each of the urease sources was included at two levels: 30 and 60 g/kg except pure urease, which was added at a level of 2.5 & 5.0 g/kg treated straw dry matter. Untreated straw without urease source was used as a control. After treatment, samples were sealed in polythene bags and stored for 2, 7, 14, 21 and 35 days at 19℃. The urease sources, their levels and treatment time produced significant effects on ammonia production ($p<0.01$). The addition of urease offered more flexibility in hydrolyzing urea in the shortest possible time. Incorporation of soya bean and jack bean meal was effective in reducing the modified acid detergent fiber (MADF) content of straw and the same time increasing organic matter (OM) digestibility. Overall effect, addition of soya bean to urea at a ratio of 1:1 appeared to be the most satisfactory urease source for the treatment of urea and wheat straw. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1063-1069*)

Key Words : Urea, Urease Sources, Wheat Straw Treatment, MADF, OM Digestibility

INTRODUCTION

The feeds available for ruminants in Bangladesh are largely agro-industrial by-products, usually rice and wheat straw, which are poor in nutrient content. Numerous attempts have been made to improve the feeding value of straw through physical, biological and chemical treatments.

Ammoniation of straw either with anhydrous ammonia directly or through urea treatment has proved beneficial to ruminants by increasing fibre digestibility and providing fermentable nitrogen (Williams, 1984; Verma et al., 1995). Urea has advantages over anhydrous ammonia in Bangladesh mainly because it can be locally manufactured, is readily available to farmers, less expensive, easier and safer to handle, and is the most practical method for use in South-East Asian countries (Saadullah et al., 1981; Jayasuriya and Perera, 1982).

At present straw treated with urea solution (as a source of ammonia) is ensiled for 3-4 weeks before being eaten by animals. Farmers in such a situation often prefer to use untreated straw rather than to treat it for long time. In early reports of such treatments (Van der Merwe, 1977; Kiangi et al., 1981), it was suggested that an exogenous source of urease was necessary in order to hydrolyse the urea to produce a more rapid improvement in the nutritive value of straw.

Addition of enzymes has been reported to hasten the process of conversion of urea to ammonia (Cafantaris et al., 1985; Munoz et al., 1991) and there is evidence to suggest that the treatment time could be successfully reduced from 3-4 weeks to 5 days by incorporating a source of urease enzyme at the time of the urea solution application (Jayasuriya and Pearce, 1983; Ibrahim et al., 1985). In general, urease enzymes catalyse the initial hydrolysis of urea to ammonium carbonate which decomposes to release ammonium ions (NH_4).

Pure urease is costly and not available to farmers, on the other hand soil might be used as a source of urease which is available to the farmers at no cost. Therefore, the study reported in this paper was carried out at a laboratory scale with wheat straw treated with urea and different common ureases sourced from plant materials and soil.

It was planned to define the optimum dose of urease source to reduce treatment time and also its overall effect upon ammonia production, fibre content, metabolizable energy content and organic matter digestibility.

MATERIALS AND METHODS

Sample preparation

Wheat straw was chaffed into lengths of 3-4 cm through a hammer mill. The sources of urease were ordinary soil, midden soil, soya bean seeds (*Glycine max. L*), jack bean seeds (*Canavalia ensiformis*) and pure urease (BDH, 1.7 EU per mg). The soils after collection were dried in an oven at a temp. of 40℃ for 48 hours and ground to pass through 1 mm sieve. Soyabean and jackbean seeds were also finely ground

* Corresponding Author: M. J. Khan.
[1] Department of Agriculture, Univ. of Aberdeen, Scotland, Aberdeen, AB24 5UA, United Kingdom.
Received October 10, 1998; Accepted February 9, 1999

Table 1. Experimental design for urea solution treatments on wheat straw (WS)

Treatment*	Replication	g/kg straw dry matter					
		Urea	Garden soil	Midden soil	Soya bean meal	Jack bean meal	Urease
WS+U	2	60	-	-	-	-	-
WS+U+GS	2	60	30	-	-	-	-
WS+U+GS	2	60	60	-	-	-	-
WS+U+MS	2	60	-	30	-	-	-
WS+U+MS	2	60	-	60	-	-	-
WS+U+SM	2	60	-	-	30	-	-
WS+U+SM	2	60	-	-	60	-	-
WS+U+JM	2	60	-	-	-	30	-
WS+U+JM	2	60	-	-	-	60	-
WS+U+UR	2	60	-	-	-	-	7.5
WS+U+UR	2	60	-	-	-	-	15

* WS: wheat straw, U: urea, GS: garden soil, MS: midden soil, SM: soya bean meal, JM: jack bean meal, UR: urease.

to pass through 1 mm sieve prior to use.

