International Journal of Research Studies in Biosciences (IJRSB)
Volume 10, Issue 1, 2024, PP 23-29
ISSN (Online) 2349-0365
https://doi.org/10.20431/2349-0365.1001004
www.arcjournals.org

The Effect of *Aspergillus niger* and *Saccharomyces Cerevisiae* on the Base Nutritional Value of Soybean Meal Used in Poultry Feed Formulation

Nqobani Ncube[1*], Cephas Mawere[2]

[1, 2]Biotechnology Department, School of Industrial Sciences and Technology, Harare Institute of Technology, P.O. Box BE 277, Belvedere, Harare, Zimbabwe.

Corresponding Author:** ***Nqobani Ncube, *Biotechnology Department, School of Industrial Sciences and Technology, Harare Institute of Technology, P.O. Box BE 277, Belvedere, Harare, Zimbabwe.*

Abstract

The availability of locally produced soybean meal (SBM) in substantial amounts compels the local poultry feed producers to use the ingredient as a major alternative to animal protein sources. The presence of anti-nutritional factors and variation of nutritional quality limits the extent to which soybean meal can be applied in poultry feed formulation. A two-step soybean meal fermentation process was carried out using Aspergillus niger and Saccharomyces cerevisiae for 48 hours at 37^{0}C and after fermentation the meal was sun dried. The fermented soybean meal (FSBM) and the soybean meal their proximate nutrient composition and amino acid concentrations were determined using AOAC standards. FSBM had the greater nutritional value and a higher total amino acid concentration than in SBM. This was due to the increase in the bioavailability of nutrients. This associated with the significant increase of small size peptides in FSBM, as long-chained proteins are broken down and reducing the levels of allergenic proteins and anti-nutritional factors in FSBM. Therefore, FSBM is a potential alternative to animal protein sources and can be used in poultry feed formulation by local poultry feed producers.

Keywords: *Soyabeans, anti-nutritional factors, Aspergillus niger, Saccharomyces cerevisiae.*

1. INTRODUCTION

Nutrition is central in poultry production because of its direct influence on broiler performance and production economics. Feed cost contributes at least 60% of the total production costs in poultry production, since the common ingredients used in poultry feed formulation include fish meal, meat meal, corn gluten which have different protein concentrations which are expensive (SMA, 2018). The Stock feeds Manufacturers Association report in 2018 report highlighted that the average monthly procurement of soybean derivatives in the first quarter of 2018 was at 11,084 metric tonnes per month (USD$6.9 million). This represented a volume increases for the first quarter 59% (51% in value) over the same period in 2017 and a 29% increase from the last quarter of 2017 (SMA, 2018). This revealed the soybean derivatives preference level and its acceptability in terms of usage by poultry feed manufacturing mills in Zimbabwe. Apart from being expensive, most protein sources from animal origin are generally highly infested and carry heavy microbial loads (Choct *et al.*, 2010).

Aspergillus niger and *Saccharomyces cerevisiae*, as microbial agents, have demonstrated potential in enhancing the nutritional quality of soybean meal through various mechanisms. Studies have indicated that these microorganisms can effectively degrade anti-nutritional factors such as phytic acid, oligosaccharides, and protease inhibitors present in soybean meal (Smith and Bedford, 2000). Decreased levels of these factors present in SBM, including trypsin inhibitors, lectins, and phytic acid factors can enhance nutrient utilization and reduce the risk of digestive disturbances in poultry (Bai *et al.*, 2017). Additionally, they contribute to the synthesis of enzymes such as phytase and xylanase, which facilitate the breakdown of complex carbohydrates and enhance nutrient availability (Bedford, 2000).

Furthermore, research suggests that microbial fermentation with *Aspergillus niger* and *Saccharomyces cerevisiae* can lead to increased protein digestibility and amino acid availability in soybean meal (Adebokun *et al.*, 2008)). This process may also result in the production of beneficial metabolites,

including vitamins and organic acids, which could further augment the nutritional value of the feed (Lückstädt *et al.,* 2013). Fermentation processes can increase the availability of nutrients such as phosphorus, calcium, and trace minerals in SBM through enzymatic breakdown and chelation mechanisms (Niu *et al.,* 2021). Overall, these findings underscore the potential of microbial fermentation techniques involving *Aspergillus niger* and *Saccharomyces cerevisiae* to optimize the base nutritional composition of soybean meal, thereby improving its suitability for poultry feed formulation.

