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Research Article
Yeast Biomass Production from Milk Permeate with Enrichment Application of Dairy Animal's Diets

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Abstract

Background and Objective: Dairy wastes are sources of environmental pollution and efficiently used for production of added value products is an important issue. The aim of this study was to use milk permeate for production of *Saccharomyces cerevesia* biomass, as baker's and feed yeast. **Materials and Methods:** The fermentation process was conducted with 250 mL conical flasks in shacked cultures using different volume ratios of culture medium with different agitation rates (150 or 200 rpm) for achieving the aeration test. The effect of incubation time on the production of yeast biomass was investigated. Applying the optimum parameters in lab scale fermenter (7.5 L) were carried out. The produced yeast biomass was used in enrichment of the feeding value of lactating animal's diets containing agricultural wastes. **Results:** The optimum parameters for the production of yeast biomass were using 1:10 ratio of fermented medium to the entire volume of the fermentation vessel (250 mL conical flask) which yielded at agitation rate of 150 and 200 rpm and a yield reached at 8.22 and 8.84 g L^{-1}, respectively. The optimum incubation period was 72 h with yield reached at 9.12 g L^{-1}. High biomass yield was produced in lab scale fermenter reached to a maximum level of 116 g L^{-1}. When the produced yeast was added to dairy animal's diets, the Dry Matter (DM), Natural Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose and hemicellulose degradability was increased, with no impact on ruminal total gas production and ammonia concentration. **Conclusion:** Hence, it was concluded that yeast biomass produced on permeate hydrolysate give high yield and improves the degradability of dairy animal's diet.

Key words: Yeast production, enzymatic hydrolysis, milk permeate, agricultural wastes, dairy animal diets

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yeast production has many applications in food processing, animal feeding and other industrial applications. The use of low cost substrates as milk permeate for yeast production may play an active role for economical production of yeast biomass to be used as baker's or fodder yeast. This will caters the needs for production of animal feed required for developing the animal wealth and milk production.

The continuous increase of population size in Egypt creates a big gap between the native production of milk and its market demands, which led to continuous increase in prices of milk and dairy products annually[1]. To grow the animal production market, this situation requires an integrated strategies. The most difficult problems facing the development of animal production sector in Egypt are the continuous increase in feed of ingredient prices and the large gap between animal's requirements and available feeds[2]. In this concern, agricultural wastes can play an important role to minimize the feed gap[3]. The annual production of agricultural residues estimated to be around 30 million t of dry materials/year[4]. Also there is a huge amount of dairy wastes (e.g.), cheese whey and UF-permeate) produced annually and represent another source of environmental pollution[5]. Cheese whey is produced during cheese manufacture and represents about 85-95% of the milk content. Liquid whey has been reported to retains about 55% of milk nutrients, the most abundant of which is lactose (4.5-5% w/v)[6]. Removal of valuable whey proteins leaves whey permeate, which on dry matter basis can contain up to 85% lactose. Lactose is largely responsible for whey's high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)[5,6] and thus whey permeate as a waste poses a significant environmental burden.

The bio-conversion of such wastes into value added products is one of the most prominent topics in scientific research now-a-days[7]. Recent developments in fermentation technology have allowed for large scale production of biologically active substances (e.g., yeast biomass)[8]. Yeast (*Saccharomyces cerevisiae*) is one of the oldest industrial fermentation products. Due to its various industrial uses, it is still one of the most important biological products[5]. Yeast production under variable cultivation conditions have been largely studied[9,10]. In the fermentation process, the raw materials are the major contributors to the cost of microbial products[11]. Whey permeate is inexpensive, but uncommonly used as a source of sugars for producing yeast biomass[10]. The use of whey permeate in yeast biomass production represent a challenge as *Saccharomyces cerevisiae* is unable to assimilate whey sugar (lactose)[9]. Therefore, the permeate must be hydrolyzed firstly by β-galactosidase to glucose and galactose and then used it as a substrate for yeast biomass production. Several factors affect the yeast fermentation process, including the carbon source and it's concentration, temperature, pH, agitation and dissolved oxygen[12]. By the end of the fermentation process the resultant biomass is treated with many downstream treatments, which may include washing, disintegration of cells, protein extraction and purification process.

