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Lactational performance of dairy buffaloes affected by replacing soybean meal with an alternative microbial protein source

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A R T I C L E I N F O

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A B S T R A C T

Thirty dairy buffaloes were used for 90 days to evaluate the impact of a novel protein source (HI-PRO®), a feed alternative rich in protein (made from Saccharomyces and Bacillus), on the productivity of nursing buffaloes. The nursing water buffaloes had an average weight of 550 ± 11.2 kg. The treatments consisted of diets containing two distinct protein sources: (1) soybean meal (44 % as the control group); and (2) HI-PRO® product. Throughout the trial, measurements of nutritional digestibility and blood metabolites (total protein, albumin, urea, and creatinine) were conducted. Quantity and composition of the milk were measured to determine the content of milk proteins, lipids, and lactose. The outcome of the results showed insignificant decrease in the buffaloes receiving HI-PRO® feed *versus* control group. Furthermore, there was enhancement in the fiber digestibility by approximately 2.5 % in the HI-PRO® group compared to the control group. The levels of protein, albumin, globulin, urea, and creatinine fall within the normal range for animals in good health. Using HI-PRO® resulted in a marginal improvement in milk production, increasing it by approximately 4.8 % compared to soybean meal. Furthermore, the buffaloes fed HI-PRO® showed a slightly elevated 4 % fat-corrected milk output and milk composition. To summarize, nursing buffaloes can utilize HI-PRO® as an efficient protein source in their diets, replacing soybean meal.

Abbreviations

ADF	Acid detergent fiber
NDF	Amylase-neutral detergent fiber of organic matter
CF	Crude fiber
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
DDGS	distillers' dried grain with soluble
DM	Dry matter
ECM	energy corrected milk
EE	Ether extract
FCM	fat corrected milk
MY	milk yield
NDF	Neutral detergent fiber
NEg	net energy for growth
NEl	net energy for lactation
NEm	net energy for maintenance

(*continued on next column*)

(*continued*)

SCP	Single cell protein
SNF	Solids not fat
TDN	Total digestible nutrient
TS	Total solids

1. Introduction

Feeding animals is essential in the animal sector to produce meat, milk, eggs, fiber, and other valuable goods [1]. The fodder is a large determinant of costs and final product quality. For instance, it is well known that dairy animals experience weight loss and a decrease in milk production as a result of insufficient food supply. The National Research Council documented in 2001 [2] that long-term restriction of caloric

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intake and protein impedes fertility. Dietary techniques optimize rumen function, maximizing milk output and its constituents. Specifically for cows in the early lactation stage, methods that affect the constituents of milk involve ensuring an adequate amount of forage NDF (neutral detergent fiber) and rumen-degradable protein in their diet [3]. Proteins provide one of the primary nutritional constituents in all varieties of feedstuffs. Proteins are crucial nutrients that significantly influence the productive performance of animals [4]. The environmental consequences of animal feed production are associated with the "protein gap," which has consequently resulted in the importation of substantial quantities of raw protein ingredients for animal feeds and subsequently increased the price of delivering these feeds [5]. soybean meal remains the preferred option for protein meals due to its low cost, acceptable quality and widespread availability [6]. Common sources of high-protein feed include cereals, legumes, distillers' dried grain with soluble, fish and animal byproducts (fishmeal), oilseed (rapeseed meal) and its derivatives, co-products of biofuel manufacturing, and formulations incorporating amino acids. In addition to conventional sources, alternative feed proteins can be derived from insects, microalgae, single-celled organisms, and waste streams from other businesses such as e.g. potato peels, although these sources are rarely exploited today.