Improved nutrient bioavailability can promote optimal growth performance and overall health in poultry (Zhang *et al.,* 2019). The use of *Aspergillus niger* and *Saccharomyces cerevisiae* in SBM fermentation can positively influence the composition and activity of gut microbiota in poultry (Zhang *et al.,* 2020). Despite the potential benefits, the economic feasibility of incorporating fermented SBM into poultry feed formulations should be carefully evaluated, taking into account the cost of fermentation and potential improvements in performance and health parameters (Wang *et al.,* 2021).

Layer and broiler production in most countries aims at closing the gap of animal protein deficiency (Lee *et al.,* 2009); therefore this led to a tremendous improvement in the past few decades in poultry production. This great improvement encompassed almost all aspects of poultry production such as nutrition and genetic selection. Hence this study investigated the effect of fermented soybean meal on the of soybean meal.

2. MATERIALS AND METHODS

Soybeans procured from Plot 16, Kent were ground to a particle size (<500 μm) by screen diameter to produce a meal. Heat sterilization was performed by spreading approximately 1kg of soybean meal on an aluminum tray and heated in the oven dryer for 30 minutes at 125 °C (González-Vega *et al*.,2011). Treated soybean meal was removed from the oven, covered in aluminums foil, and stored in a previously autoclaved glass jar until it was needed. The fermentation culture medium was in a ratio of (97%) soybean meal (SBM): (2%) maize meal (2%) and (1%) glucose. The moisture content of culture medium was adjusted to about 60% and the substrate was mixed with the *A. niger*spore suspension (2×107 spores/mL) per flask.

The inoculated substrate was incubated at 30°C for 72 hours. Triplicate samples were taken for analysis after every 6 hours of fermentation. After *A.niger* fermentation process, the one step fermented soybean meal was dried so as to inactivate the *A.niger*. The one-step fermented soybean meal was inoculated with active cells of *S.cerevisiae*. The initial concentration of the yeast in the flask was approximately 0.002g/ml. The second step of fermentation was at 37°C for 10 hours and after the fermentation process, the fermented soybean meal (FSBM) was sun dried. The dried FSBM was ground through a 2 mm mill and stored in a dry environment.

Determination of the proximate chemical composition, that is, crude protein lipids, ash and crude fiber and the determination of the amino acid concentration in FSBM and SBM were determined following the standard methods of AOAC (2005). The content of intestines was collected in sterile containers as a bulk sample from each of the analyzed groups. Then 20g of collected contents were placed into bottles containing 180ml of the dilutions, and homogenized. The next step was to make a series of decimal dilutions and plate prepared samples for microbiological growth.

3. RESULTS

3.1. Organoleptic Characteristics of FSBM

The SBM,that is, (A) during the fermentation process turned from pale yellow colour to a golden yellow colour,that is,(B) as shown in Figure 1 below. The following were the oragnoleptic characteristics of the fermented soybean meal: distinctive smell (sweet smelling aroma), golden yellow colour and rough texture (after drying).

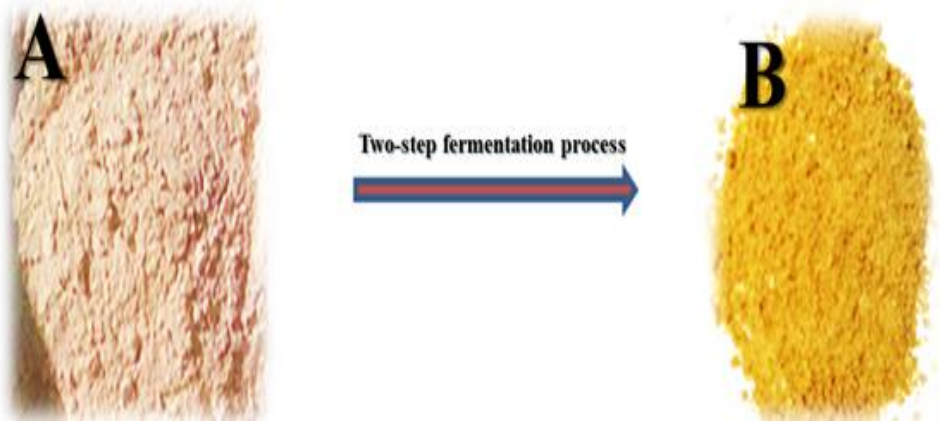

Figure 1. *The colour change of SBM before and after fermentation*

3.2. Proximate Nutrient Composition of FSBM and SBM

The CP, CF, NDF, Fat, Ash and P (33.5, 4.54, 47.41, 16,48, 4.10 and 0.39 respectively) was higher in FSBM than in the SBM, while the latter had a higher dry matter and calcium content (90.6 and 0.67 respectively) than the FSBM as show in Table 1 below. FSBM had the greater nutritional value across all test nutrient parameters per 100g as shown in Figure 3.

