



Administrating Different Diet Formula with Probiotics and their Effect on Milk Production in the Feeding of the Dairy Mesopotamian Water Buffaloes (*Bubalus bubalis*) of Al-Chibayish Marshes (Central Marshes)

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Abstract

This study was carried out in Al-Chibayish wetland within Thi-Qar province, Iraq from 15th May to 1st July 2023. The goal was to calculate balanced diets using available ingredients, which results in better costs and benefits with respect to health performance (hematological and microbiota), milk composition and production. Twelve adult female dairy water buffalo (*Bubalus bubalis*) which were randomly selected. The buffalos' age range was (4 -5) years and weight about 400-500 Kg. The experiment was conducted under the climatic conditions of temperature (37–48 °c) and humidity (15–40 %). The animals were divided into 3 groups (4 female buffalos for each), control was the first group that fed on traditional diet (floor and wheat bran) 3% B.W. and allows grazing reeds and typhus. Second group (local ingredient) was a new balance formula prepare from the available ingredient in local markets (1.5% of B.W. concentrated feed + 3% B.W. corn silage) and allow grazing reeds and typhus. while, third group (local ingredient + probiotic), animals fed on local ingredient formula (1.5% of B.W. concentrated feed + 3% B.W. corn silage) supplemented with probiotics 0.05% and allows grazing reeds and typhus from marshes. The experimental period lasted for 45 days, blood and milk samples were taken every 15 days throughout the experimental period and the faecal samples were taken in 2 intervals during the experimental period. The field observations revealed the enhancement in animal palatability, animal welfare and productivity with regard to buffaloes fed on local ingredients and probiotics groups. Furthermore, significant differences ($P \leq 0.05$) in milk production between the animal fed on probiotics supplemented diet and the control animal were found. There are no significant differences ($P \leq 0.05$) among the hematological and leukocytic indices among the diet groups and intervals. Chemical characteristics analysis of milk revealed a significant elevation ($P \leq 0.05$) in protein, fat and total solid materials percentage in milk of buffaloes fed on local ingredients formula with or without probiotics compared to dairy buffaloes fed on a traditional diet. whereas, reduces in lactose, salt and moisture. The physical properties of milk indicated decrease ($P \leq 0.05$) in the acidity of milk in local ingredients formula containing probiotics and no significant change ($P \leq 0.05$) was detected in the freezing point and specific gravity in other formula groups compared with local ingredients group. Probiotics supplementation helped maintaining balanced bacterial population inside the digestive system indicated by the existence of *Bacillus coagulans* (*Lactobacillus sporogenes*) over the total bacterial count in fecal samples by rtPCR throughout the experiment. From the above results it can be concluded that feeding the optimal ingredients with the addition of probiotics, increase the consumption of feed ingredients, milk production, and chemical and physical characteristics of buffalo milk in the central marshes of Al-Chibayish city.

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Introduction:

The marshes are the natural environment for buffaloes to live in, and it is not possible for them to live in other areas. The Iraqi Ministry of Agriculture / Department of Animal Wealth 2017 mentioned through a survey done in different marshes areas that the feeding of buffaloes in Iraq is within the middle level (69.28%), and often depends on floor and wheat bran and some poor-quality feed, while the good nutrition level constituted 20.81% (Alrawi,2017). During dry seasons in the central marshes of southern Iraq there is a shortage of concentrated and/or green fodder available to breeders of Buffalo. In addition, the cost of feed during the dry season (summer season) was also elevated, floor ton (700,000 ID) and wheat bran may reach to (600,000 ID), As a result, many buffalo breeders left their animals due to disease and lack of interest, or gave them up at a low price.

During dry season, dairy buffalo were not provided with nutritional requirements that negatively affected their body condition and may result in decreased milk production, body weight and immunity level (Ivanova and Markov,2021). The imbalance and reduce quality of feed was found as the main cause for the reduction in the performance of buffaloes by Sarwar et al. (2009). Buffaloes like other ruminant need the main feed component like protein, fat, vitamins, minerals and water to maintain their life and performance (Zicarelli,2007). Physiological state of the animals as indicated by the different stages, age and body weight, previous nutritional status and environmental conditions are multifactorial aspects that affects these requirements (Paul and Lal, 2010).

