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Development of Nutrient Enriched Animal Feed from Jackfruit (*Artocarpus heterophyllus* L.) Waste through Solid State Fermentation

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ABSTRACT

Jackfruit (Artocarpus heterophyllus L.) is an important underutilized fruit of the tropics constitutes three parts namely bulbs, seed and rind or waste constitutes 50 - 55 per cent of the bulk of the fruit and is a good source of pectin and minerals. An experiment on solid state fermentation of jackfruit waste supplemented with organic and inorganic sources of nitrogen and fermented by probiotic yeast (Saccharomyces boulardii) and lactic acid bacteria (Lactobacillus acidophilus) was studied for the nutrient enrichment of the jackfruit waste as animal feed. The results revealed that jackfruit waste supplemented with 2 per cent ammonium sulphate and combined fermentation by yeast and lactic acid bacteria showed significant increase in nutrient contents of crude protein (23.37%), crude fibre (22.26%) carbohydrates (70.99%), titrable acidity (2.17%), total sugars (1.22%) with pH (3.97), moisture content (5.53%), yeast (1.5x10^6 cfu/g), LAB population of (1.8x106 cfu/g). The results conclude that supplementation and fermentation of jackfruit waste helps to enrich the nutrients which could be a very good source of animal feed supplement which otherwise being discarded.

Keywords: *Jackfruit waste, Solid state fermentation, Organic nitrogen, Inorganic nitrogen, Nutrient enriched feed, Animal feed*

INTRODUCTION

There is a chronic shortage of feed and fodders in India and in many of the developing countries, the food grains mainly produced for human consumption are also in shortage, so it became necessary to depend on unconventional feeds to sustain the feeding of livestock. Efforts are focused on determining the seasonal availability and nutritive value of locally available fruit and vegetable wastes / by-products to formulate adequate feeding system to bridge the gap between the demand and supply of nutrients of livestock, (Anil, 2012).

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Solid-state fermentation has emerged as an efficient technology for the production of microbial products such as feed, fuel, food, industrial chemicals and pharmaceutical products (Pandey, 2003). Few studies reviewed on solid state fermentation of apple pomace with *Candida utilis* significantly increased 2.5 fold in protein content, 3.4 fold in niacin, 1.5 fold in thiamine (Joshi & Sandhu, 1996). Supplementing of fruit and vegetable waste with different organic sources like azolla, groundnut cake, soybean cake are known to increase the feed intake and digestibility in cattle (Kusmartono, 2007).

Jackfruit (*Artocarpus heterophyllus* L.) is an important underutilized fruit of the tropics, native to the India and grows widely in the south western rain forest of India. Karnataka produces about 2, 13,800 MT with the area of 5,250 ha, and productivity of 30-35 t/ha (Anon, 2000). Fruit industry waste and by-products are available in large quantity with low nutritional value. The by-product of fruit waste can be a valuable material for animal feed. Nearly 60 per cent of the jackfruit is discarded after the fleshy parts are taken out. The jackfruit wastes, which consists of aerial part, skin, seed and heart, will be a feed resource with high organic matter (OM) digestibility (70-78%), with low crude protein (6 to 7% in DM) as reported by Kusmartono (2001). Subburamu et al. (1992) studied the utilization of jackfruit waste as a source of cattle feed. Thus, there is a possibility that jackfruit wastes can be processed into byproducts like animal feed through solid state fermentation technology.

MATERIALS AND METHODS

The experiment was conducted for the development of nutrient enriched animal feed from jackfruit waste through solid state fermentation (Plate 1). The treatment details are as follows.