Table 1. *Proximate nutrient composition of FSBM and SBM*

Test Parameter (g/100g)	DM	CP	CF	NDF	Fat	Ash	Ca	P
	%	%	%	%	%	%	%	%
FSBM	89.6	33.5	4.54	47.41	16.48	4.10	0.65	0.39
SBM	90.6	27.1	4.46	44.73	10.73	3.60	0.67	0.24

Notes: Ca-Calcium; P- Phosphorus; Nacl -Sodium Chloride; CP- Crude Protein; Fat; DM-Dry Matter; NDF-Neutral Detergent Fiber; ME-Metabolisable Energy

3.3. Amino Acid Concentrations of SBM and FSBM

Table 2 below shows the amino acids concentration in FSBM and SBM. The total amino acid concentration of FSBM is higher than SBM amino acid concentration as shown in Figure 3. The essential amino acids concentrations for poultry such as glycine, histidine, leucine, isoleucine, lysine, methionine, cysteine, phenylalanine, threonine and tryptophan were higher in FSBM than in SBM. The glutamic acid concentration was the highest in both SBM and FSBM, however between the two FSBM had the glutamic acid concentration, as show in Figure 2 below.

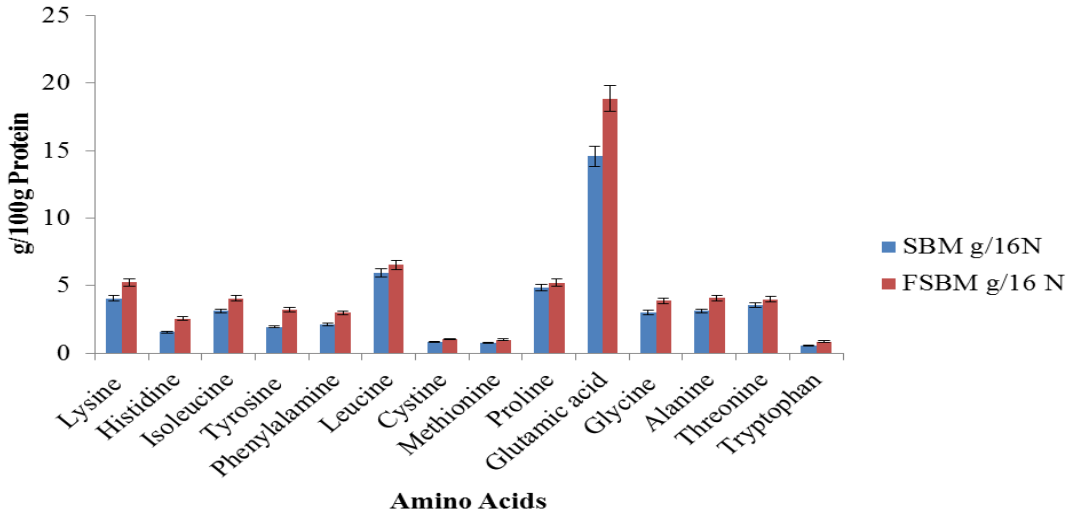

Figure 2. *Amino acid profile of SBM and FSBM.*

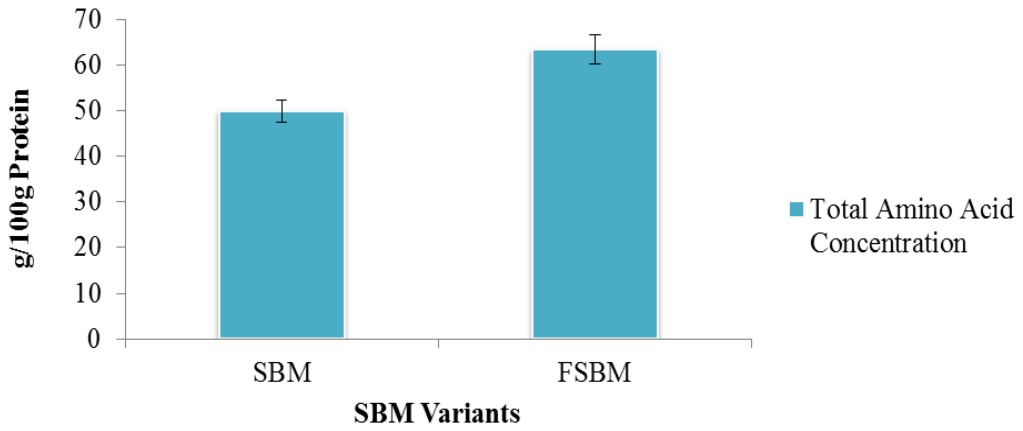

Figure 3. *Total Amino Acid concentration of SBM and FSBM.*

Table 2. *Amino acid concentrations of SBM and FSBM*

Amino Acid	SBM (g/16N)	FSBM (g/16N)
Lysine	4.05	5.23
Histidine	1.56	2.56
Isoleucine	3.12	4.05
Tyrosine	1.95	3.21
Phenylalanine	2.09	2.98
Leucine	5.94	6.52
Cysteine	0.81	1.02
Methionine	0.75	0.99
Proline	4.84	5.21
Glutamic acid	14.56	18.85
Glycine	3.01	3.87
Alanine	3.11	4.09
Threonine	3.54	3.98
Tryptophan	0.56	0.86
Total Amino Acid Concentration	49.89	63.42

3.4. Feed Microbiological Safety