Buffaloes have been part of Asian agriculture for more than 5000 years, to provide meat, milk, hide, and power, and today, the stock is more than 170 million animals [7,8]. The species (*Bubalus bubalis*) therefore is of high relevance. Researchers have observed a difference in the buffalo's consumption of nitrogen in the ration compared to cattle, primarily due to variations in the microbial population in the rumen [9]. The production of animal protein, such as meat and milk, relies on the availability of plant protein. This plant protein might come from sources like grass from grazing or animal feed. However, to obtain enough plant protein for animal production, significant amounts of land are required, which is partly due to the fact that cattle have a very disadvantageous feed conversion ratio on the order of 10, and approx. 7 for buffaloes [10]. The cultivation of feed and fodder necessitates the application of nitrogenous fertilizers, which, if not utilized in the recommended quantities, can lead to a range of issues like eutrophication and excess costs. Mineral fertilizer production is also a major contributor to climate change [11]. Likewise, the storage and preparation of animal feeds can present economic and environmental challenges. Specifically, the issue lies in the provision of protein, as essential amino acids are irreplaceable. A potential resolution to this issue is the synthesis of single-cell protein (SCP). According to Nalage et al. [4], SCP are becoming increasingly popular due to their ability to grow on a small area of land, with minimum water and energy footprint, and they can grow on a wide variety of substrates, amongst which several waste and side streams are found. Microorganisms have very high growth rates and are non-demanding, which opens the possibility to produce protein more efficiently than from conventional sources.

However, the SCP (*i.e.,* single-cell proteins) are obtained by extracting microorganisms with a high protein content, either in the form of dried cells or as pure proteins [12]. The nutritional value and utility of SCP from any source depend on its composition [13], particularly protein content, amino acid profile and other constituents. That feedstuff contains proteins (up to typically 70 %), carbohydrates, lipids, nucleic acids, non-protein nitrogenous compounds, vitamins, and inorganic substances [4]. Animal feed incorporates of SCP from a wider variety of sources than those approved for human consumption. Some SCP for food – e.g. mycoprotein Quorn™ and Spirulina and Chlorella microalgae have been on the market before the introduction of Novel Food legislation (in the EU in 1997), while a few selected others have received Novel Food Approval in the last years. In the EU, the EU feed catalogue EU 68/2013 is the relevant feed legislation.

Key drivers to replace soybean protein and fishmeal protein are sustainability, but also performance issues at high dosages; For instance, Lim et al. [14] studied the replacement of fish meal by defatted soybean meal with supplemental monocalcium phosphate and limiting amino acids lysine and methionine and found a negative impact at 40 % and 60 % dosing (by mass). The SCP has also been considered as an alternative protein source in case of a global food catastrophe since its production is decoupled from primary agricultural operations [15,16]. The aim of this study was to investigate the impact of substituting soybean meal with a novel protein source in the diet of dairy buffaloes. The study focused on evaluating the effects on digestibility, milk yield, and milk composition.

2. Material and methods

The present study was conducted at a privately owned farm, specifically the Noubaria dairy buffalo farm, located in the Beheira Governorate of Egypt.

2.1. HI-PRO® Protein

The HI-PRO® is a new, advanced product developed using fermentation technology to achieve a highly concentrated protein formulation. This is a liquid that is easy to consume and contains a high amount of protein (almost 80 % on dry mass basis). Some ingredients of this product (per 1 L): L-arginine (90,000 mg); lysine (75,000 mg); methionine hydroxy analogue (80,000 mg); vitamin B3 (5000 mg); vitamin B5 (1500 mg); and Excipients q.s.p (Milli-Q water, 1 L), without any antibiotics. The material uses agricultural waste streams as feedstocks and has a protein content (Kjeldahl) of 30 % (dry matter). It is obtained from non-genetically modified microorganisms, *Saccharomyces cerevisiae* and *Bacillus* sp.

The specific formulation was found to increase food intake, promote weight gain, and enhance milk production in ruminants and chicken, according to preliminary studies by the authors. It provides energy to support the activity of microorganisms in the rumen and helps prevent the separation of feed components in the diet. Because of its high protein concentration, the original super protein concentrate is an excellent substitute for all other protein concentrates. Additionally, it includes sucrose, which makes up 29 % of the total carbohydrates, as well as glucose (12 %) and fructose (13 %). The presence of mannan and glucan in it enhances animal immunity [17]. HI PRO™ is *Saccharomyces cerevisiae* and *Bacillus* sp. based.