In the other side, the major food source for wetland buffaloes is grasses or straw, although it is considered as low-quality feed (depending on its contents of cellulolytic materials) but it served as low-cost digestible energy of the fibers of this grasses (Paulino et al.,2008). Hence, giving concentrated feed to buffaloes' farms will be done so as to assure that the main nutrition requirement of buffaloes will be satisfied (Sabia et al., 2015). The effect of feed composition in ruminant on the microbial population of the rumen was previously investigated (Carberry et al.,2012). Thus, adding various ingredients to the ruminant results in a diverse rumen microbial population which enhances the digestibility and increases conversion of low quality of feedstuff into protein and energy that promotes buffaloes productivity (Cui et al.,2019). Various supplements have been proved to be beneficial in livestock feeding. These are represented by using byproduct either industrial or agricultural, fermentation modifiers, and addition of protein or fat source that enhance productivity of livestock (Sarwar et al.,2009). In recent years, probiotic gained a lot of attention to be used as a functional feed or dietary supplement for cattle. The effects of the studied probiotics were beneficial to cattle performance and milk productivity and health status (Verma et al.,2022; Branco-Lopes et al.,2023).

Hence, the present work aims to prepare and compare the different types of buffalo diet in the form of concentrated feed and inclusion silage in dairy buffalo feed with extension to grazing from the reeds and typhus available spontaneously in the central marshes (Al-Chibayish area) and to investigate the effect of using buffalo probiotic in the above diets on some productive and physiological traits.

Objective of the study: -

Calculation of balanced diets with available ingredients aids in milk production and composition with improved costs and benefits.

Material and Methods

Protocol of the study and animal management

The study conducted in Al-Chibayish Marshes, a monotonous wetland of aquatic ecosystem consists majorly from dense vegetations of reed and other submerged plants situated in western Thi Qar Governorate, southern Iraq. The study was conducted from 15th of May to 1st of July 2023, and includes 12 adult female dairy Mesopotamian Water buffalo (*Bubalus bubalis*) which were randomly selected by the owner inside the central Marshes (N°30.999657 E°47.015286, elevation 9 m a.s.l.). The buffaloes' age range was (4 -5) years and weight about 400-500 Kg. The experiment held under climate temperature ranges (37–48 °c) and humidity (15–40 %). All buffalo females were selected as non-pregnant and at early lactation stage, and breed in semi-open field. The barn is divided into three (3) sections, each section consists from a group of (4) four animals and containing a water-drinking trough and a feed basin under the shed.

The animals were divided into 3 groups (4 female buffaloes for each) as the following:

1. Control group (Traditional diet): Animal fed on floor and wheat bran (3% B.W.) and allows grazing reeds and typhus from marshes for 45 days.

2. Local ingredients formula group: Animals fed on new balance formula prepared from the available ingredients in local markets (1.5% of B.W. concentrated feed + 3% B.W. corn silage) and allowed grazing reeds and typhus from marshes along 45 days of the experiment.

3. Local ingredient + probiotic formula group: Animals fed on local ingredient formula (1.5% of B.W. concentrated feed + 3% B.W. corn silage) supplemented with probiotics 0.05% and allowed grazing reeds and typhus from marshes along 45 days of the experiment.