Treatment of straw

The chaffed straw in a batches of 1 kg, was treated with 60 g urea dissolved in 500 ml water. The urea solution was applied as a fine spray with a hand sprayer and the straw was mixed concurrently to achieve uniform wetting. Treated straw in 100 g lots was then placed into double layered polythylene bags, alone or with one of the urease sources. As a crude sources of urease 3.0 & 6.0 g of fine garden soil, midden soil, soya bean meal and jack bean meal were mixed to give a urea:urease source ratio of 1:0.5 or 1:1. Pure urease was added to 100 g of straw to give urea:urease ratio of 1:0.125 or 1:0.250. Bags were squeezed sufficiently to expel excess air and sealed tightly to ensure anaerobic condition. The sealed bags were kept in an oven at a temperature of 37℃ for one and half hours and subsequently stored at room temperature (19℃) for 2, 4, 7, 14, 21 and 35 days. Moisture content of treated straw was 40%. Urea treated straw without any urease source was held as control. After the specified period of storage, the bags were opened for the analysis of ammonia (soluble nitrogen) without any loss by volatilization. The treatment procedure in detail is given in table 1.

Chemical analysis

Immediately after opening the bags, 2 gms of treated straw was stirred with 20 ml 0.1 N HCl in a 50 ml beaker for 10 minutes and then filtered through Whatman 41 filter paper into a Kjeldahl distillation tube. Ammonia was determined according to AOAC (1990). After opening the remaining sample was dried at 45℃ to constant weight, then ground through 1 mm mesh sieve and stored for subsequent chemical analysis. Dry matter was determined by drying the sample at 103℃ for 48 hrs. Samples were analysed for ash, total nitrogen (AOAC, 1990), MADF (Kirk and Sawyar, 1991). Organic matter digestibility (OMD) and metabolizable energy (ME) were estimated adopting the following formulla:

1. OMD%= 93.49 - (0.971×MADF%) and
2. ME MJ/kg dry matter= 0.15×OMD.

Statistical analysis

The effect of the urease sources, their levels and treatment times on the parameters studied were subjected to analysis of variance using factorial design with replicates for each treatment using Minitab 10 for Windows. When significant differences occurred, Tukeys comparison procedure was used to compare means (Steel and Torrie, 1980).

RESULTS

Chemical composition of wheat straw and different urease sources used in the experiment is given in table 2. Among the urease sources soya bean meal had the highest (6.03%) and garden soil the lowest (0.23%) nitrogen content. Both soils contained a high ash (93.80%) whereas the ash content of the bean meal samples was very low.

Table 2. Chemical composition of wheat straw and urease sources (g/100 g dry matter)

Material	Dry matter	Ash	Total nitrogen	MADF
Wheat straw	94.18	6.75	0.82	51.88
Garden soil	95.01	93.83	0.23	76.81
Midden soil	94.86	90.87	0.33	68.59
Soya bean meal	90.86	4.66	6.03	10.78
Jack bean meal	88.83	3.19	4.43	10.62

Table 3. Effect of sources of urease enzyme on the soluble ammonia nitrogen production of urea-treated ensiled wheat straw (g/100 g)