2.2. Animals

A total of thirty nursing buffaloes, with an average weight of 550 ± 11.2 kg, were divided into two groups, each consisting of fifteen animals. The animals commenced ingesting the experimental meal approximately 24 h subsequent to parturition. The lactation experiment lasted approximately 90 days. The individualized care and accommodations were provided for each animal in the enclosed barn. Animals were nourished in accordance with the ICAR [2] requirements tables, catering to their production and maintenance needs.

2.3. Experimental design, treatments, and feedings

Buffaloes were separated into said two groups, each containing fifteen animals, and animals were subjected to two treatments, which included various sources of protein: (1) soybean meal at a concentration of 44 %; and (2) HI-PRO® products, which were both mixed with the diet in a feed mixer to uniformity, and to get a good texture with a suitable palatability to the animals. The experiment was designed on the basis of CRD (completely randomized design) and the experiment lasted for a total of 90 days, with the initial 7 days dedicated to adapting to the new conditions. However, offered feed during the experiment was based on the average measured daily intake for those 7 days. Rations were developed using CPM-Dairy 3.0.10, which is based on the Cornell Net Carbohydrate and Protein System (CNCPS) 5.0, as described by Fox et al. [18].

Table 1 displays the constituent composition of the rations, while

Table 1
Ingredient and chemical composition of total mixed ration of experimental lactating buffaloes and HI-PRO® ration.

Ingredients (% = starch)	Rations, kg			
	Control		HI-PRO® formulation	
	Fresh matter	Dry matter	Fresh matter	Dry matter
Corn grain, 69 % starch	2	1.75	2	1.75
Barley grain ground, 58 %	3	2.66	3	2.66
Sugarcane molasses, 49 %	0.25	0.18	0.25	0.18
Soybean meal mech, 44 %	0.40	0.38	0	0
HI-PRO® SCP	0	0	0.20	0.18
Alfalfa hay, 53.16 %	3	2.65	3	2.65
Wheat bran	1.10	0.98	1.10	0.98
Beet pulp pellets	0.70	0.62	0.70	0.62
Corn silage	10	3.50	10	3.50
Wheat straw	1.5	1.29	1.5	1.29
Rice straw	1.3	1.16	1.3	1.16
Limestone, ground	0.13	0.13	0.13	0.13
Salt white	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.07	0.07	0.07	0.07
Trace mineral premix	0.04	0.04	0.04	0.04
Vitamin premix ADE[a]	0.02	0.02	0.02	0.02
Totals	23.61	15.52	23.41	15.32

[a] Containing 141 g/kg of Ca, 27 g/kg of P, 65 g/kg of Mg, 14 g/kg of S, 120 g/kg of Na, 6 g/kg of K, 944 mg/kg of Fe, 1613 mg/kg of Zn, 484 mg/kg of Cu,1748 mg of Mn, 58 mg/kg of I, 51 mg/kg of Co, 13 mg/kg of Se, 248,000 U/kg of vitamin A, 74,000 UI/kg of vitamin D3 and 1656 IU/kg of vitamin E.

Table 2
Chemical analysis and nutritive value of total mixed ration of experimental lactating buffaloes and HI-PRO® ration.

	Rations	
	Control	HI-PRO® formulation
Nutrient, %[a]		
Dry matter	65.72	65.45
Crude protein	10.52	10.53
Crude fiber	15.77	15.82
Ether extract	2.66	2.51
Ash	7.68	7.62
NDF	40.48	40.68
ADF	25.25	25.38
ADL	4.35	4.38
Nutritive value, Mcal/kg[b]		
Digestible energy	2.05	2.03
Metabolizable energy	2.27	2.25
NEm NRC	1.50	1.49
NEg NRC	0.91	0.90
NEl 3x NRC	1.39	1.38
TDN 1x, %	65.4	65.2

[a] NDF: Amylase neutral detergent fiber of organic matter. ADF: acid detergent fiber, ADL: Acid detergent lignin, NEm: net energy for maintenance, NEg: net energy for growth, NEl 3x: net energy for lactation, TDN 1x, %: Total digestible nutrients.
[b] Calculated using published values of feed ingredients [2].