The microbial count in the intestinal content of broilers fed with feed containing FSBM was lower than in those fed with feed containing SBM as shown in Figure 4. SBM feed had a higher, number of coliforms present, the total overall number of fungi and the total number of microorganisms in terms of the colony forming units per gram (cfu/g) at Log 10, 3.56, 1.65 and 5.27 respectively compared with 2.96, 1.13 .4.29 respectively in FSBM as shown in Table 3 below.

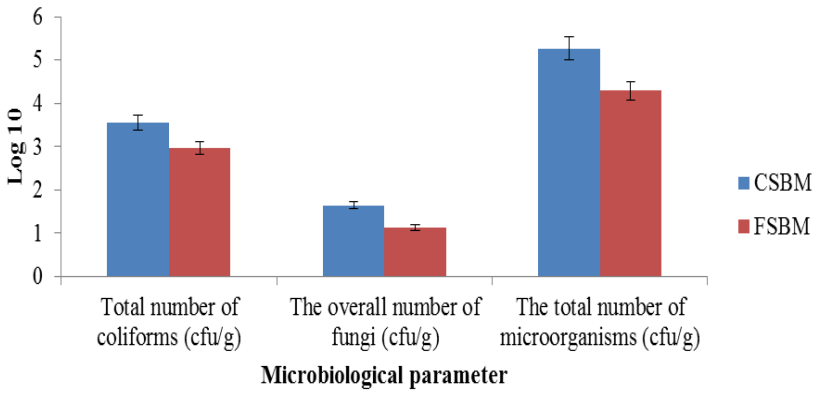

Figure 4. *Microbiological Analysis.*

Table 3. *Feed microbiological research*

Microbiological safety parameters	SBM		FSBM	
	Result	Log 10	Result	Log 10
Total number of coliforms (cfu/g)	3636	3.56	909	2.96
The overall number of fungi (cfu/g)	45	1.65	14	1.13
The total number of microorganisms(cfu/g)	184545	5.27	19545	4.29

Colony forming units per gram (cfu/g)

4. DISCUSSION

4.1. Organoleptic Characteristics of FSBM

Fermentation of soybean meal resulted in a golden yellow, sweet smelling aroma of the fermented soybean meal. This is due to the presence of organic acids such as lactic acids present in FSBM. Also the colour change is attributed to the sun drying of FSBM that caused a golden yellow colour.

4.2. Proximate Nutrient Composition of FSBM and SBM

The FSBM had higher crude protein and fat content than the SBM. Thus 19% crude protein increase was observed after a two-step fermentation of soybean meal with *Aspergillus niger* and *Saccharomyces cerevisiae.* This is attributed to the *Aspergillus* species and *Saccharomyces cerevisiae's* capability to produce enzymes such as hemicellulases, hydrolases, pectinases, protease, amylase, lipases, and tannases(Orleans and Green, 2011). The fermentation by *Aspergillus species* and *Saccharomyces cerevisiae* increases the bioavailability of nutrients (Mo *et al*., 2016).This is associated with the significant increase of small size peptides in FSBM (Spanghero *et al.*, 2017), as long-chained proteins are broken down.

