RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.1.59-63

Assessment of growth responses of Wistar rats fed with a diet containing composite flour premix#

R. L. Beena[1]*, S. N. Rajakumar[2], Sajith Purushothaman[3], P. Sudheerbabu[4], A. K. Beena[5] and M. P. Divya[6]
Department of Dairy Technology,
Verghese Kurien Institute of Dairy and Food Technology (VKIDFT),
Mannuthy, Thrissur- 680651,
Kerala Veterinary and Animal Sciences University, Wayanad, India

Citation: Beena, R. L., Rajakumar, S. N., Purushothaman, S., Sudheerbabu, P., Beena, A. K. and Divya, M.P. 2023. Assessment of growth responses of Wistar rats fed with a diet containing composite flour premix. *J. Vet. Anim. Sci.* **54**(1):59-63
DOI: https://doi.org/10.51966/jvas.2023.54.1.59-63

Received: 20.07.2022 *Accepted: 25.10.2022* *Published: 31.03.2023*

Abstract

Composite flour technology involves the process of mixing cereals and legumes to facilitate the usage of locally available raw materials to produce high-quality food products economically. The present investigation evaluated the growth responses of a composite flour blend formulated using selected cereals, pulses and oil seed (a total of nine grains) were evaluated in two-month-old growing Wistar rats. The feeding trial was conducted for four weeks after an acclimatization period of five days. Daily feed intake and weekly body weight were recorded and parameters such as weight gain, feed conversion efficiency and digestibility of protein were estimated. A significantly higher average final live weight, weekly weight gain and better feed conversion efficiency ($p<0.05$) were observed in the treatment group. The observed values of digestibility of protein were 84.74 ± 0.85 % and 65.16 ± 0.89 % respectively for the treatment and control diets. Data were analyzed statistically using Analysis of Variance in SPSS 24 software. The investigation revealed that the formulated composite flour premix promotes growth with good protein digestibility in rats and could be used for nutraceutical fortifications to support human life and good health.

Keywords: *Growth responses*, *feed conversion efficiency, composite flour premix, protein digestibility*

#Part of PhD thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. *Assistant Professor*, Dept. of Dairy Technology, CDST, Thiruvananthapuram*
2. *Professor and Head, Dept. of Dairy Technology and Dean, VKIDFT*
3. *Assistant Professor, Dept. of Animal Nutrition, College of Veterinary and Animal Sciences*
4. *Professor, Dept. of Dairy Engineering, VKIDFT and Registrar, KVASU*
5. *Professor and Head, Dept. of Dairy Microbiology and Associate Dean, VKIDFT*
6. *Assistant Professor, Dept. of Dairy Chemistry, VKIDFT*

**Corresponding author: beenarl@kvasu.ac.in, Ph: 9947367580*

Malnutrition or malnourishment is a common problem for people of all ages. As per the recent report of the National Family Health Survey (NFHS-5) during 2019-21, a high percentage of undernourished women aged between 15 and 49 exist in India (NFHS, 2022). Cereal grains are good sources of energy with a considerable amount of carbohydrates, protein, minerals and sulfur-containing amino acids (Kavi *et al*., 2021), but deficient in certain essential amino acids, especially lysine (Iqbal *et al.,* 2006). Legumes which are regarded as poor man's protein, are rich in lysine but poor in methionine (Chardigny and Walrand, 2016). A judicious selection of ingredients will provide all the essential nutrients (Kushi *et al*., 1999; Kumari and Sangeetha, 2017) to ensure balanced nutrition. The composite flour technology is based on this concept which encourages the use of nutrient-rich cereals and other grains to produce high-quality food products economically. A composite flour pre-mix was formulated based on the nutrient requirement of Indian women as per the guidelines of the Indian Council of Medical Research (ICMR, 2020), the optimization process of which is discussed elsewhere. The current investigation was undertaken to evaluate the effect of dietary incorporation of the optimized composite flour as a feed resource in the diet of Wistar rats on their growth response.