Table 2 presents the chemical composition and nutritional value of the experimental rations. The authors achieved this by giving the buffaloes an unlimited amount of food for 7 days before the experiment started, and then measuring the amount of dry matter each buffalo had consumed. Animals were fed twice at 7:00 a.m. and 6:00 p.m., and it was ensured that they had unlimited access to fresh water. The buffaloes were milked twice a day, at 5:00 a.m. and 3:00 p.m.

2.4. Apparent digestibility

Digestibility was assessed according to Van Kulen and Young's [19] guidelines, using silica as an internal marker. Oats samples were collected from the rectum of each buffalo in the morning after feeding and around 4 h later for three consecutive days in week 12 of the study. The total weight of the collected feces was approximately 100 g each. Subsequently, the samples were merged by buffalo. The feces samples underwent a 48-h drying process at a temperature of 55 °C prior to chemical analysis. After that, the material was crushed into fine particles using a feed mill and filtered to obtain particles with a diameter of max. 1 mm. The following equation was used to calculate the digestibility of the nutrient:

Digestibility (%) = 100 * (Nutrient in Feed - Nutrient in Feces)/(Marker in Feed - Marker in Feces).

2.5. Measurement, sample collection, and preparation

The following parameters were determined: DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), EE (ether extract), and AC (ash content) in both the ration and feces samples using the established AOAC [20] protocols. Buffaloes' milk production was assessed twice a day, both in the morning and in the evening, the milk analysis data is based on the average composition of two daily samples. An ultrasonic milk analyzer was used, specifically the Milkotester Master from Milkotester LTD in Belovo, Bulgaria, to analyze milk samples. This analysis was conducted daily during the final week. The purpose of the analysis was to determine the composition of the milk, including its protein, fat, and lactose content. Each buffalo underwent blood sampling from the jugular vein using heparinized syringes on the last day of the experiment. The collected samples were immediately centrifuged. The plasma was aliquoted, separated, and stored at a temperature of −20 °C, prior to analysis of total protein, albumin, and urea.

2.6. Statistical analysis

For statistical analysis, the data underwent statistical analysis using one-way ANOVA in SPSS software (version 20). The statistical techniques of analysis of variance and Duncan's multiple range tests were employed to identify significant differences ($P < 0.01$) among the various treatments using the following model:

$$Y_i = \mu + R_i + e_{ij}$$

Where:

Y_i: is the dependent variable; μ: the overall mean, R_i: the effect of ration, e_{ij}: experimental error.

3. Results

3.1. Feed intake and digestibility

For meals' digestibility coefficients and feeding values, no statistically significant differences were observed in neither the control group nor the HI-PRO® group. Nevertheless, the feed intake of buffaloes fed with HI-PRO® was marginally lower compared to the control group by about 1.3 %. In the HI-PRO® group, the digestibility of fibers showed a negligible improvement of around 2.5 % (Table 3). This improvement may be due to the effect of fibrolytic microorganisms and their exogenous enzymes in HI-PRO®.

3.2. Blood metabolites

The data of plasma metabolite concentrations indicated that the inclusion of HI-PRO® in dairy buffalo feeds did not have a significant

Table 3
Effect of different feeding levels on feed intake and nutrient digestibility of dairy buffaloes.