Diet of buffaloes:

The animals of were fed diets that were formulated according to the availability of ingredients in the local markets, taking into consideration that farmers feed buffalo on traditional feed (floor and wheat bran only). Therefore, the study designed concentrated diet that used the same traditional diet (floor and wheat bran) and added other ingredients to make diet formula easier, accepted and more beneficial for farmers animals (scientific and psychological vision). As well as, corn silage was introduced as the main component (3% of B.W.) to elevate the nutritional value of the diet and reduce the cost of feeding. Probiotics were supplemented to the third group diet by 0.05% of the diet (Biolac) from Achaepharm Company, Vietnam, that contained *Bacillus subtilis* (10^9 CFU) and *Lactobacillus sporogenes* (*Bacillus coagulans*) (10^9 CFU) to enhance milk production and the immunity status of animals. All animals' diets were formulated according to the recommendations of the NRC (2001). Administration of feed for animals were modified by introduce concentrated ration in early morning and let the animal to grazing until evening in reeds and typhus marshes, then animals' intake the silage. Feed composition was analyzed by the AOAC (2002), and the composition of the traditional diets and local ingredient formulas used in this experiment is shown in Table 1.

Table 1: chemical composition of the experimental diet.

Ingredients	Traditional diet %	Local ingredients formula %	Local ingredients formula + probiotic %
yellow corn	0	10	10
wheat bran	60	35	35
Floor	40	25	25
Chickpeas	0	30	30
Mineral-vitamin premix	0	0.5	0.5
Salt	0	0.5	0.5
Probiotics	0	0	0.05
Total	100%	100%	100%
Corn silage	0	100%	100%
Chemical composition % DM of concentrated feed			
Dry matter	90.58	90.19	90.19
Crude protein	11.29	13.38	13.38
Crude fiber	5.27	4.28	4.28
Total energy	2445	2631	2631
Chemical composition DM% of corn silage			
Dry matter		46.5	46.5
Crude protein		8.4	8.4
Crude fiber		3.6	3.6
metabolic energy		10.79	10.79

Table 2: cost of experimental formulas for dairy buffalos.

	Traditional diet	Local ingredients formula	Local ingredient formula + probiotics
Concentrated feed cost ITD / ton	560,000	525,000	550,000
Corn silage	0	250,000	250,000
Total cost ITD / head	8,400	7,680	7,875
Reed and Typhus	0	0	0

Blood Samples and hematological analysis

Blood samples were collected from all experimental buffalos three times (each 15 day) during the experimental period. Blood was aspirated from the ear vein of buffalos by using a disposable syringe (5ml) that was put in a special tube that contained EDTA (an anticoagulant) for hematological analysis that was done by an auto-hematological analyzer (Exigo H400, Sweden) in the Bio-vet laboratory for veterinary diagnosis and molecular research which include (RBC, MCV, HCT, HGB, MCH, MCHC, WBC, Neutrophils, lymphocytes, Monocytes, and basophils).

Milk samples and composition analysis

For milk chemical and physical analysis, milk was collected directly from the buffalo udder into a 50-ml disposable container and analyzed in the Food Research and Consumer Protection Unit, College of Agriculture, University of Basrah, by using a Lactoplus Milk Analyzer (India). The analysis parameters include (Protein, Fat, Lactose, Non-Fatty Solid materials NFS, salt, Total solid materials, Moisture, Ph, Freezing point, and Specific gravity).

Fecal samples and bacteriological studies

Fecal samples were taken using sterile plastic bags (From the inside of the rectum of animals). All samples were transported directly to the laboratory for analysis in a suitable, cooled environment. Total DNA was isolated from fecal samples using the Presto™ Stool gDNA Extraction Kit- GENE AID. The total coliform bacterial count and determination of *Lactobacillus sporogenes* bacteria in feces were done in The Bio-vet laboratory by using Real-time PCR (thermo Fisher, USA) and published primers (B.coag-F : 5'-TACGGCATTGGCAAGTATCA3' ; B.coag-R: 5'-CGACATGATTTGGTTTTCCA -3) against a positive control standard using SYBR green dye. The coliform bacteria were enumerated by the conventional culture method using MacConkey agar (Difco, USA). Plates containing between 30 - 300 colonies were counted and expressed as colony-forming units (CFU) per gram of fecal content (Table 10).

Statistical analysis: Using SPSS software version 27, the dataset was statistically analyzed using a two-way analysis of variance (ANOVA) in a completely randomized design (CRD). To determine differences between means, post-hoc comparisons were performed using the least significant difference method (LSD). $P \leq 0.01$ and $P \leq 0.05$ were the thresholds for significance.