Treatments

T1 = JF waste only

T2 = Jackfruit waste + 10% azolla + yeast

T3=Jackfruit waste + 2% ammonium sulphate + yeast

T4 = Jackfruit waste + 5% glyricidia leaves + yeast

T5 = Jackfruit waste + 10% azolla + LAB

T6 = Jackfruit waste + 2% ammonium sulphate + LAB

T7 =Jackfruit waste + 5% glyricidia leaves + LAB

T8 = Jackfruit waste + 10% azolla + yeast + LAB

T9 = Jackfruit waste + 2% ammonium sulphate + yeast + LAB

T10 = Jackfruit waste + 5% glyricidia leaves + yeast + LAB

Replications: 3, Fermentation period: 7 days fermentation

Plate 1: Experimental set up for SSF of jackfruit waste supplemented with different organic and inorganic sources

Collection and Preparation of jackfruit waste for experiment:

Well matured and healthy ripe jackfruits were collected from the Zonal Agricultural Research Station Farm, University of Agricultural Sciences, GKVK, Bangalore for the experimentation. The jackfruits were cut and peeled and removed the edible bulbs from the core rind. The jackfruit waste includes rind with arils and perigones used as a jackfruit waste for the experiment (Plate 2).

Plate 2. Preparation of jackfruit waste for Experiment

Jackfruit waste includes rind, arils, perianth cut into small pieces (size-1 inches) and filled into the autoclavable polypropylene bags and samples were pasteurized at 90-100^{0}C for 10 minutes. After pasteurization the jackfruit waste samples were transferred into polythene bags of 400 gauges for fermentation process.

Microbial cultures used

Authenticated microbial cultures of probiotic lactic acid bacteria *Lactobacillus acidophilus* (MTCC 10307) was brought from Microbial Type Culture Collection Centre, Chandigarh, India in the form of lyophilized cultures and same were revived in the form of agar based slant cultures. Whereas probiotic yeast *Saccharomyces boulardii* was isolated from the sachets of commercially available in the medical shops using Sabourauds culture media as per standard procedure.

Preparation of probiotic yeast starter culture

Purified and authenticated loop full of inoculums of probiotic yeast *Saccharomyces boulardii* was inoculated to conical flask containing Sabourauds Hi-media broth. The inoculated flasks were kept for 48 h incubated at 28^{0}C. The broth culture of probiotic yeast was inoculated at 5 per cent containing 10^{7}cfu/ml to 500 g of supplemented jackfruit waste contained in polythene bags under solid state fermentation process

Preparation of probiotic LAB starter culture

Loop full inoculum of purified and authenticated probiotic lactic acid bacteria *Lactobacillus acidophilus* (MTCC 10307) transferred to conical flasks containing 100 ml of Manns Rogasa Sharp broth (MRS). Inoculated flasks were incubated for 48 h at 37^{0}C. These broth cultures of LAB was inoculated at 5 per cent containing 10^{7}cfu/ml to 500g of supplemented jackfruit waste, contained in polythene bags under solid state fermentation process for 7 days under room temperature (27–30^{0}C).

Supplementation of different nitrogen sources to the jackfruit waste

Ammonium sulphate: 2 per cent of ammonium sulphate was supplemented to the jackfruit waste wherever it is required in the treatments.

Azolla: 10 per cent of azolla was supplemented to the jackfruit waste wherever it is required in the treatments.

Glyricidia leaves: 10 per cent of glyricidia leaves were supplemented to the jackfruit waste wherever it is required in the treatments.

Tray drying: After completion of 7 days fermentation, the samples were subjected to

tray drying at 50^{0}C for 48 hours. Samples were spread uniformly in the tray for effective drying.

Grinding: Dried pieces were grinded in mixer/ grinder to get powder and rind powder was subjected for biochemical and microbiological analysis by following standard procedures.

*Biochemical analysis: T*he biochemical composition pH, titrable acidity , total sugars, protein, crude fat, crude fibre, carbohydrates, total sugars, ash, and microbiological analysis of the fermented jackfruit waste in the form of dried powder as influenced by supplementation and fermentation (Plate 3) were analyzed for as per standard AOAC procedures.