Treatment	Ratio* Urea:Source	Treatment times (days) 2	4	7	14	21	35	Mean
Urea treated straw alone		0.37	0.59	1.16	1.45	1.72	1.87	1.19
Garden soil	1:0.5	0.40	0.81	1.25	1.75	1.95	1.89	1.34
	1:1	0.44	0.83	1.24	1.75	2.01	1.85	1.35
	Mean	0.42	0.82	1.25	1.75	1.98	1.87	1.35
Midden soil	1:0.5	0.38	0.81	1.07	1.18	1.22	1.18	0.97
	1:1	0.40	0.80	1.16	1.22	1.32	1.20	1.01
	Mean	0.39	0.80	1.11	1.20	1.27	1.19	0.99
Soya bean meal	1:0.5	0.85	0.98	1.31	1.21	1.05	1.25	1.10
	1:1	1.04	1.41	1.82	1.73	1.45	1.43	1.48
	Mean	0.94	1.19	1.57	1.47	1.25	1.34	1.29
Jack bean meal	1:0.5	1.26	1.35	1.23	1.21	1.27	1.27	1.26
	1:1	1.19	1.74	1.67	1.66	1.37	1.15	1.46
	Mean	1.22	1.54	1.45	1.43	1.32	1.21	1.36
Urease	1:125	1.09	1.11	1.23	1.23	1.14	1.10	1.15
	1:250	1.14	1.21	1.36	1.27	1.21	1.15	1.22
	Mean	1.12	1.16	1.29	1.25	1.17	1.13	1.18

* The ratio of urea and urease source.
Level of signifincance for, treatment ($p<0.01$), level ($p<0.01$) and time ($p<0.01$).

Table 4. Effect of sources of urease enzyme on the total nitrogen content of urea-treated wheat straw (g/100 g DM) after drying under various treatment conditions

Treatment	Ratio* Urea:Source	Treatment times (days) 2	4	7	14	21	35	Mean
Urea treated straw alone		2.54	2.27	1.40	1.64	1.95	1.74	1.92
Garden soil	1:0.5	2.01	1.86	1.25	1.38	1.48	1.60	1.59
	1:1	1.62	1.72	1.18	1.58	1.67	1.87	1.60
	Mean	1.81	1.79	1.21	1.48	1.57	1.73	1.60
Midden soil	1:0.5	2.05	2.08	1.37	1.19	1.17	1.32	1.55
	1:1	1.64	1.86	1.43	1.25	1.06	1.23	1.41
	Mean	1.85	1.97	1.40	1.22	1.11	1.28	1.47
Soya bean meal	1:0.5	2.16	1.94	1.78	1.54	1.58	1.53	1.75
	1:1	2.05	1.66	1.70	1.60	1.76	1.77	1.75
	Mean	2.10	1.80	1.74	1.57	1.67	1.65	1.75
Jack bean meal	1:0.5	1.17	1.22	1.30	1.38	1.52	1.55	1.35
	1:1	1.28	1.42	1.37	1.46	1.52	1.70	1.45
	Mean	1.22	1.32	1.33	1.42	1.52	1.62	1.40
Urease	1:125	1.77	1.37	1.33	1.32	1.18	1.16	1.35
	1:250	1.67	1.31	1.31	1.31	1.22	1.18	1.33
	Mean	1.72	1.34	1.32	1.32	1.20	1.17	1.34

* The ratio of urea and urease source.
Level of signifincance for, treatment ($p<0.01$), level (NS) and time ($p<0.01$).
Nitrogen content of untreated wheat straw, 0.82 g/100 g DM.

The ammonia nitrogen content of undried samples of treated straw is shown in table 3. Within two days of the straw having been treated, urea was hydrolysed and ammonia was evolved and the differences between urea treated straw alone and urease treated groups were significant ($p<0.01$). Addition of an external source of urease increased the rate of urea hydrolysis significantly ($p<0.01$) and reduced the time needed to obtain the same level nitrogen in straw treated with urea alone. The largest increase in nitrogen production

Table 5. Effect of sources of urease enzyme on the modified acid detergent fibre (MADF) content of urea-treated wheat straw (g/100 g DM)