Results:

The dry season in this region of Iraq (the marshes of southern Iraq) is very painful, as the scarcity of water and the lack of resources affect the inhabitants of the region and consequently their animals, making them loss weight, unproductive and suffering from recurring diseases and infection. When the above project was initiated, the animals were monitored a long day to follow up behavioral of animal any digestive disorders that might occur as a result of changing the feed given, despite it being given gradually. Field observation revealed enhancement in animal palatability toward the feed (no residue feed), which improved the buffalo's health status and appeared to improve milk production. Also, this trail investigated whether administration of concentrated feed in the early morning and letting animals graze before introducing silage improved their welfare, productivity, and performance. According to Table 1, referring to the composition of local ingredients, the formula had a higher crude protein percentage and energy than the traditional diet that was introduced to buffaloes. Whereas, the cost of local ingredient formula (7.680 ID/animal) is cheaper than that of traditional diet (8.400 ID/ animal) table 2. However, the results revealed a significant increase ($P \leq 0.05$) in milk production for the buffalo fed on formula supplemented with probiotics when compared with other studied groups. Also, local ingredients formula showed a significant increase ($P \leq 0.05$) in milk production compared to dairy buffalo fed on a traditional diet (table 3). On the other hand, the period of study had an effect on milk production. This appeared in buffaloes fed formula supplemented with probiotics, but did not show significant differences ($P \leq 0.05$) between traditional and local ingredient formula intervals (tables 3).

Erythrocytes and hemoglobin indices referred to non-significant differences ($P \leq 0.05$) among the groups and intervals of the study, this indicates that using the formula for buffaloes was safe and did not have any deleterious effect. (Table 4,5). Similarly, total and differential leukocytes also revealed non-significant differences ($P \leq 0.05$) among the groups and intervals of the study, these also indicate safety using the formula (table 6, 7).

Chemical characteristics analysis of milk revealed a significant elevation ($P \leq 0.05$) in protein and fat percentage in milk of buffaloes fed on local ingredients formula with or without probiotics compared to dairy buffaloes fed on a traditional diet. Whereas the group of buffalo fed on a traditional diet showed a significant elevation ($P \leq 0.05$) in lactose percentage when compared with other studied groups (Table 8). These are very important points when protein and fat percentages increase in milk. This means an increase in dairy products and will lead to an increase in farmer income. While, salt percentage in milk is reduced ($P \leq 0.05$) when buffaloes were fed local ingredients formula with or without probiotics when compared with dairy buffalo fed on a traditional diet. This gave milk a better taste and increased acceptance for use (Table 9). Nevertheless, probiotic supplementation in the formula of local ingredients increases the

percentage of total solid materials at the expense of moisture, which in turn increases the quality of the milk (Table 10).

The physical characteristics of milk in the present study showed a reduction ($P \leq 0.05$) in the acidity of milk in local ingredients formulas with probiotics compared with other studied groups. These made the milk more acceptable for human consumption (Table 11).

The addition of probiotics helped maintaining balanced bacterial population inside the digestive system indicated by the existence of *Bacillus coagulans* (*Lactobacillus sporogenes*) over the total bacterial count in fecal samples by rtPCR throughout the experiment and these beneficial bacteria assist in enhance performance and metabolic status of animals (table 12).

Discussion:

The most important approach for the present study was modulation of traditional concentrated feed based on availability of ingredient in the market with acceptable price. Respecting their beliefs about the necessity of using flour and wheat bran, chickpeas were used instead of soybeans or a high-protein feed ingredient for the purpose of balancing the feed and also the final cost, without impaired productive and reproductive performance of buffalos. (Serrapica *et al.*, 2020). Route of feed administration for buffaloes' animals by introduce concentrated feed in the morning and let animals to grazing reeds and typhus, after that silage was introduce in the afternoon led to enhance growth and performance of the buffalos (Mohd *et al.*, 2021). These changing and modification in feed and route of administration gave their results on quantity and quality of milk production. Probiotic supplemented group showed significant increase ($P \leq 0.05$) in milk production volume compared with traditional and local ingredient diet groups, while, local ingredient group recorded significant elevation ($P \leq 0.05$) in milk production when compared with traditional diet group. These improvement in volume of milk production explained by the enhancement in quality of diet after re-formulation and increase protein and energy values for the diet (Manish, 2021). The beneficial effect of balanced energy and protein content in diet, explanation the enhancement in growth, performance and production due to enhancement in dry matter intake, weight gain, growth hormones, carcass traits and milk quantity and quality (Singh *et al.*, 2017). Also. Wang and his team (2017) demonstrated that increase ruminal amino acids, volatile fatty acids and ammonia attributed to changes in bacterial population in rumen and enhancement in crude protein and energy level in the diet. Therefore, results recorded enhancement in daily milk volume for the local and probiotic ingredient groups. Several studied explained the role of probiotic in yield production of ruminant animals. They found that additive of *Bacillus subtilis* and *Lactobacillus sporogenes* in the diet as probiotic improved intestinal mucosa for better nutrients absorption (Wang *et al.*, 2022), enhancement of immunity status via competitive pathogenic bacteria and stimulating immunoglobulins (Sun *et al.*, 2019), and improve rumen fermentation and reduce the stress that encouraged animal for increase milk production (Deng *et al.*, 2020).

Hematological analysis for the buffaloes that fed on three different formula appeared non-significant difference ($P \leq 0.05$) for the RBC, HGB and WBC indices. These indicated that modification and changes in the animal's nutritional content and habits did not show any harmful effect on the animal's health. A study on buffalo calves concluded that increase in dietary energy improve hemoglobin and HCT level (Kumar *et al.*, 2020). Because the change in the amount of protein and energy of the dairy buffalo feed was slight, no significant changes were observed in the blood parameters.

As shown by feeding trials, dairy buffaloes exhibited remarkable variances in milk composition after being fed switch diets of traditional, local ingredient, and local with probiotic ingredient diets. Protein and fat milk concentration in the probiotic supplemented fed group reached 4.90-5.11% and 8.67-8.73%, respectively, which was significantly higher than in the rest of the groups. This suggests the Probiotics ability to enhance nitrogen efficiency and protein biosynthesis in dairy buffaloes (Elghandour *et al.* 2020). Khan *et al* (2019), on the other hand, showed that local ingredient diet improved protein and fat milk content compared with traditional diet, which is in line with the statement in the previous paragraph that points to an imbalanced diet and its symptoms that was set by Khan *et al* (2019). Throughout the trial period, all groups showed an increase in protein and fat content of buffalo milk, with the probiotic supplemented group showing the most significant increase in composition or concentration, as seen in the works of Sharma *et al* (2020), and Sun *et al* (2019). They mentioned that this is mostly due to the increased availability of basic fatty acetyl groups as well as a positive influence on metabolism and feed efficiency. On the other hand, the local ingredient and probiotic supplemented fed groups showed the lowest lactose concentration in milk for the duration of the study. This might explain the increased protein and fat content in the milk (Bogdan *et al.*, 2008). The probiotic supplemented fat and protein were reflected on the milk non-fat solid materials in buffaloes which were significantly (11.15 11.18%) higher than the traditional and local ingredients diet groups. This indicates that the probiotic improves the retention of fat, protein and minerals in the milk of dairy buffaloes (Elghandour *et al.*, 2020). In contrast, the salt content of the milk increased significantly in the traditional diet in relation to the other groups over the study period

(0.95-1.02%) which is higher than the other studied groups due to the balance of minerals in other diet formulas of the study (Ahmad et al., 2013). The Total Solids (%) and Moisture (%) content in buffaloes over the 15, 30, and 45 days across three dietary treatments shows that there was a difference in the composition of milk over the period of feeding based on the formulation of the feed, which was added to the feeding formulation and period to feeding duration. Probiotic supplementation shows probiotic added diets proved to have the highest total solids content (18.25-19.82%) and therefore higher than the traditional diets (13.07-16.55%) and local-ingredient-only diets (14.00-17.40%).