Observations: pH, titrable acidity, total sugars, protein, crude fat, crude fibre, ash, moisture carbohydrates.

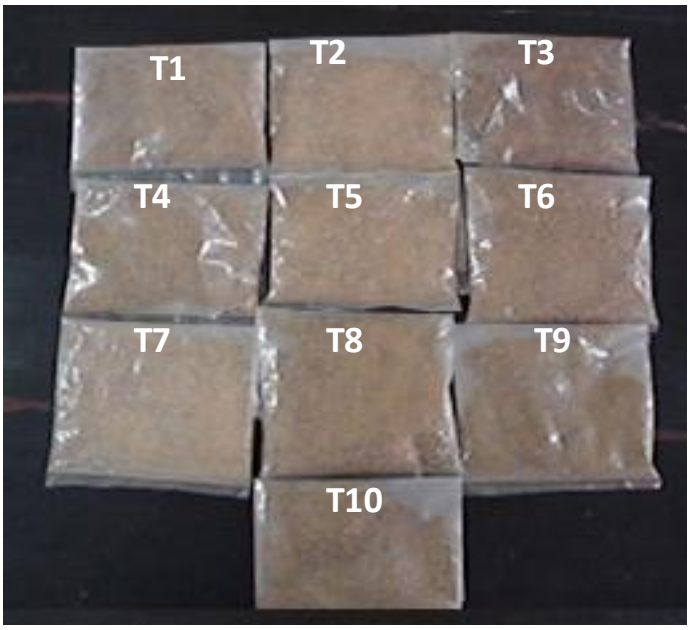

Plate 3: Fermented dried jackfruit waste in the form of powder as influenced by supplementation and fermentation

pH: pH of the fermented jackfruit waste samples were measured using digital pH meter of analog model (Pocket Refractometer Pal-1, made in Japan 100%, (0-53) ATAGO).

Titrable acidity(%): Titrable acidity of the samples were estimated as per the procedure followed by Srivastava and Kumar, 1993.

Protein: The protein content was determined from the organic nitrogen content by Micro-Kjeldahl method. (Sadasivam & Manickam, 1996).

Total sugars: Total sugar content of the samples were estimated according to the Fehling's method (Sadasivam & Manickam, 1996).

$$\text{Total ash percent (\%)} = \frac{W1 \times 100}{W}$$

Where; W = Weight of the sample (g)

W1 = Weight of the residue after ashing (g)

Crude fat : Fat content of the samples were estimated as crude ether extract of the dry material. The dry sample (3-5g) was weighed accurately in a thimble and plugged with cotton. The thimble was then placed in the Automatic Soxhlet Apparatus for further process (Sadasivam & Manickam, 1996).

Crude fibre: The crude fibre content of samples was estimated using the procedure described in AOAC (1980).

Ash : (AOAC, 1980), The samples were charred carefully on a heater or flame, and then ignited in a muffle furnace maintained at 525-550^{0}C. It is then cooled in a desiccator and weighed.

Carbohydrates: (AOAC, 1980), The content of the availale carbohydrate is detemined by the difference.

Carbohydrate (%) = 100 - [Protein (%) + Fat + Fibre (%) + Ash (%) + Moisture (%)]

Moisture: (AOAC, 1980), The average moisture content on wet basis of the samples was calculated using the following equation and the mean of 3 such reading was recorded as average moisture content.

Statistical analysis: The data obtained from the investigation was subjected to analysis of variance by completely randomized design using AGREES-C and AGDATA. The treatments difference was separated at 5 per cent significance level using Duncan's multiple range test.