Treatment	Ratio* Urea:Source	Treatment times (days)						
		2	4	7	14	21	35	Mean
Urea treated straw alone		51.47	50.36	50.34	49.65	49.90	48.74	50.08
		(51.51)	(50.81)	(50.67)	(50.55)	(50.48)	(49.63)	(50.60)
Garden soil	1:0.5	50.49	50.84	52.44	51.75	51.51	51.11	51.35
	1:1	50.76	51.82	52.13	52.20	50.28	49.90	51.18
	Mean	50.62	51.33	52.28	51.97	50.89	50.50	51.27
Midden soil	1:0.5	50.79	50.44	52.70	50.98	52.15	52.61	51.61
	1:1	51.09	51.71	52.05	52.42	52.71	53.30	52.21
	Mean	50.94	51.07	52.37	51.70	52.43	52.95	51.91
Soya bean meal	1:0.5	48.54	49.99	47.25	47.76	47.58	49.32	48.40
	1:1	47.90	47.97	47.65	48.00	47.23	48.00	47.79
	Mean	48.22	48.98	47.45	47.88	47.40	48.66	48.10
Jack bean meal	1:0.5	50.67	48.33	48.36	49.34	51.89	50.46	49.84
	1:1	49.67	47.32	47.15	49.21	49.19	49.00	48.59
	Mean	50.17	47.82	47.75	49.27	50.54	49.73	49.21
Urease	1:125	49.87	50.83	51.09	51.50	51.89	51.78	51.61
	1:250	51.08	50.22	50.60	50.43	50.99	50.86	50.69
	Mean	50.47	50.52	50.84	50.96	51.44	51.32	50.93

* The ratio of urea and urease source.
Level of signifincance for, treatment ($p<0.01$), level (NS) and time (NS).
Nitrogen content of untreated wheat straw, 0.82 g/100 g DM.

by the addition of 6% jack bean meal and soya bean meal was at storage times 4 and 7 of days respectively, but to produce the same amount of nitrogen urea-treated straw alone took 35 days. Maximum nitrogen (2.01%) production was obtained by using garden soil at 21 days of incubation. Inclusion of pure urease enzyme significantly ($p<0.01$) increased the ammonia nitrogen production up to 7 days of treatment and thereafter decreased.

As expected, urea treatment increased the total nitrogen content of dried wheat straw (table 4). The increment varied up to 3 times the value of untreated wheat straw, but it decreased with treatment times. Similarly, addition of ground soya bean showed the highest nitrogen content at two days of treatment but the values decreased rapidly with increase of stroage times. At any given time, straw treated with urease sources had a lower nitrogen content than treated only with urea straw. Level of urease sources did not affect ($p>0.05$) the nitrogen content of wheat straw.

Table 5 showed the effects of urease on MADF content of urea treated wheat straw under various treatment conditions. MADF content was decreased by the urea treatment with the advancement of incubation time. Addition of soya bean and jack bean had a small, although significant effect ($p<0.01$) in decreasing fibre content. The effect was higher at the ratio 1:1 at 4 days for jack bean and 7 days for soya bean meal. On the other hand, MADF content unchanged after 7 days at both urease levels. Treatment time did not significantly affect ($p<0.05$) MADF content. Addition of soil to urea treated straw increased the MADF content of straw.

As illustrated in table 6, addition of ground soya bean and jack bean significantly reduced the treatment time needed to attain maximum organic matter (OM) digestibility. The effect of level of urease application was particularly pronounced ($p<0.05$) and it would appear that the 1:1 ratio was the most effective. The OM digestibility of straw was significantly ($p<0.01$) increased by the addition of soya bean and jack bean meal, but longer the treatment times gave no additional improvement in OM digestibility. Treating straw for two days with 6% urea and urease from soya bean gave a digestibility value higher than that given by 6% urea without urease addition after 35 days of incubation. There was little improvement in OM digestibility by the addition of soil as well as pure urease with treated straw.

The effect on metabolizable energy (ME) content of adding urease sources with urea treated straw is given in table 7. Results showed significant differences for treatment level ($p<0.01$) but for treatment time the differences were not significant ($p<0.05$). The use of soya bean and jack bean as urease source improved the feeding vlue of straw, particularly at the upper level (1:1) of their use, which were as highest as 7.17 MJ/kg wheat straw.