This significant enhancement supports the notion that probiotics increase retention of all constituents of milk, such as proteins, fats, minerals, and even lactose (Gaggia et al., 2010; Elghandour et al., 2020). The local-ingredient diet without probiotics resulted in intermediate total solids values, while the traditional diet showed the lowest values. This suggests that local ingredients are beneficial in improving total solids, as opposed to traditional feeding, but the addition of probiotics to the diet augments these solids to an even greater extent (Sharma et al., 2020). On the contrary, the probiotic group had the lowest moisture content (80.17-83.45%), which is the range of their highest total solids, and suggests an inverse relationship. This indicates that there is an increase of valuable components of milk, and the water content is greatly minimized which means probiotic supplementation is beneficial (Sun et al., 2019).

Within a 45-day puppy period, this experimental work focused on the impact of three dietary treatments (Traditional diet, Local ingredients formula, Local ingredients formula + probiotic) on the physical consistency, including pH, freezing point, and gravity of buffalo milk. The data obtained show significant variation across the periods and the treatments in regard to milk pH: At 15 days, all treatments pH values were the same, and by 30 days, the diet with probiotics (6.85 ± 0.02) pH value significantly ($p < 0.05$) exceeded the pH value of the other treatments. This observation was maintained at 45 days, with probiotic milk having a pH of 6.83 ± 0.02 while milk from the traditional diet pH was 6.66 ± 0.03 . These probiotics might be the cause of increased microbial fermentation in the rumen while the milk reduced the buffering capacity because of a decreased lactic acid concentration. These results are consistent with other works on probiotics and the pH of dairy products (Gaggia et al., 2018). The freezing point depression values suggest traditional diet milk possessed greater concentrations of solutes, whereas probiotic supplementation, may be osmoregulatory to the mean values of the buffalo milk (Ahmad et al., 2020). Specific gravity was attributed to the increased concentration of other milk components, with the highest values specific gravity being attributed to traditional diet and probiotic milk.

Table (3): Milk production L/day of dairy buffaloes fed on different diet formula. (Mean \pm S.E)

*Capital letters represent significant differences vertically at ($P \leq 0.05$) level.

Treatments	Milk Quantity L/Day		
	15 days	30 days	45 days
Traditional diet	$1.28 \pm^{Cb} 0.06$	$1.97 \pm^{Cb} 0.06$	$2.08 \pm^{Ca} 0.04$
Local ingredients formula	$2.64 \pm^B 0.02$	$2.83 \pm^B 0.04$	$2.85 \pm^B 0.03$
Local ingredients formula + probiotic	$3.88 \pm^{Ab} 0.05$	$4.86 \pm^{Aa} 0.04$	$4.85 \pm^{Aa} 0.06$

*Small letter represents significant differences horizontally at ($P \leq 0.05$) level

Table (4): Erythrocyte indices of dairy buffalos fed on different diet formula (Mean \pm S.E).

Treatments/ Weeks	RBC 10 ¹² /L			MCV fl			HCT %		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	5.78 \pm 0.88	4.15 \pm 1.01	5.82 \pm 0.94	48.65 \pm 3.50	51.57 \pm 1.09	50.31 \pm 2.95	27.27 \pm 1.93	21.32 \pm 5.09	23.27 \pm 3.84
Local ingredients formula	6.98 \pm 1.11	5.35 \pm 1.04	6.75 \pm 1.87	45.92 \pm 4.48	48.52 \pm 2.91	51.72 \pm 3.26	30.62 \pm 2.13	25.22 \pm 4.05	27.85 \pm 4.91
Local ingredients formula +probiotic	6.73 \pm 1.28	5.40 \pm 0.47	6.36 \pm 1.73	47.47 \pm 5.19	53.72 \pm 0.87	53.87 \pm 4.49	30.05 \pm 2.63	23.95 \pm 4.66	25.76 \pm 4.52

Table (5): Hemoglobin indices of dairy buffalos fed on different diet formula (Mean \pm S.E).