The incorporate of yeast biomass in dairy animal diets had been evaluated by several workers[12,13]. It has been reported that yeast alone or with fibrolytic enzymes can play an important role in the improvement of dairy animal's digestion and metabolism through provided factors that make rumen environment more stable[12-18]. Stable rumen environment is a key factor for achieving optimum milk production and a source of good animal's health[4,15]. Accordingly, this study was conducted to: (1) Investigate the effect of aeration, agitation and incubation time on the production of yeast biomass in medium containing hydrolyzed permeate and (2) Evaluate the impact of adding resultant yeast biomass to dairy animal's diets containing agricultural residues on diet degradation and rumen fermentation characteristics (*in vitro*).

MATERIALS AND METHODS

This study was carried out in the period from 5 May-3-October, 2018 at the Dairy Department-National research center, Egypt. Permeate and β-galactosidase were obtained from company of Gohina, Egypt. The hydrolyzed permeate by β-galactosidase (5000 μ mL^{-1} at 37°C) was employed as a medium for yeast biomass production using isolated *Saccharomyces cerevisiae* as described by Murad *et al.*[5]. The purity of the isolated *S. cerevisiae* was confirmed by16S rRNA analysis[19]. The chemical composition (moisture, protein, ash, total solid and total lipid content) for the resultant yeast biomass was determined according to AOAC[20]. The fermentable sugars were determined according to Miller[21], while, ethanol content of the fermented samples was measured by Ebulliometer[22]. The total viable plate count was used for measuring the yeast viability after its enumeration on PDA medium at 30°C for 48 h[23]. Biomass production efficiency was calculated as gram biomass yield g^{-1} consumed fermentable sugars in fermentation medium divided on theoretical biomass yield[5] multiply by 100.

Yeast biomass production optimization

Impact of aeration level on yeast biomass production at different shaking speeds: Flasks (250 mL) contain 25, 50 and 75 mL of enzymatically hydrolyzed permeate medium (35 g L^{-1} of reducing sugar, 0.5 g magnesium sulfate, 0.1 g zinc sulfate, 0.2% peptone, 0.2% K_2HPO_4, 0.1 ppm biotin, 3 ppm pantothenic acid, tween 80 and 0.4 ppm m-inositol) were inoculated by the isolated *S. cerevisiae* and incubated for 48 h at 30°C for test of different shaking speeds (150 and 200 rpm) impact on yeast biomass production.

Influence of incubation period on yeast biomass production: The yeast culture was incubated for 24, 48, 72 and 96 h at 30°C under shaking at 200 rpm.

Role of dilution ratio on the yeast biomass production: Yeast production was carried out in 250 mL conical flasks each contained:

- 50 mL of enzymatically treated permeate (control)
- 25 mL of enzymatically treated permeate+25 water (1:1)
- 16.5 mL of enzymatically treated permeate+33.5 water (1:2)
- 12.5 mL of enzymatically treated permeate+37.5 water (1:3)

The fermentation experiments were carried out in 250 mL conical flasks contain 50 mL of fermentation medium and were inoculated with 1% (v/v) with active viable culture of *S. cerevisiae* then incubated in a rotary shaker adjusted to 30°C and 200 rpm for 48 h.

Production of yeast biomass under optimum conditions: The fermentation experiments were carried out in 250 mL conical flasks each contain 25 mL of fermentation medium and were inoculated with 1% (v/v) with active viable culture of *S. cerevisiae* under shaking 200 rpm at 30°C for 72 h.

Pilot scale production of yeast biomass: The enzymatically hydrolyzed permeate was concentrated by evaporation till obtaining a syrup contains 20% glucose. A fed batch fermentation system was employed on a fermenter Bio Flo 310 (Appendorf, Inc.) 7.5 L capacity for 24 h for production of *Saccharomyces cerevisiae* biomass. Temperature, agitation and aeration were controlled at 30°C, 200 rpm and 1 vvm, respectively. The initial volume of growth medium was 3 L inoculated by 10% liquid inoculum. A feeding volume of 1.5 L was added to the fermenter batch wise by input of 100 mL of the medium every 2 h.