Materials and methods

Composite flour premix

Four locally available cereals (wheat, barley, foxtail millet and finger millet), pulses (green gram, horse gram, soyabean and green peas) and an oil seed (black sesame) were used in the study. The composite flour was optimized based on the central composite rotatable design (CCRD) of response surface methodology (RSM). It was optimized at an ingredient composition of 62.25% cereal blend, 33.99 % pulse blend and 3.76 % oilseed in the composite flour premix.

Animal bioassay

The experiment was carried out as per the protocol approved by the Institutional Animal Ethical Committee (IAEC/CVASMTY 7/2021). Fourteen numbers of two-month-old female Wistar rats were selected from the small animal breeding station at the College of Veterinary and Animal Sciences, Mannuthy, Thrissur. They were randomly allotted to two groups of seven animals each. All animals were kept in individual cages under uniform ambient conditions. An acclimatization period of five days was provided before the start of the trial. The control group was fed with basal feed and the treatment group was fed with a diet in which 50 per cent of the basal feed protein was replaced by the composite flour premix. Both diets were formulated as per BIS standards (BIS, 2012) with similar protein content. Feed and water were offered to all the animals *ad libitum*. The ingredient composition of both diets is given in Table 1. The required quantity of each ingredient was weighed separately from respective flours, dry blended and stored in air-tight containers.

Feeding trial

The feeding trial was conducted for a period of four weeks. Weighed quantities of the feed were given individually to all animals every morning at 9 AM. Representative samples of the feed given and the leftover were collected and weighed daily for analyzing the dry matter (DM) intake. It was recorded for the entire experimental period. The body weight of all animals was recorded once weekly and the feed efficiency ratio was determined as done by Manjula *et.al*. (2016).

Digestion trial

A digestion trial for three days was carried out at the end of the feeding trial by the total collection method. Daily feed offered, the residue leftover and the quantity of faeces and urine were recorded at 9 AM every day. The dry matter content of the feed offered as well as the residue was determined daily. The faeces of each animal was collected in polyethylene bags and stored separately in a deep freezer (-20°C) for further analysis. Daily urine was transferred quantitatively to plastic vials, added with a drop of formaldehyde and stored in a refrigerator (4-5°C). At the end of the trial, the faecal matter collected for three consecutive days from each animal was pooled, mixed and analyzed for

Table 1. Ingredient composition of the diets offered to the experimental animals

Ingredients	Control	Treatment
Wheat (g)	16.56	1
Corn gluten fiber (g)	19.99	1
Gingelly oil cake (g)	24.98	8.99
Soybean meal (g)	11.99	17.99
Black gram husk (g)	24.98	6
Tallow (g)	1	5
Supplementary vit M (g)	0.25	0.25
Salt (g)	0.25	0.25
Composite flour pre-mix (g)	----	59.53
Total (g)	100	100

nitrogen content (AOAC, 2016) to determine the crude protein output. The crude protein of the feed offered was also analyzed to observe the apparent digestibility of protein in the feed. Statistical evaluation of the data obtained was done by the analysis of variance (Snedecor and Cochran, 1994) using SPSS software version 24.

Results and discussion

The chemical composition of the optimized composite flour premix and that of the two animal diets are presented in Tables 2 and 3, respectively. The amount of composite flour premix added in the treatment group was 59.53g per 100g of animal feed (Table 1). The diets of the control and treatment groups had 23.92 and 23.24 per cent of crude protein respectively on a dry matter (DM) basis.

The average body weight of animals recorded at weekly intervals is shown in Table 4. The mean initial weight of the control and treatment animals were108.04±6.59g and 113.05±5.84 g respectively and was not statistically different. Between ($p > 0.05$). But a significantly higher final body weight of 181.32±6.6 g was observed for the treatment group than the control animals which had 157.15±8.21g ($p < 0.05$). The result indicated that the high-quality nutrients in the test diet were readily absorbed by the body and was in accordance with the findings of Jood *et al.*, 1992, who found a similar increase in body weight of animals fed with cereal-based composite flour. The average daily body weight gain was 1.75 ± 0.09g and 2.44 ± 0.07g respectively for animals fed on control and treatment diets.