RESULTS AND DISCUSSION

Influence of yeast and lactic acid bacteria with organic and inorganic nitrogen sources on pH, titrable acidity and total sugars of the fermented jackfruit waste is presented in Table 1.

pH: The results revealed that the slight variation in pH among different treatments both by yeast and bacteria was due to the fermentation potential of the microorganisms. Fermentative activity of yeast reduced the pH level compared to un-inoculation, mainly due to the utilization of sugars and carbohydrates in the substrate. The lower pH levels by bacterial strain with or without supplementation indicate the low fermentative characteristics in relation to changes in pH. However, higher reduction was observed by dual inoculation in all the three sources of nitrogen supplemented. This may be due to the pH depends upon the acids and sugars utilized by the microorganisms in the supplementing substrates. A pH range of 3.7-4.2 is generally considered beneficial for whole-crop cereal preservation (Kung & Shaver, 2001) and that of our study was pH of 3.97, indicative good for animal feed. Combined inoculation to jackfruit waste to stimulate lactic acid fermentation by accelerating the decrease in pH and thus improving jackfruit waste preservation. These results support the work of Mc Donald et al. (1991) in sweet sorghum silage.

Titrable acidity: The initial acidity of the jackfruit waste was 1.12 per cent, when it was supplemented with different sources and fermentation by yeast and lactic acid bacteria, titrable acidity varied from 0.89 to 2.27 per cent between the treatments. Upon completion of yeast and lactic acid fermentation of supplemented jackfruit waste, production of titrable acidity in terms of lactic acid is more important in the fermented products. In the present study (Table 1) the highest titrable acidity (2.27%) was produced in the jackfruit waste supplemented with 2% ammonium sulphate fermented by yeast and bacteria fermentation (T9). Similarly, single LAB fermentation of supplemented jackfruit waste showed highest titrable acidity (2.17%) was produced in the jackfruit waste supplemented with 2% ammonium sulphate fermented by yeast (T6). Combined fermentation of inorganic sources supplemented jackfruit waste did not influence on the enhancement of titrable acidity in any of the nitrogen sources.

Total sugar: The initial total sugar content of the jackfruit waste was 1.26 per cent, when it was supplemented with different sources and fermentation by yeast and lactic acid bacteria, total sugar content varied from 0.93 to 2.24 per cent between the treatments. However, jackfruit waste supplemented with ammonium sulphate (2%) and bacterial fermentation (T6) and combined fermentation (T9) showed significant increase in total sugar 2.24 per cent and 2.01 per cent respectively. Utilization of sugar is indicative of growth and fermentative efficiency of the yeast and lactic acid bacteria. Low total sugar is due to high fermentative character of the yeast than lactic acid bacteria. Supplementing with ammonium sulphate (2%) has further enhanced the yeast activity during fermentation, the lowest total sugar (0.93%) was produced in the jackfruit waste supplemented with 2% ammonium sulphate fermented by yeast (T3).

Effect of supplementation and fermentation on crude fibre, crude fat and crude protein of jackfruit waste is presented in Table 2

Crude fibre: The initial crude fibre content of the jackfruit waste was 22.72 per cent, when it

was supplemented with different sources and fermentation by yeast and lactic acid bacteria, crude fibre content varied from 21.53 to 22.35 per cent between the treatments. The slight reduction in fibre content of the fermented jackfruit waste was observed by the influence of supplementation and fermentation. Crude fibre of fermented jackfruit waste showed non significant with the enhancement of crude fibre by yeast and LAB strains (Table 2). However, crude fibre content is more in the yeast and combined inoculation compared to LAB fermentation of jack fruit waste supplemented with different nitrogen sources. This results support the work of Joshi and Sandhu (1996) who reported that the solid state fermentation (SSF) with 3 different yeast strains enriched the crude fibre content of dried apple pomace powders.