Impact of the produced and commercial yeasts on dairy animal's diets digestibility and rumen fermentation characteristics (*in vitro*): The *in vitro* experiment was conducted to evaluate impact of the laboratory produced yeast (yeast biomass produced from *S. cerevisiae*, each gram of it contains 1×10^9 CFU g^{-1}) compared with Yea-Sacc 1026 (A commercial yeast product each gram of it contains 1×10^9 CFU g^{-1}, (Alltech Inc, Lexington, KY, USA) on dairy animals diet degradability and rumen fermentation characteristics. According to Ismail *et al.*[24], a 500 mg sample of the control diet (its feed ingredients and chemical composition was shown in Table 1) was weighed into 120 mL serum bottles. The bottles (3 replicates) were separately supplemented with rumen liquor, buffer solution and laboratory yeast and Yea-Sacc1026 solutions at different levels (0, 1, 2 and 3 g kg^{-1} DM) of the diet. Rumen liquor was collected from the rumen of slaughtered rams then moved directly to the laboratory in separate warmed oxygen-free plastic jars. The obtained liquor was mixed with the buffer solution at 39°C under carbon dioxide continuous flushing[25]. The bottles were sealed and maintained at 39°C in a shaking water bath (20 oscillations/min) for 24 h. After 24 h of incubation the pH value, total gas production volume, NH_3 and total volatile fatty acids concentrations were determined[26]. The *in vitro* Dry Matter (DM) degradability was determined according to the AOAC[20] methods, while Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) degradability were determined according to Van Soest *et al.*[27] methods.

Statistical analysis: Data obtained from this study were statistically analyzed by IBM SPSS Statistics for Windows[28] using one way ANOVA procedure to compare means. The significance among means were tested through Duncan's multiple range tests with probability level ($\leq$%5).

RESULTS

Impact of aeration on yeast biomass production: Data illustrated in Table 2 showed that by increasing the volume of the flask head space (fermented volume 25 mL with aeration ratio 1:10), the yeast biomass yield and fermentation efficiency (%) were increased but ethanol yield and residual sugar content were decreased. By elevation the shaking speed to 200 rpm the yeast biomass was reached to its highest yield (8.84 g L^{-1}). It's obvious that increasing rate of agitation is leading to increase of the aeration level at which the

Table 1: Chemical composition of feed ingredients and the control diet

	DM (g kg^{-1})							
Feed ingredients	DM	OM	NDF	ADF	CP	EE	Ash	NFC
Corn grain	887	985	187	37	83	55	15	660
Soybean meal	890	932	152	65	390	48	68	342
Wheat bran	895	955	355	99	155	40	45	405
Wheat straw	905	918	815	575	32	20	82	51
Clover hay	925	865	410	270	175	40	135	240
Control diet	899.52	937.64	373.16	202	136.68	42.36	62.36	385.44

DM: Dry matter, OM: Organic matter, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, CP: crude protein NFC: Non-fiber carbohydrate and EE: Ether extract, Control ration: Consisted of 36% corn, 12% soybean meal, 12% wheat bran, 20% Berseem hay and 20% wheat straw

Table 2: Aeration impact on production of yeast from enzymatically hydrolyzed permeate under shaking speed 150 and 200 rpm

	Yeast biomass (g L^{-1})		Consumed fermentable sugars (g L^{-1})		Residual fermentable sugars (g L^{-1})		Ethanol yield (v/v)		Yeast biomass efficiency (%)	
Fermented volume (mL)	150 rpm	200 rpm	150 rpm	200 rpm	150 rpm	200 rpm	150 rpm	200 rpm	150 rpm	200 rpm
25	8.22	8.84	27.80	28.20	7.20	6.80	4.80	4.60	46.10	49.52
50	7.74	7.98	26.90	27.90	8.10	7.10	5.10	5.00	43.40	44.71
75	7.22	7.42	25.30	27.40	9.70	7.60	5.90	5.32	40.45	41.57

Table 3: Effect dilution ratio of enzymatically hydrolyzed permeate on yeast biomass production