Table 6. Effect of sources of urease enzyme on the organic matter digestibility (OMD) of urea-treated wheat straw (g/100 g DM)

Treatment	Ratio* Urea:Source	Treatment times (days)						
		2	4	7	14	21	35	Mean
Urea treated straw alone		43.51	44.55	44.60	45.28	45.04	46.16	44.85
		(43.48)	(44.15)	(44.29)	(44.40)	(44.47)	(45.30)	(44.30)
Garden soil	1:0.5	44.55	44.12	42.57	43.24	43.47	43.86	43.63
	1:1	44.20	44.22	42.87	42.81	44.66	45.04	43.96
	Mean	44.38	44.17	42.72	43.02	44.07	44.45	43.80
Midden soil	1:0.5	44.19	44.52	42.32	43.98	42.85	42.41	43.38
	1:1	43.89	43.27	42.96	42.59	42.21	41.74	42.77
	Mean	44.04	43.90	42.64	43.28	42.53	42.07	43.07
Soya bean meal	1:0.5	45.85	44.95	47.61	47.12	47.29	46.73	46.59
	1:1	46.97	46.91	47.85	46.88	47.63	46.88	47.17
	Mean	46.41	45.93	47.73	47.00	47.46	46.81	46.88
Jack bean meal	1:0.5	44.29	46.56	46.48	45.59	43.10	44.49	45.08
	1:1	45.26	47.54	47.70	45.91	45.54	45.91	46.34
	Mean	44.77	47.05	47.09	45.75	44.41	45.20	45.66
Urease	1:125	45.07	44.14	43.87	43.48	43.11	43.21	43.81
	1:250	43.82	44.72	44.36	44.52	43.98	44.11	44.25
	Mean	44.44	44.43	44.12	44.00	43.54	43.66	44.03

* The ratio of urea and urease source.
Level of signifincance for, treatment ($p<0.01$), level ($p<0.05$) and time (NS).
Nitrogen content of untreated wheat straw, 0.82 g/100 g DM.

Table 7. Effect of sources of urease enzyme on the metabolizable energy (ME) content of urea-treated wheat straw (g/1000 g DM)

Treatment	Ratio* Urea:Source	Treatment times (days)						
		2	4	7	14	21	35	Mean
Urea treated straw alone		6.52	6.63	6.68	6.79	6.75	6.92	6.69
		(6.51)	(6.61)	(6.64)	(6.65)	(6.66)	(6.79)	(6.64)
Garden soil	1:0.5	6.68	6.62	6.38	6.48	6.52	6.57	6.54
	1:1	6.63	6.63	6.42	6.41	6.69	6.75	6.58
	Mean	6.65	6.62	6.40	6.44	6.60	6.66	6.56
Midden soil	1:0.5	6.63	6.67	6.35	6.59	6.42	6.35	6.50
	1:1	6.58	6.49	6.44	6.38	6.34	6.26	6.41
	Mean	6.60	6.58	6.39	6.49	6.38	6.30	6.45
Soya bean meal	1:0.5	6.95	6.74	7.13	7.06	7.09	7.01	6.99
	1:1	7.04	7.03	7.17	7.02	7.14	7.03	7.07
	Mean	6.99	6.88	7.15	7.04	7.11	7.02	7.03
Jack bean meal	1:0.5	6.64	6.98	6.97	6.83	6.46	6.67	6.75
	1:1	6.79	7.12	7.15	6.88	6.86	6.88	6.94
	Mean	6.71	7.05	7.06	6.86	6.66	6.77	6.85
Urease	1:125	6.75	6.62	6.58	6.52	6.46	6.47	6.56
	1:250	6.57	6.70	6.65	6.67	6.59	6.61	6.63
	Mean	6.66	6.66	6.61	6.59	6.53	6.54	6.59

* The ratio of urea and urease source.
Level of signifincance for, treatment ($p<0.01$), level ($p<0.05$) and time (NS).
Values in parenthesis are for water soaked straw only.

DISCUSSION

The results obtained in this study showed that urea applied in solution to chopped wheat straw was hydrolysed even in the absence of an exogenous urease source .