The total DM intake and the total weight gain of the animals fed with the control diet during the experimental period were 408.60 ± 23.33g and 49.11±2.51g respectively (Table 5). Deepak *et al.*, 2020 also made similar observations in a study of one month in Wistar rats. The treatment group had a significantly lower ($p<0.01$) total dry matter intake of 312.90±8.37g, but observed a significantly high ($p<0.01$) total body weight gain of 68.27±1.94g during the experimental period. Data on

Table 2. Proximate analysis of the composite flour pre-mix

Parameter	Composite flour pre-mix (%)
Dry matter	93.99
Crude protein (on dry matter basis)	17.06
Ether extract (on dry matter basis)	5.21
Crude fibre (on dry matter basis)	3.67
Ash (on dry matter basis)	2.96
Nitrogen-free extract (on dry matter basis)	65.09

Table 3. Proximate composition of the diets of experimental animals

Parameter	Groups	
	Control (%)	Treatment (%)
Dry matter	90.63	92.04
Crude protein (on dry matter basis)	23.92	23.24
Ether extract (on dry matter basis)	6.96	8.62
Crude fiber (on dry matter basis)	10.44	4.94
Ash (on dry matter basis)	8.29	4.52
Nitrogen-free extract (on dry matter basis)	50.39	58.68

Table 4. Weekly average body weight and average daily weight gain in Wistar rats

Period	Average body weight (g)		p-value	F-value
	Control	Treatment		
Initial	108.04±6.59	113.05±5.84	0.58	0.32 ns
First week	115.31±11.55	141.16±6.11	0.07	3.92ns
Second week	134.53±14.07	158.55±5.84	0.141	2.49 ns
Third week	146.95±10.88	173.32±6.28	0.058	4.41 ns
Fourth week	157.15±8.21	181.32±6.6	0.041	5.27*
Average daily gain	1.75±0.09	2.44±0.07	0.001	36.6**

Figures are the mean of seven replicates
ns- non-significant at p>0.05, *- significant at p<0.05, **- significant at p<0.01

Table 5. Average daily and total DM intake and feed conversion efficiency

Particulars	Groups		p-value	F-value
	Control	Treatment		
Total dry matter intake (g/animal)	408.60±23.33	312.90±8.37	0.002	14.91**
Total weight gain (g)	49.11±2.51	68.27±1.94	0.001	36.6**
Average daily dry matter intake (g)	14.59±0.83	11.18±0.30	0.002	14.9**
Feed conversion efficiency (g feed intake / g weight gain)	8.44±0.64	4.61±0.18	0.001	33.56**

Figures are the mean of seven replicates
**- significant at p<0.01

Table 6. Summarized data on crude protein intake and digestibility of experimental diets

Parameters	Groups		p-value	F-value
	Control	Treatment		
Total protein intake (g)	9.74±0.51	7.29±0.34	0.002	15.8**
Protein output (g)	3.38±0.15	1.11±0.07	0.001	177.5**
Net protein intake (g)	6.36±0.39	6.18±0.31	0.72	0.14ns
% Digestibility of protein	65.16±0.89	84.74±0.85	0.001	252.5**

Figures are the mean of seven replicates
ns-non-significant at a five per cent level (p<0.05)
**-significant at one per cent level (p<0.01)

cumulative feed conversion efficiency (kg feed/ kg weight gain) of the animals were 8.44±0.64 and 4.61±0.18g respectively for the control and treatment groups. A significantly lower (p<0.01) feed conversion efficiency observed in the treatment group indicates better body utilization of the nutrients in the feed (Manjula *et al.,* 2016).

Data on net protein intake and protein digestibility observed in the metabolic study are given in Table 6. The crude protein intake (protein content in the feed offered- protein content in the leftover) and the protein content in the faeces were determined by micro Kjeldahl method (conversion factor 6.25). The digestibility of the protein in the feed was calculated as follows;

$$\% \text{ Digestibility} = \frac{(\text{Crude protein intake} - \text{Crude protein output}) * 100}{\text{Crude protein intake}}$$

It was observed that the total crude protein intake by the control group, 9.74±0.51 g, was significantly higher (p<0.01) than in the treatment group (7.29±0.34 g). The protein output was 3.38±0.15 g for the control group and that of the treatment group was 1.11±0.07g. Though the total protein intake and output were lower in the treatment group, no significant difference was observed in the net protein intake by the two groups. This indicates that most of the protein in the treatment diet was absorbed into the body. It was also indicated by a higher protein digestibility of 84.74±0.85% in the treatment group against 65.16±0.89 in control animals. The result is in accordance with Aremu *et al*., 2011, who suggested that high faecal output is an indicator of reduced digestibility. Sarwar *et al.* (1989) studied protein digestibility in selected foods containing legumes like soya beans by rat balance method and reported about 70-85% of digestibility.