Dilution ratio	Yeast biomass (g L^{-1})	Consumed fermentable sugars (g L^{-1})	Residual fermentable sugars (g L^{-1})	Ethanol yield (v/v)	Yeast biomass efficiency (%)
Control	7.98	27.40	7.60	5.00	44.71
1:1	11.20	28.63	6.37	2.30	62.75
1:2	12.60	29.33	4.80	1.22	70.60
1:3	13.65	30.80	4.20	0.67	76.50

Table 4: Effect of incubation period on the production of yeast biomass

Incubation period (h)	Biomass (g L^{-1})	Consumed fermentable sugars (g L^{-1})	Residual fermentable sugars (g L^{-1})	Ethanol yield (v/v)	Yeast biomass efficiency (%)
24	5.24	11.20	23.8	0.90	29.40
48	7.98	27.40	7.60	5.00	44.71
72	9.12	28.64	6.34	4.60	51.10
96	8.90	28.86	6.14	4.76	50.00

yield reached 8.84 g L^{-1}. This reflected enhancement of fermentation efficiency through increase rate of sugars consumption and reduction of alcohol production.

Impact of the dilution ratio on the yeast biomass production: Data illustrated in Table 3 showed that by increasing the dilution ratio of permeate, the yeast biomass yield was increased and ethanol production was decreased. The fermentation efficiency were increased and the residual sugars were decreased which indicated more sugar consumption.

Incubation period impact on the production of yeast biomass: Data illustrated in Table 4 showed that the optimum incubation period for the production of yeast biomass was 72 h at which the biomass yield was reached to 9.12 g L^{-1} and the fermentation efficiency were elevated to reach 51.1%, respectively.

Production of yeast under the optimum conditions: Data in Table 5 exhibited the production of yeast from enzymatically hydrolyzed permeate under the optimum conditions. Data showed that after employing the above conditions it was noticed that the yeast biomass reached 16.1 g L^{-1} with reduced ethanol yield as 0.38 v/v compared with control (10.15 g L^{-1}, 4.62 v/v, respectively) accordingly the yeast biomass efficiency attained a maximum level as 90.2% compared with control (57%).

Production of yeast biomass using lab fermenter: Data illustrated in Fig. 1 showed that by extending the fermentation time to 24 h, the yeast biomass was increased to reach its maximum (116 g L^{-1}).

Chemical composition of the produced yeast (on fresh weight basis): The chemical composition of the produced yeast on the basis of fresh yeast (70.12% moisture) exhibited

Table 5: Production of yeast biomass from enzymatically hydrolyzed permeate under optimum conditions

Dilution ratio	Yeast biomass (g L^{-1})	Consumed fermentable sugars (g L^{-1})	Residual fermentable sugars (g L^{-1})	Ethanol yield (v/v)	Yeast biomass efficiency (%)
Control	10.15	28.74	6.26	4.62	57.0
1:3	16.10	33.11	1.89	0.38	90.2

Table 6: Chemical composition of yeast biomass and total viable count (log CFU g^{-1}) versus factory reference strain

Constituents (%)	*S. cerevisiae*	Reference strain
Moisture	70.12	64.40
Dry matter	29.50	30.60
Protein	15.20	14.90
Ash	2.12	4.20
Fat	0.04	0.05
Total carbohydrate	11.75	12.10
Trehalose (mg g^{-1})	4.001	4.60
Total viable cell (log CFU g^{-1})	10.06	9.17

Table 7: Yeast effects on degradability parameters of experimental diets

Treatments	Yeast level (g kg^{-1})	Diet degradability parameters (%)					
		DM	NDF	ADF	Cellulose	Hemicellulose	Yeast efficiency
Control	0	55.67^{c}	34.20^{c}	31.11^{c}	37.64^{c}	38.51^{c}	0.00
Yea-Sacc1026	1	58.52^{b}	41.28^{b}	40.05^{b}	45.97^{b}	42.98^{b}	5.12
	2	60.03^{b}	43.16^{b}	43.10^{b}	50.28^{a}	43.37^{b}	7.83
	3	65.23^{a}	47.61^{a}	47.40^{a}	51.78^{a}	47.92^{a}	17.17
Produced yeast	1	57.56^{c}	37.1b^{c}	31.78^{c}	44.11^{b}	44.32^{b}	3.40
	2	62.77^{b}	43.89^{b}	44.03^{b}	51.77^{a}	43.68^{b}	12.75
	3	65.09^{a}	45.60^{ab}	45.81^{ab}	50.02^{a}	45.3a^{b}	16.92