The fungal fermentation also increases the nutritional value of the soybean meal by increasing the fat, ash, phosphorus and crude protein (CP) contents (Divate *et al*., 2016). This is in agreement with the results obtained after the two-step fermentation of soybean meal by *Aspergillus niger* and *Saccharomyces cerevisiae* as shown in Table 1. The phosphorus content increased by 69% in FSBM, as shown in Table 1.The ability of *Aspergillus niger* and *Saccharomyces cerevisiae* to produce phytase which digests phytic acid, releasing phosphorus. Hence, effectively increases the bioavailability of phosphorus (Jongbloed *et al*., 1992). Thus *Aspergilli* almost completely eliminates phytate, resulting in FSBM with highly available phosphorus (Soomro *et al*.,2017).

4.3. Amino Acid Concentrations of SBM and FSBM

The total amino acid concentration of FSBM is higher than SBM amino acid concentration as shown in Figure 3. Thus 27% amino acid concentration increase was observed after a two-step fermentation of soybean meal with *Aspergillus niger* and *Saccharomyces cerevisiae.* This is associated with the significant increase of small size peptides in FSBM (Spanghero*et al.*, 2017). Since the long-chained proteins are broken down by proteases produced by the *Aspergillus niger* and *Saccharomyces cerevisiae*. According to Hong *et al*., (2004), soybean meal fermented with *Aspergillus* species for 48 hours, successfully degrades the large allergenic proteins. Thus increasing crude protein content and improving the essential amino acid profile of soybean meal. The glutamic acid concentration was the highest in both SBM and FSBM, however between the two FSBM had the glutamic acid concentration, as show in Figure 2 below. Glutamic acid is present in largest concentration in 100grams, hence this contributed to glutamic acid having the highest concentration.

Fermentation of SBM with *A.niger* increased the concentrations of histidine, isoleucine, tyrosine, phenylalanine, glutamic acid and glycine as shown in Figure 2. This finding is in partial agreement with the results of Bonvini *et al*., (2018), in which the contents of most of essential AAs improved. However some essential AA profile remained significantly unchanged such as for cysteine, tryptophan and methionine after fungal fermentation. This is due to the *Aspergillus niger* and *Saccharomyces cerevisiae* preferentially using specific AAs rather than all. These findings are in agreement with the suggestion by Soomro *et al.*, (2017), who suggested that concentrations of certain

AAs increases, as well as the changes in AA profile of FSBM could be attributed to microbial metabolism that takes place during SBM fermentation.

4.4. Feed Microbiological Safety

The microbial count in the intestinal content of broilers fed with feed containing FSBM was lower than in those fed with feed containing SBM as shown in Figure 4. This is due to the increase in lactic acid concentration in the FSBM hence reducing or limiting the number of pathogenic microorganisms in the FSBM.

5. CONCLUSION

FSBM had a higher amino acid concentration and crude protein compared to the SBM, after the hence a proximate composition and amino acid concentrations, hence it can be concluded that fermentation of the SBM improves the nutritional value and amino acid concentration of the SBM. This is associated with the significant increase of small size peptides in FSBM as long-chained proteins are broken down by proteases enzymes and other enzymes such as hemi-cellulases, hydrolases, pectinases amylase, lipases, and tannases during the two step fermentation process. Thus reducing and eliminating the anti-nutritional factors and allergenic proteins present in the SBM. However there is need for optimization of the solid state SBM fermentation, so as to monitor and maintain fermentation activity conditions. Based on the findings, FSBM is a potential alternative to animal protein sources such that can be used in poultry feed formulation by local poultry feed producers and poultry feed users.

6. DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

7. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

8. ACKNOWLEDGEMENT

This work was partially funded by the Harare Institute of Technology.

REFERENCES

[1] Adedokun, S. A., Adeola, O., andParsons, C. M. (2008). Comparative Efficacy of a Multicarbohydrase Complex and Phytase in Improving Nutrient Utilization of Diets Containing Low-Phosphorus Corn and Soybean Meal for Young Broilers. Poultry Science, 87(11), 2244–2253.

[2] AOAC., (2005). Official Methods of Analysis.Association of Official Analytical Chemists. Arlington, VA.