Treatments/ Weeks	HGB g/dl			MCH pg			MCHC g/dl		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	10.07 \pm 0.71	9.27 \pm 0.36	10.16 \pm 0.95	17.97 \pm 1.29	26.75 \pm 6.04	21.67 \pm 3.81	36.95 \pm 0.94	51.87 \pm 12.07	43.18 \pm 3.97
Local ingredients formula	11.12 \pm 0.98	9.85 \pm 0.47	12.83 \pm 0.74	16.50 \pm 1.28	21.02 \pm 4.58	25.93 \pm 2.69	36.17 \pm 0.74	42.72 \pm 7.64	40.43 \pm 5.19
Local ingredients formula +probiotic	10.82 \pm 1.12	9.60 \pm 0.40	11.38 \pm 0.54	16.97 \pm b 1.60	32.97 \pm a 3.64	30.64 \pm a 5.24	35.97 \pm 0.64	53.05 \pm 9.42	48.68 \pm 5.74

Treatments/ Weeks	WBC 10 ⁹ /L			Neutrophils 10 ⁹ /L			Lymphocyte 10 ⁹ /L		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	7.25 ± 0.59	6.07 ± 1.11	6.17 ± 0.72	1.50 ± 0.38	1.27 ± 0.63	1.43 ± 0.72	5.07 ± 0.64	4.15 ± 0.29	4.21 ± 0.47
Local ingredients formula	7.55 ± 0.35	6.55 ± 0.98	5.94 ± 0.51	2.12 ± 0.23	2.62 ± 1.23	2.53 ± 0.29	4.67 ± 0.57	3.35 ± 0.55	3.76 ± 0.52
Local ingredients formula +probiotic	5.95 ± 0.32	5.25 ± 0.35	6.32 ± 0.61	0.70 ± 0.23	2.27 ± 0.77	1.84 ± 0.65	4.85 ± 0.32	3.90 ± 0.32	3.93 ± 0.56

Table (6): Total and differential leukocytes values of dairy buffalos fed on different diet formula (Mean ± S.E).

Table (7): Monocyte and basophils values of dairy buffalos fed on different diet formula (Mean ± S.E).

Treatments	Monocyte 10 ⁹ /L			Basophil 10 ⁹ /L		
	10 days	20 days	30 days	10 days	20 days	30 days
Traditional diet	0.67 ± 0.14	0.65 ± 0.23	0.64 ± 0.18	0.20 ± 0.08	0.35 ± 0.07	0.12 ± 0.04
Local ingredients formula	0.75 ± 0.06	0.57 ± 0.14	0.62 ± 0.09	0.22 ± 0.001	0.27 ± 0.02	0.75 ± 0.26
Local ingredients formula +probiotic	0.40 ± 0.10	0.45 ± 0.06	0.51 ± 0.13	0.21 ± 0.03	0.70 ± 0.05	0.50 ± 0.12

Table (8): The protein%, fat% and lactose% in milk of dairy buffalos fed on different diet formula (Mean \pm S.E).

Treatments	Protein (%)			Fat (%)			Lactose (%)		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	3.60 \pm ^B 0.18	4.35 \pm 0.16	4.27 \pm ^B 0.13	2.60 \pm ^B 0.57	3.47 \pm ^B 0.78	3.92 \pm ^B 0.84	5.75 \pm ^A 0.09	5.85 \pm ^A 0.06	5.87 \pm ^A 0.07
Local ingredients formula	3.80 \pm ^B 0.25	4.42 \pm 0.20	4.81 \pm ^{AB} 0.27	3.32 \pm ^B 1.12	6.55 \pm ^A 1.01	6.68 \pm ^A 1.23	5.77 \pm ^A 0.02	5.52 \pm ^B 0.10	5.56 \pm ^B 0.12
Local ingredients formula +probiotic	4.90 \pm ^A 0.04	4.87 \pm 0.37	5.11 \pm ^A 0.64	8.67 \pm ^A 0.19	8.70 \pm ^A 1.58	8.73 \pm ^A 1.29	5.37 \pm ^B 0.04	5.70 \pm ^B 0.09	5.68 \pm ^B 0.14

Capital letters represent significant differences vertically at ($P \leq 0.05$) level.