*Means with different letter (a, b and c) in the same column are significantly different at p<0.05, DM: Dry matter, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 8: Yeast effects on ruminal parameters (*in vitro*)

Treatments	Yeast level (g kg^{-1})	TGP (mL)	pH	NH_3 (µmol L^{-1})	TVFA (meq dL^{-1})
Control	0	158	6.44	40.85	6.13^{c}
Yea-Sacc1026	1	155	6.46	38.75	6.18^{c}
	2	157	6.41	39.87	6.43^{b}
	3	157	6.45	38.98	6.50^{a}
Produced yeast	1	157	6.45	39.85	6.45^{b}
	2	161	6.47	40.49	6.30^{bc}
	3	160	6.45	37.84	6.17^{c}

*Means with different letter (a, b and c) in the same column are significantly different at p<0.05, TGP: Total gas production volume, NH_3: Ammonia and TVFA: Total volatile fatty acids

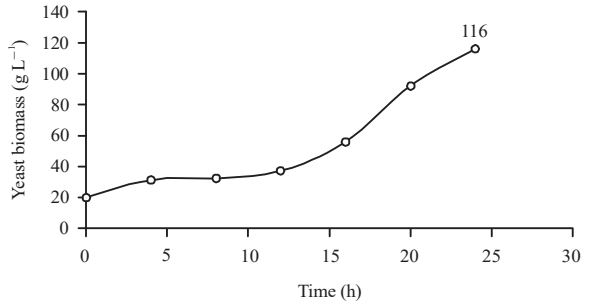

Fig. 1: Yeast biomass during fermentation time in lab fermenter

a high level of protein with very low total lipid content, compared with factory reference strain. The total viable plate count was 10.06 log CFU g^{-1} (Table 6).

Impact of the produced and commercial yeasts on diet's digestibility parameters and rumen fermentation characteristics (*in vitro* study): Data of Table 7 showed that, by increasing yeastes (produced yeast and Yea-Sacc 1026) addition level to 3g kg^{-1} DM of diet, degradability of the treated diet's DM, NDF, ADF, cellulose and hemicellulose increased. No significant differences in diet degradability parameters have been detected due to source (produced or commercial) of yeast. Also, no significant changes in ruminal gas production-pH and NH_3 concentration due to produced and commercial yeast's addition to tested diets (Table 8). The ruminal total Volatile Fatty Acids (VFA) concentration increased slightly after produced yeast and Yea-Sacc 1026 addition to the tested diets.

DISCUSSION

In this study, factors affecting production of yeast biomass such as aeration, incubation time and dilution ratio were investigated. The results showed the effect of aeration which influenced by the volume of the fermented medium in relation to the volume of the fermentation vessel (flask 250 mL) with changing the shaking speeds. As the shaking speed was increased from 150-200 rpm, the biomass increased and ethanol yield decreased. These results were due to an increase in the aeration level with increasing the agitation rate therefore, sufficient oxygen must be supplied by increasing the air space in the fermentation vessel with increasing the agitation rate. These result agreed with those of Olson and Johnson[29], who found that a liquid volume of 25 mL in the 500 mL Erlenmeyer flask gave maximum yield. Similar data of Kamble *et al*.[30] indicated that, agitation below 200 rpm resulted in lower biomass production. So, increased agitation level resulted in increased growth of cell mass. The use of stirrers in fermenter leads to better air bubbles dispersion and thus larger oxygen transfer surfaces.

Yeast cultures which having a large size resulting in relatively low densities of biomass, while a lower medium volume in the same size of bioreactor allowed for more aeration and consequently higher yeast growth rates. Similarly, Estela-Escalante *et al*.[31] showed that, the most important parameter determining the balance between the fermentation and respiratory activity in many yeasts is oxygen, but these results disagree with other researchers[32] who reported that the maximum growth of yeast was observed at 120 rpm.