[3] Bai, K. W., Huang, Q. C., Zhang, J. Y., He, J. H., Zhang, L. L., Wang, T., ... and Zhang, N. N. (2017). Effect of fermented rapeseed meal on the growth performance and immune index of yellow-feathered broilers. Animal Nutrition, 3(4), 340-345.

[4] Bedford, M. R. (2000). Exogenous Enzymes in Monogastric Nutrition – Their Current Value and Future Benefits. Animal Feed Science and Technology, 86(1–2), 1–13.

[5] Bonvini, E., Bonaldo, A., Mandrioli, L., Sirri, R., Dondi, F., Bianco, C., Fontanillas, R., Mongile, F., Gatta, P.P. and Parma, L., 2018.Effects of feeding low fishmeal diets with increasing soybean meal levels on growth, gut histology and plasma biochemistry of sea bass. *Animal*, *12*(5), pp.923-930.

[6] Choct, M., Dersjant-Li, Y., McLeish, J. and Peisker, M., (2010). Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australasian journal of animal sciences, 23(10), 1386-1398.

[7] Divate, R, D., Wang, C.C., Chou, S.T., Chang, C.T., Wang, P.M., Chung, Y.C., (2016).Using wheat bran and soybean meal as solid state fermentation substances for the production of *Xylarianigripes* with bioactivities. J Taiwan InstChem E 70:127-133.

[8] Hong, K. J., Lee, C. H., Kim, S. W., (2004). *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. Journal of medicinal food, 7(4), 430-435.

[9] Jongbloed, A. W., Mroz, Z., Kemme, P. A., (1992). The effect of supplementary *Aspergillusniger*phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. Journal of Animal Science, 70(4), 1159-1168.

[10] Lückstädt, C., Mellor, S., Weitzel, J. M., and Breves, G. (2013). Effects of Different Yeast Products on Growth Performance and Intestinal Enzyme Activities of Broiler Chickens. Archives of Animal Nutrition, 67(3), 248–259.

[11] Niu, D. Y., Liu, Y. J., Dong, B., Liu, Q., and Wang, Z. X. (2021). Effects of fermented soybean meal on growth performance, serum biochemical indexes, immune function and microflora in weaned piglets. Animal Husbandry and Veterinary Medicine, 53(4), 76-82.

[12] Orleans, C.T and Green, L.W., (2011). Evidence-Based Prevention and Health Reform: Opportunities and Challenges for Behavioral Medicine and Sbm. Ann Behav Med 41:123-133.

[13] Smith, T. K., and Bedford, M. R. (2000). Enzymes and Enzyme Interactions. In A. G. P. J. Lewis and M. R. Bedford (Eds.), Enzymes in Farm Animal Nutrition (pp. 267–291). CABI Publishing.

[14] Soomro, R.N., Yao, J., Hu, R., Memon, A., Abbasi., (2016). Effects of Dietary Fat Supplementation on Hematology and Growth Trait in Broiler Chickens. Advances in Animal and Veterinary Sciences 4:518-526.

[15] Spanghero, M., Mason, F., Zanfi, C., Nikulina, A., (2017). Effect of diets differing in protein concentration (low vs medium) and nitrogen source (urea vs soybean meal) on in vitro rumen fermentation and on performance of finishing Italian Simmental bulls.

[16] Wang, S., Zhang, W., Liu, C., and Zhang, Y. (2021). Effects of Aspergillus niger-fermented soybean meal on growth performance, antioxidant capacity, immunity and gut microflora in weaned piglets. Journal of Henan Agricultural Sciences, 50(3), 96-103.

[17] Zhang, J. Y., Zhang, L. L., Wang, T., Huang, R. L., Li, L. L., Lu, Z. X., ... and Yu, D. Y. (2019). Effects of Aspergillus niger-fermented rapeseed meal on growth performance, nutrient digestibility, and intestinal health in broiler chickens. Poultry Science, 98(11), 5577-5585.

[18] Zhang, Y. B., Chen, J., Li, S. X., Tang, Y. Q., Zhu, W. Y., and Shi, S. R. (2020). Effects of fermented soybean meal on growth performance, intestinal development and immune response of broilers. Journal of Gansu Agricultural University, 55(4), 33-38.

Citation: *Nqobani Ncube, Cephas Mawere. The effect of Aspergillus niger and Saccharomyces cerevisiae on the base nutritional value of soybean meal used in poultry feed formulation. International Journal of Research Studies in Biosciences (IJRSB). 2024; 10(1):23-29. DOI: http://dx.doi.org/10.20431/2349-0365.1001004.*