This results coincide with those reported by Oji and Mowat (1977) and Dias da silva et al. (1988). But use of urease sources from soil and plant origin accelerated the rate of hydrolysis. Besle et al. (1990 a, b) also state that urea hydrolysis is favoured by addition of external urease. According to Mahapatra et al. (1977), plant material contains enzymes capable of hydrolyzing urea and releasing ammonia and bacteria originating from contamination of straw with soil are also capable of hydrolyzing urea. In general, urease enzyme catalyses the initial hydrolysis of urea to ammonium carbonate which on further hydrolysis, decomposes to release, ammonium ions and carbon dioxide. Urease from soya bean and jack bean appeared to assist in producing abundant ammonia immediately after treatment. Total nitrogen can be divided into two fractions - soluble and insoluble nitrogen. Ammonia is usually the major part of soluble nitrogen. Much of the soluble nitrogen obtained by urea treatments can be used by ruminal microflora as pointed out by Williams et al. (1984a), Mandell et al. (1988), Chermiti et al. (1989) and Ben Salem et al. (1994). Urea nitrogen was retained slightly better in straw treated only with urea than urease source treatments, which are in agreement with the findings of Kiangi and Kategile (1981). In the present experiment, urea treatment was carried out at a temperature of 19℃. In an NH_3 treatment of straw, Alibes et al. (1984) refer to the necessity of high ambient temperature to produce optimum results, particularly for better nitrogen retention. Hadjipanayiotou (1982) observed a nitrogen fixation of 44% after 30 days treatment period. On the other hand Chermiti et al. (1989) noted a nitrogen retention of between 25 and 31%. Although the published values for the retention of nitrogen vary somewhat but the values obtained in our study are within this range. According to Hassen and Chenost (1992) retained nitrogen is quickly degraded in ammonia-treated straw, and increases in the excretion of fecal nitrogen.

MADF content were depressed significantly by the addition of plant urease from soya bean and jack bean. Kiangi and Kategile (1981) also reported a decrease in cell wall constituents with ammonia treatment when urease was added in the treatment process.

Organic matter digestibility of urea treated samples increased with time and the results obtained in the present experiment are similar to the changes in digestibility reported by Sundstol et al. (1978) and Keran et al. (1979) but higher than those of Shultz et al. (1974) who reported no improvement with urea treatment without urease enzymes. The samples treated with soil as a source of urease exhibited lower OMD than urea treated samples. Addition of soya bean and jack bean showed positive effects on OMD.

On the other hand pure urease had no influence on OMD after two days of preservation time. Addition of urease sourced from soya bean and jack bean reduced the treatment time for OM digestibility, although the degree of reduction in treatment time did not differ between the two sources. Jayasuria and Pearce (1983) and Williams et al. (1984b) have also reported that inclusion of urease can successfully reduce the time required to achieve a given level of digestibility in rice straw. The overall positive effect of soya bean and jack bean addition on OMD was quite small from a nutritional point of view. In deed, after 14 days of treatment it was absent for jack bean treated samples. Kiangi et al. (1981), Wanapat et al. (1985) and Ibrahim et al. (1986) have found very little effects on digestibility and other parameters by adding a urease source. Other authers including Horton (1983) and Han et al. (1983) noted that the improved digestibility resulting from the treatment is due to the solubilization of the hemicelluse and alteration of the crystalline structure of cellulose. Ibrahim et al. (1985) used different sources of urease enzyme in an experiment with urea treated rice straw and found a very little difference between the different seeds tested as urease sources. But they reported that all the seeds had sufficient urease to hydrolyse 4 g urea in one day. In the present study the addition of soya bean seeds resulted in maximum OM digestibility, the results corresponding well with the findings of Ibrahim et al. (1986). An increase in the level of inclusion of soyabean produced higher OMD. A similar trend was observed by Jayasuria & Pearce (1993) in their study.

CONCLUSION

Results indicate that urea can improve the nutritive value of wheat straw in terms of nitrogen retention and digestibility. Addition of soya bean meal and jack bean meal as urease sources reduced treatment time. The procedure to maximize the nutritive value of urea treated wheat straw involved the addition of soya bean meal at the ratio of 1:1 (urea:SM). This combinition allows significant improvement in reducing MADF content and improving OMD in 2 to 7 days of treatment. This treatment could be applied in a tropical climate, but more research is needed to evaluate this treatment in farm scale silos in terms of animal performance.

ACKNOWLEDGEMENT

The authors wish to thank the laboratory staff of the Animal Sciences, Dept. of Agriculture, University of Aberdeen, particularly Mike and Moira for their assistance in analytical works. The research work was financed by the Commonwealth Commission and was conducted while the senior author was the recipient of the Post doctoral academic staff fellowship.

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