The presence of glucose above 0.2% in the yeast production medium may led to switch the respiratory growth metabolism of yeast to a fermentative one and let for excretion of intermediate metabolites like ethanol. So, in this study, it was important to overcome the high level of sugar, by dilution of whey to reach an optimum level of sugar supporting the best yield of yeast biomass. The biomass yield was increased by increasing of dilution ratio as shown in Table 3. The reason for adopting the fed batch fermentation was due to using a fermentation system more effective for avoiding crabtree effect[33]. In fed batch fermentation, the optimum parameters were applied because it is the only practical system that allows production of yeast biomass without simultaneous formation of significant alcohol quantities. Sugar must be supplied at slow rate incrementally and continuously to allow the cells to consume it continuously and not to surpass the critical concentrations[34].

In this study, the best incubation time for yeast production was 72 h and then the yield decreases. This can be due to nutrients shortage, products inhibitory accumulation of growth or deviation from optimum pH. The optimum incubation period for biomass production from *Myrothecium verrucaria* and *Trichoderma viride* were 3 and 5 days, respectively[35] while, Fadel and Degheidi[36] reported an optimum incubation period of 48 h using *K. fragilis*. In other reports after 36 h of incubation, the yeast produced the maximum cell mass and lowest residual reducing sugar[37].

During fed batch cultivation of yeast, levels of carbohydrate fed and dissolved oxygen were used for growth rate and high biomass regulation. Limited sugars with presence of O_2 allow *S. cerevisiae* to grow in full respiratory, which produces higher biomass yields in the batch phase[38]. In this study, the enzymatically hydrolyzed permeate was concentrated by evaporation till obtained a syrup of 20% glucose. A feeding volume of this syrup was provided periodically to the fermenter during the first 10 h from the fermentation process. Growth was allowed for 24 h during which *S. cerevisiae* biomass was monitored. A relatively short lag phase has been observed for about 6 h. This was accompanied by gradual transition to an exponential phase showing a maximum growth rate between 16-20 h from the incubation period. A relatively high yeast biomass (116 g L^{-1}) was released at the end of the growing period (24 h). The previous described process allowed high consumption of sugar with avoiding the majority of alcohol formation and thus recommended for production of high biomass yield. Similar result has been reported by El-Helow *et al*.[39]. The viability reached to log 10.06 CFU g^{-1}, which indicating a good level of viability. The chemical composition and fermentation power of the produced *S. cerevisiae* were comparable to those of the factory reference strain. The most important demand for efficient commercial production of baker's yeast is high biomass yield accompanied by good dough leavening efficiency[39].

As application, the produced yeast was used as a feed additive for improving dairy animal's productivity. The occurred improvement of DM, NDF, ADF, cellulose and hemicellulose degradability after yeastes addition may be attributed to provide the nitrogen requirements for ruminal microbes which may have been partially or completely met due to protenic biomass of *S. cerevisiae*. Also, yeast biomass can positively effect the rumen environment through provide factors that stimulate proteolytic bacteria causing Cerebral Palsy (CP) digestion to increase[8]. Also, increase microbial protein synthesis[12]. As well as, reduce the concentration of

oxygen in rumen fluid and improve utilization of diet's starch[13]. Moreover, increase total number of microorganism's especially cellulolytic bacteria and increase rate or extent of ruminal fiber digestion[12]. Higher, Total Volatile Fatty Acids (TVFA) concentration for yeasts treated diets than those of control is a direct result for improvement of rumen environment with enhancement in diets carbohydrates degradability[13].

CONCLUSION

Production of yeast biomass on permeate basal medium has 2 main advantages: The first one is production of an important product essential for bread manufacture on a cheap substrate and this represents an economical advantage. The second one is reducing the pollution resultant from the misdisposal of permeate and this contributes in improving the environmental and health conditions. In this study, the yeast biomass was produced successfully from hydrolyzed permeate after investigation of the optimum production conditions including aeration, agitation, incubation period and dilution ratio for the yeast (*S. cerevisiae*) growth.

SIGNIFICANCE STATEMENT

This study discovered the suitability of employing dairy wastes as cheaper substrates for the production of yeast biomass in large scale and applied this product in several applications. This study will help the researchers to uncover the critical areas of recycling many agro-industrial wastes into added value products to overcome the gap between the native production of milk and its demands by minimizing the feed gap.

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