Crude fat: The initial crude fat content of the jackfruit waste was 5.89 per cent, when it was supplemented with different sources and fermentation by yeast and lactic acid bacteria, fat content varied from 5.48 to 6.35 per cent between the treatments. The organic nitrogen sources enriched the crude fat content of the inoculated jackfruit waste compare to supplementing with inorganic nitrogen sources. The highest crude fat (6.35%) was produced in the jackfruit waste supplemented with 10 per cent azolla fermented by yeast (T2). Similarly, LAB fermentation of jackfruit waste showed highest crude fat (6.13%) supplemented with 10 per cent azolla fermented by LAB (T5). In combined fermentation of jackfruit waste supplemented with 10 per cent azolla enhanced the crude fat (6.16%) (T8). The organic nitrogen supplement includes 10 per cent azolla and 5 per cent glyricidia leaves enriched the fat content of jackfruit waste compare to jackfruit waste supplemented with 2 percent ammonium sulphate by individual and combined inoculation. This may be due to the addition of sugars and carbohydrates degradation by microorganisms in the organic substrates helps in enhancing the crude fat.

Crude protein*:* The initial crude protein content of the jackfruit waste was 9.37 per cent, when it was supplemented with different sources and fermentation by yeast and lactic acid bacteria, crude protein content varied from 11.76 to 23.37 per cent between the treatments. The increasing in the protein content of jackfruit waste is due to utilization of sugars and nitrogen sources by the probiotic yeast and LAB. The highest crude protein (23.37%) was produced in the jackfruit waste supplemented with 2 percent ammonium sulphate fermented by combined yeast and lactic acid bacteria (T9) followed by single inoculation with lactic acid bacteria (T6) which are not significant (22.24%). Jackfruit waste supplemented with 2 percent ammonium sulphate in individual and combined inoculation enriched the protein content of jackfruit waste compared to the organic nitrogen supplement *viz,* 10 per cent azolla or 5 per cent glyricidia, The significant increase in protein and fat content of the fermented jackfruit waste sample may be attributed to the fact that the microorganisms degrade the sample as well as microbial biomass (Odetokum, 2000) and also due to the addition of nutrients from blending of different sources. This data supports the work of Correia *et al.,* (2006) in pineapple waste supplemented with 2.5 per cent of ammonium sulphate fermented by yeast *Saccharomyces cereviceae* enriched the protein content from 6.4 to 22 per cent, Similarly, reported by Joshi and Sandhu (2012) revealed that the protein content of apple pomace supplemented with 2.5 percent ammonium sulphate fermented by *Candida utilis* increased from 4.18 to 13.1 per cent.

Effect of supplementation and fermentation on ash, carbohydrates and moisture content of the fermented jackfruit waste is presented in Table 3.

Ash: The initial ash content of the jackfruit waste was 5.58 per cent, when it was supplemented with different sources and fermentation by yeast and lactic acid bacteria, fat content varied from 6.22 to 7.27 per cent between the treatments (Table 3). Yeast fermentation of jackfruit waste with 5% glyricidia leaves (T4) recorded more ash content (6.96%) differed significantly from treatments T3 (6.27%) and T2 (6.22%). Similarly, LAB fermentation of jackfruit waste supplemented with 5 % glyricidia leaves (T7) showed more ash content (7.05%). Whereas,

combined fermentation of jackfruit waste supplemented with 10% azolla (T8) showed highest ash content (7.27%) which is significantly differ from the treatments T10 and T9. Among three different sources of nitrogen, the organic source of azolla and fermented by combined inoculation was more influenced on ash enhancement in the jackfruit waste. This may be due to the addition of minerals in terms of ash present in the supplemented organic sources degraded by both yeast and bacterial fermentation helps in enhancement of ash content.

Carbohydrate: The initial carbohydrate content of the jackfruit waste was 73.73 per cent, when it was supplemented with different sources and fermentation by yeast and lactic acid bacteria, carbohydrate content varied from 63.21 to 76.15 per cent between the treatments. Results indicated that the jackfruit waste supplemented with 10% azolla fermented by single inoculation and combined inoculation by yeast and bacteria showed highest carbohydrate (76.15%) followed by treatments T2, T5 and T8 respectively. Jackfruit waste supplemented with inorganic source ammonium sulphate fermented by both individual and combined inoculation resulted in decrease in carbohydrate content in the jackfruit waste. The results indicated that inoculated jackfruit waste with organic supplements enriches the carbohydrate content than jackfruit waste supplemented with inorganic nitrogen sources. Oboh and Akindahunsi (2003) reported that fermentation of cassava pulp with *Saccharomyces cerevisiae* shows significant decrease in the carbohydrate contents of the cassava products.