Acknowledgement

The authors would like to acknowledge the College of Veterinary and Animal Sciences, Mannuthy for providing the facilities at the Small Animal Breeding Station (SABS) to carry out the work.

Conclusion

In the present investigation, a composite flour pre-mix formulated using selected cereals, pulses and an oil seed was analyzed *in vivo* in female Wistar rats of 2 months old. The results revealed good protein digestibility and enhanced feed conversion efficiency in the composite flour premix, which promotes growth. Hence, it can be concluded that the composite flour premix can be considered a good choice for nutraceutical fortifications in human food.

Conflict of interest

The authors declare that they have no conflict of interest.

References

ICMR [Indian Council of Medical Research]. 2020. Report of National Institute of Nutrition. Department of Health Research, Ministry of Health and Family Welfare, Government of India.

A.O.A.C. [Association of Official Analytical Chemists]. 2016. *Official methods of Analysis*, (20th Ed). Washington, DC.

Aremu, M.O., Osifade, B.G., Basu, S.K. and Ablaku, B.E. 2011. Development and Nutritional Quality Evaluation of Kersting's Groundnut-Ogi for African Weaning Diet. *Am. J. Food Technol*. 6(12): 1021-1033.

BIS [Bureau of Indian Standards]. 2012. *Specification for compounded feeds for laboratory animals:* IS 5654-1: New Delhi. 17p.

Chardigny, J.M. and Walrand, S. 2016. Plant protein for food: opportunities and bottlenecks. *OCL*. **23**(4): 6.

Deepak, C., Jasmine Rani, K., Shyama, K. and Ally, K. 2020. Effect of dietary incorporation of ksheerabala residue on growth performance in wistar rats. *J. Vet. Anim. Sci.* **51**(2): 179-183.

Iqbal, A., Khalil, I.A., Ateeq, N. and Khan, M.S. 2006. Nutritional quality of important food legumes. *Food Chem*. **97**(2): 331-335.

Jood, S., Kapoor, A.C. and Singh, R. 1992. Biological evaluation of protein quality of maize as affected by insect infestation. *J. Agric. Food Chem.* **40**(12): 2439-2442.

Kavi Kishor, P.B., Anil Kumar, S., Naravula, J., Hima Kumari, P., Kummari, D., Guddimalli, R., Edupuganti, S., Karumanchi, A.R., Venkatachalam, P., Suravajhala, P. and Polavarapu, R. 2021. Improvement of small seed for big nutritional feed. *Physiol. Mol. Biol. Plants*. **27**(10): 2433-2446.

Kumari, P.V. and Sangeetha, N. 2017. Nutritional significance of cereals and legumes-based food mix-A review. *Int. J. Agric. Life Sci*. **3**: 115-122.

Kushi, L.H., Meyer, K.A. and Jacobs, D.R. 1999. Cereal, legume and chronic diseases risk reduction: Evidence from epidemiologic studies. *Am. J. Clin. Nutr*. **70**: 451-458.

Manjula, K., Raj, M.A. and Krishna, R. 2016. Feed efficiency and serobiochemical profile of wistar rats fed with spirulina as functional food. *Curr. Res. Nutr. Food Sci*. **4**(2): 135-140.

NFHS [National Family Health Survey]. NFHS-5 Report 2019-21. Ministry of Health and Family Welfare. Govt. of India [March 2022].

Sarwar, G., Peace, R.W., Botting, H. G.and Brulé, D. 1989. Digestibility of protein and amino acids in selected foods as determined by a rat balance method. *Plant Foods Hum Nutr.* **39** (1): 23-32.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. (8th Ed). The Iowa State University Press, Ames, USA. ■