Item	Rations		SEM[a]	P-value
	Control	HI-PRO®		
Feed intake				
Dry matter, kg/d	15.52	15.32	0.18	0.41
Crude protein, g/d	1631	1613	11.3	0.22
Net energy for lactation, Mcal/d	21.64	21.16	0.33	0.27
Nutrients digestibility, %				
Dry matter	59.01	59.95	0.65	0.23
Organic matter	64.23	64.98	0.92	0.41
Crude protein	66.11	66.74	0.84	0.25
Crude fiber	53.34	54.67	0.42	0.19

[a] Slandered error of the mean.

impact on plasma protein, albumin, globulin, creatinine, and urea levels. The blood metabolite concentration is within the usual range for animals in good health. Globulin level slightly increased within the normal range in the HI-PRO® group compared to the control group (2.63 *vs.* 2.33, respectively), which may be due to the presence of mannan and glucan in HI-PRO® which enhances animal immunity. The results demonstrated that the inclusion of the HI-PRO® product in the diets of nursing buffaloes did not have any adverse effects on kidney and liver function or the overall health of the animals (Table 4).

3.3. Milk yield and its composition

The results of milk production and composition indicated that there were no statistically significant differences seen in both the control group and the HI-PRO® group. Furthermore, the use of HI-PRO® improved milk production, with a 4.8 % increase in comparison to the use of soybean meal. Milk composition (fat, protein, lactose) in HI-PRO® and control groups were almost equal, which indicates that HI-PRO® has the same effect as soybean meal on milk production rates. Feeding soluble protein and amino acids in HI-PRO® can lead to an increase in milk output and improve milk composition (Table 5).

4. Discussion

The high-quality forage contributes to the increased digestibility values of various nutrients in the experimental rations, as observed by Öz, and Küçükersan [21]. This forage stimulates cellulolytic bacteria and other bacteria in the rumen, leading to improved digestion. Furthermore, Hashemian et al. [22] found that single-cell proteins often exhibit substantial protein content and excellent digestion. These findings are in agreement with the results of this study whereas the HI-PRO® diet improved fiber digestibility. According to Yin et al. [23], the use of soybean meal protein is limited due to the presence of anti-nutritional components, which negatively affect protein solubility and digestion. One of the main limiting factors of soybean meal is the proteinaceous trypsin inhibitors (TIs), which cause the inactivity of trypsin and chymotrypsin and impair protein digestibility [24]. Therefore, it was necessary to provide alternative sources of protein to meet the needs of dairy animals, and the results of this study showed that the HI-PRO® product s the milk yield by about 400 g/day compared with soybean meal. Kalscheur et al. [25] found in their study that to ensure an adequate supply of proteins the rumen can break down to meet the needs of the present microorganisms is an important initial step in formulating diets for lactating dairy cows. Additionally supplemented protein can be used to meet the cow's total protein requirement when microbial protein synthesis alone is insufficient. Ravindra [13] stated that single-cell protein (SCP) has a high nutritional value and is a potent source of proteins, amino acids, carbohydrates, lipids, minerals, and vitamins. This is what is available in the HI-PRO® according to the analysis of the product as it contains many amino acids (L-arginine, lysine, methionine) and vitamins (B_3 and B_5). The HI-PRO® product can meet the specific needs of certain dairy animals. Multiple studies conducted on nursing dairy cows have demonstrated that increasing the provision of dietary protein leads to an augmentation in milk production [9]. It is advisable to refrain from overfeeding dairy cows with soybean integral seeds, as it may alter the fatty acid composition of their milk and have adverse effects on the texture and shelf life of their butter [26]. In contrast, the HI-PRO® product contains a diverse range of amino acids as well as minerals, mannan, and glucan. Therefore, the nutritional quality of the product relies entirely on the amino acids and their levels. These results in agreement with Bratosin et al. [12], these authors found that the chemical composition of SCP, which includes amino acids, nucleic acids, minerals, enzymes, and vitamins, determines its nutritional value. Additionally, SCP is inexpensive when compared to other types of alternative protein such as lab-grown meat or insect protein. Olsen et al. [27] found that the type and amount of protein in the feed play a crucial role in determining the qualities of cheese and the amount of cheese produced. They observed that yeast, as a single-cell protein, exhibited superior milk coagulation capabilities compared to soybean meal in dairy cow diets. It is important to take this into account when designing dairy cow diets, as it has a significant impact on the financial aspects of the overall dairy industry. Nalage et al. [4] found that the composition of yeast protein is nearly indistinguishable from that of soybean protein. Microbial species have a significant content of protein, making them a unique and non-traditional source of protein, Compared with producing protein from plant sources such as grass, whether for grazing or animal feed, microbial protein requires a significantly lower amount of land. This land usage is essential for the production of other conventional crops to human nutrition. In addition to, the production and storage of animal feeds pose significant environmental and financial difficulties [4]. Microorganisms that produce biomass with high protein levels encompass bacteria, yeasts, fungi, and algae. In addition, they can utilize waste as an inexpensive raw material. Suman et al. [28] have