Moisture: The moisture content of fermented Jackfruit waste varied from 5.28 to 6.13 per cent after 7 days of fermentation by Yeast and LAB strains with different nitrogen sources. The results indicated that moisture content did not much vary between the treatments. However, the jackfruit waste supplemented with organic sources such as azolla and glyricidia showed slightly more in moisture content compared to inorganic source fermented by single or combined inoculation of yeast and LAB fermentation which are non significant each other. More moisture in the jackfruit waste supplemented with organic sources may be the additional moisture present in the supplemented sources of azolla and glyricidia includes may be results in more moisture.

Table 1: Effect of supplementation and fermentation on pH, titrable acidity and total sugars of the fermented jackfruit waste

Strains	Treatments	pH	Titrable acidity (%)	Total sugars (%)
Yeast strain	T_1 = Jackfruit waste only	4.84^{d}	1.12^{b}	1.26^{b}
	T_2 = Jackfruit waste + 10 % azolla + yeast	4.18^{bc}	0.89^{a}	0.93^{a}
	T_3 = JF waste + 2 % Ammonium sulphate + yeast	4.21^{bc}	1.23^{b}	1.22^{b}
	T_4 = JF waste + 5 % glyricidia leave + yeast	4.39^{c}	1.05^{ab}	1.06^{ab}
LAB strain	T_5 = Jackfruit waste + 10 % azolla + LAB	4.02^{ab}	1.67^{c}	1.68^{cd}
	T_6 = JF waste + 2 % Ammonium sulphate + LAB	3.96^{ab}	2.17^{f}	2.24^{f}
	T_7 =JF waste + 5 % glyricidia leaves + LAB	4.26^{bc}	1.98^{de}	1.91^{e}
Yeast and LAB strains	T_8 = JF waste + 10 % azolla + yeast + LAB	3.83^{a}	1.69^{c}	1.60^{c}
	T_9 = JF waste + 2 % Ammonium sulphate + yeast + LAB	3.97^{ab}	2.27^{ef}	2.01
	T_{10} = JF waste + 5 % glyricidia leaves + yeast + LAB	3.99^{ab}	1.92^{d}	1.83^{de}
	S.Em±	**0.10***	**0.06***	**0.06***
	CD (at 5%)	**0.29***	**0.19***	**0.18***

Note: Fermentation period 7 days,

Yeast: *Saccharomyces boulardi*i

LAB: *Lactobacillus acidophilus*

Table 2: Effect of supplementation and fermentation on crude fibre, crude fat and crude protein content of the fermented jackfruit waste

Strains	Treatments	Crude fibre (%)	Crude fat (%)	Crude protein (%)
	T_1 = Jackfruit waste only	22.72^{c}	5.89abc	9.37^{a}
Yeast strain	T_2 = Jackfruit waste + 10 % azolla + yeast	22.01^{ab}	6.35a	11.85^{ab}
	T_3 = Jackfruit waste + 2 % $(NH_4)_2SO_4$ + yeast	21.56^{a}	5.63bc	15.33^{b}
	T_4 = Jackfruit waste + 5 % glyricidia leaves + yeast	22.35^{bc}	5.81bc	12.97^{ab}
LAB strain	T_5 = Jackfruit waste + 10 % azolla + LAB	21.54^{a}	6.13ab	11.76^{ab}
	T_6 = Jackfruit waste + 2 % $(NH_4)_2SO_4$ + LAB	21.53^{a}	5.48c	22.24^{c}
	T_7 =Jackfruit waste + 5 % glyricidia leaves + LAB	21.62^{a}	5.63c	12.16^{ab}
Yeast and LAB strains	T_8 = Jackfruit waste + 10 % azolla + yeast + LAB	21.75^{a}	6.16^{ab}	11.00^{ab}
	T_9 = JF waste + 2 % $(NH_4)_2SO_4$ + yeast + LAB	22.26^{bc}	5.49^{c}	23.37^{c}
	T_{10} = JF waste + 5 % glyricidia leaves + yeast + LAB	21.74^{a}	5.86^{abc}	12.99^{ab}
S.Em±		**0.16**	**0.18**	**1.47**
CD (at 5%)		**0.47**	**0.53**	**4.36**