Table (9): Non-fat solid material% and salt% in milk of dairy buffalos fed on different diet formula (Mean \pm S.E).

Treatments	NFS (%)			Salt (%)		
	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	10.47 \pm 0.24	10.70 \pm 0.22	10.67 \pm 0.07	1.02 \pm ^{Aa} 0.02	0.95 \pm ^b 0.02	0.97 \pm ^{Ab} 0.07
Local ingredients formula	10.67 \pm 0.19	10.77 \pm 0.10	10.67 \pm 0.46	1.00 \pm ^{Aa} 0.04	0.82 \pm ^b 0.04	0.73 \pm ^{Bb} 0.04
Local ingredients formula+ probiotic	11.15 \pm 0.06	11.15 \pm 0.09	11.18 \pm 0.53	0.77 \pm ^B 0.02	0.92 \pm 0.07	0.72 \pm ^B 0.16

*Capital letters represent significant differences vertically at ($P \leq 0.05$) level.

*Small letter represents significant differences horizontally at ($P \leq 0.05$) level.

Table (10): Total solid material% and Moisture% in milk of dairy buffalos fed on different diet formula (Mean ± S.E).

Treatments	Total solids materials (%)			Moisture (%)		
	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	13.07± ^{Bb} 0.79	16.55± ^{Ba} 0.81	16.31 ^{Ba} ±0.48	86.67± ^A 0.91	84.60± 1.80	84.37± 0.78
Local ingredients formula	14.00± ^{Bb} 1.30	17.32± ^{ABa} 1.07	17.40± ^{AB} a 0.52	86.00± ^A 1.30	83.42± 1.29	83.20± 0.52
Local ingredients formula + probiotic	19.82± ^A 0.18	18.40± ^A 1.80	18.25± ^A 0.63	80.17± ^B 0.18	83.45± 0.81	82.15± 0.76

Capital letters represent significant differences vertically at (P≤0.05) level.
Small letter represents significant differences horizontally at (P≤0.05) level.

Table (11): physical properties in milk of dairy buffalos fed on different diet formula (Mean ± S.E).

Treatments	PH			Freezing point °C			Specific gravity gm/cm ³		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Traditional diet	6.79± 0.04	6.68± ^B 0.02	6.66± ^B 0.03	-0.70± 0.01	-0.72± ^A 0.005	-0.71± 0.006	1.037± ^A 0.001	1.036± 0.001	1.036± 0.001
Local ingredients formula	6.79± 0.01	6.70± ^B 0.05	6.75 ± ^{AB} 0.04	-0.69± 0.007	-0.68± ^B 0.006	-0.69± 0.005	1.036± ^A 0.001	1.033± 0.001	1.035± 0.001
Local ingredients formula +probiotic	6.79± 0.01	6.85± ^A 0.02	6.83± ^A 0.02	-0.68± 0.004	-0.69± ^B 0.007	-0.69± 0.006	1.031± ^{Bb} 0.001	1.035± ^a 0.001	1.035± 0.001

*Capital letters represent significant differences vertically at (P≤0.05) level.

Table (12): total bacterial account and *L. sporogenes* count in fecal sample of dairy buffalo fed on different diet formula.

Treatments/ Weeks	CFU Total Bacteria/gm		CFU <i>L. sporogenes</i>	
	22 days	45 days	22 days	45 days
Traditional diet	2×10^6	2×10^7	0	0
Local ingredients formula	4×10^9	3×10^{10}	0	0
Local ingredients formula +probiotic	2×10^{10}	3×10^{11}	4×10^6	3×10^8

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