Table 4
Effect of different feeding levels on some blood metabolites of dairy buffaloes.

Item	Rations		SEM[a]	P-value
	Control	HI-PRO®		
Total protein, g/dl	6.01	6.03	0.21	0.23
Albumin, g/dl	3.81	3.92	0.23	0.33
Globulin, g/dl	2.33	2.63	0.11	0.15
A/G ratio	1.64	1.49	0.16	0.26
Creatinine, mg/dl	0.82	0.88	0.19	0.19
Urea, mg/dl	57.3	58.9	4.6	0.41

[a] Slandered error of the mean.

Table 5
Effect of different feeding levels on milk yield, milk composition, milk content yield and feed efficiency of dairy buffaloes.

	Rations		SEM[b]	P-value
	Control	HI-PRO®		
Milk yield, kg/d				
Milk yield, kg/d	8.2	8.6	0.24	0.41
4 % FCM, kg/d [a]	11.7	11.8	0.29	0.32
ECM, kg/d [a]	12.2	12.4	0.33	0.41
Milk composition, %				
Fat	7.2	7.3	0.16	0.58
Protein	4.4	4.5	0.11	0.19
Lactose	4.72	4.78	0.45	0.62
Total solids	16.32	16.58	0.35	0.44
Solids not fat	9.12	9.28	0.74	0.26
Feed efficiency				
kg milk yield/kg DM intake	0.528	0561	0.08	0.37
kg 4 % FCM/kg DM intake	0.753	0.77	0.03	0.48
kg ECM/kg DM intake	0.786	0.809	0.13	0.23

[a] FCM: fat-corrected milk, ECM: energy-corrected milk.
[b] Slandered error of the mean.

produced SCP using conventional substrates like starch, molasses, and vegetable and fruit wastes.

5. Conclusion

The study suggests that incorporating HI-PRO® product into dairy animal rations can serve as a viable alternative source of protein, which also increases sustainability over (imported) soy protein. Furthermore, it improves digestibility, milk yield, and milk composition without compromising animal health. Single-cell protein is still in its infancy on the market, yet there is a very strong potential for it to revolutionize the entire feed and food system, providing protein from waste and side stream materials for a growing world population.

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Ethics approval

The studies involving animals were reviewed and approved by the Council of Animal Care guidelines (1995) at the Faculty of Agriculture, Ain Shams University, and informed consent was obtained from the owners for the participation of their animals in this study.

Consent for publication

Not applicable.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Code availability

Not applicable.

CRediT authorship contribution statement

Hany M. Gado: Writing – original draft, Writing – review & editing, Validation, Supervision, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hamdy M. Metwally:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Hend A. Sayed:** Writing – review & editing, Writing – original draft, Visualization, Software, Validation, Supervision, Resources, Methodology, Conceptualization, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation. **Zeinab R. Mohammed:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Pasquale De Palo:** Writing – review & editing, Project administration, Data curation, Conceptualization. **Maximilian Lackner:** Writing – review & editing, Software, Investigation, Project administration, Data curation, Conceptualization. **Abdelfattah Z.M. Salem:** Writing – review & editing, Project administration, Data curation, Software, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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