Note: Fermentation period 7 days,

Yeast: *Saccharomyces boulardii*,

LAB: *Lactobacillus acidophilus*.

Table 3: Effect of supplementation and fermentation on ash, carbohydrates and moisture content of the fermented jackfruit waste

Strains	Treatments	Ash (%)	Carbohydrates (%)	Moisture (%)
	T_1 = Jackfruit waste only	5.58^{a}	73.73^{de}	5.42^{ab}
Yeast strains	T_2 = Jackfruit waste + 10 % azolla + yeast	6.22^{b}	76.15^{f}	5.77^{bcd}
	T_3 = Jackfruit waste + 2 % $(NH_4)_2SO_4$ + yeast	6.27^{b}	68.30^{b}	5.38^{ab}
	T_4 = Jackfruit waste + 5 % glyricidia leaves + yeast	6.96^{de}	74.92^{ef}	5.28^{a}
LAB strains	T_5 = Jackfruit waste + 10 % azolla + LAB	6.62^{c}	73.70^{de}	6.01^{cd}
	T_6 = Jackfruit waste + 2 % $(NH_4)_2SO_4$ + LAB	6.74^{cd}	63.21^{a}	5.74^{bcd}
	T_7 =Jackfruit waste + 5 % glyricidia leaves + LAB	7.05^{ef}	72.93^{d}	6.13^{d}
Yeast and LAB strains	T_8 = Jackfruit waste + 10 % azolla + yeast + LAB	7.27^{f}	73.16^{d}	5.71^{bc}
	T_9 = JF waste + 2 % $(NH_4)_2SO_4$ + yeast + LAB	6.73^{cd}	70.99^{c}	5.28^{a}
	T_{10} = JF waste + 5 % glyricidia leaves + yeast + LAB	6.86^{cde}	72.59^{d}	5.53^{ab}
S.Em±		0.08	0.46	0.12
CD (at 5%)		0.23	1.37	0.37

Note: Fermentation period 7 days,

Yeast: *Saccharomyces boulardii*,

LAB: *Lactobacillus acidophilus*.

CONCLUSION

Jackfruit waste is a mixture of rind, arils and perianth with high moisture content and nutritional value remains after minimal processing of jackfruit. Presently, this valuable byproduct waste is being utilized very limited (1 – 2%) due to high moisture content leads to composted or dumped in road sides which causes environmental health hazards. Hence, an attempt was made to recycle through solid state fermentation technology for developing nutritionally enriched animal feed from this waste for livestock as feed supplements. The yeast strain *Saccharomyces boulardii* and LAB strain *Lactobacillus acidophilus* performed better for solid state fermentation of jackfruit waste with respect to enhancement of nutrients. The results revealed that jackfruit waste supplemented with 2 per cent ammonium sulphate and combined fermentation (T9) helps in drastic increase in the crude protein, acidity, fat and slight reduction in fibre in the jackfruit waste thus improving its nutritional quality. Utilization of Jackfruit waste as an animal feed through solid state fermentation by probiotic yeast and lactic acid bacteria is advantage to dairy entrepreneurs which is simple and low cost technology.

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