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EFFECT OF RUMEN PROTECTED CHOLINE SUPPLEMENTATION ON DIGESTIBILITY, RUMEN ACTIVITY AND MILK YIELD IN LACTATING FRIESIAN COWS

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ABSTRACT

Twelve lactating Friesian cows at 2nd to 5th lactating season, with body weight of 500±15 kg were fed a basal ration consisting, on a dry matter (DM) basis, of 40% concentrate feed mixture + 40% fresh berseem + 20% rice straw supplemented with rumen-protected choline (RPC) in the form of choline chloride at levels of 0, 15 and 30 g/head/day. The cows were used 8 weeks after calving in a complete switch-back design. The digestibility coefficients and nutritive values significantly increased ($P<0.05$) with RPC. Rumen-protected choline supplementation increased ($P<0.05$) the intake of total digestible nutrients (TDN) and digestible crude protein (DCP), but decreased plasma cholesterol and triglyceride concentrations ($P<0.05$). The pH, TVFA and NH_3-N concentrations of rumen liquor was not significantly ($P>0.05$) affected by RPC supplementation. Rumen-protected choline supplementation led to increased actual milk yield by 1.32 and 2.24 kg/head/day or 8.75 and 14.85%, and 4% fat corrected milk (FCM) by 1.55 and 2.60 kg/head/day or 10.86 and 18.31% for 15 and 30 g RPC compared with un-supplemented RPC, respectively ($P<0.05$). The contents of fat and total solids (TS) and the yield of all milk constituents except ash increased significantly ($P<0.05$) with RPC supplementation. Rumen-protected choline supplementation decreased the quantities of DM, TDN and DCP per kg 4% FCM ($P<0.05$). The feed cost per kg 4% FCM decreased, but the average income of milk yield increased by 3.11 and 5.20 LE or 10.94 and 18.28% for 15 and 30 g RPC compared with un-supplemented RPC, respectively ($P<0.05$). It could be concluded that rumen-protected choline supplementation at 30 g/head/day to lactating Friesian cows improved digestibility, milk yield and composition, feed conversion, and economic efficiency.

Key words: rumen, feed conversion, economic efficiency, choline, milk yield

INTRODUCTION

Choline is a vitamin-like compound whose metabolism interacts very closely with methionine and vitamin B12 metabolism. Feedstuffs for dairy cattle contain free choline and phosphatidylcholine because the content of these compounds in plants is relatively small and their ruminal degradation is extensive (Sharma and Erdman, 1989). Intestinal supply is not enough to meet tissue requirements. The microbial populations in the rumen quickly degrade dietary choline; therefore, the only practical means of increasing choline to the periparturient dairy cow is to feed it in a rumen-protected form (Atkins et al., 1988). Recently, a rumen-protected choline product that could potentially be used to supplement the needs of dairy cows has become available.

There is an estimated requirement for gram quantities of choline for normal tissue metabolism and milk production in lactating dairy cows and, because very little dietary choline escapes rumen degradation, choline may limit milk production (Erdman, 1992). Increasing the post-ruminal supply of choline by an infusion of choline into the abomasum has increased milk production and milk fat yield (Erdman and Sharma, 1991).

Rumen-protected choline (RPC) products have been fed to pre-parturient dairy cows to increase the

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supply of choline to the small intestine with the goal of increasing milk or component yields or alleviating the development of fatty liver syndrome (Hartwell *et al.*, 2000; Piepenbrink and Overton, 2003; Pinotti *et al.*, 2003; Overton and Waldron, 2004).

The objective of this study was to investigate the effect of RPC supplementation on digestibility, rumen fermentation, blood plasma constituents, milk yield and composition, feed conversion and economic efficiency of lactating Friesian cows.

MATERIALS AND METHODS

Experimental animals

Twelve lactating Friesian cows at the 2nd to 5th lactating season with a mean body weight of 500±15 kg were used 8 weeks after calving. The cows were free from any disease, with a normal healthy appearance, and were housed individually under open sheds.

Feeding system and management

The cows were fed a basal ration which consisted, on a DM basis, of 40% concentrate feed mixture + 40% berseem + 20% rice straw supplemented with RPC in the form of choline chloride at the levels of 0, 15 and 30 g/head/day. The cows were individually fed to meet the recommended requirements for dairy cows according to NRC (2001). Feed refusals were recorded once daily. Feeding allowances were adjusted every week according to changes in body weight and milk production. Rumen-protected choline in the form of choline chloride was procured from Qingdao Worldwide International Trade Co. Ltd., China.

Concentrate feed mixture, berseem and rice straw were offered twice daily in two almost equal meals at 8 am and 3 pm. Choline chloride was added to the concentrate feed mixture at the morning feeding. Cows were watered three times daily at 7 am, 12 am and 5 pm. Chemical composition of the feedstuffs and basal ration is presented in Table 1.

Table 1: Chemical composition of feedstuffs and calculated composition of basal ration

Item	Concentrate feed mixture*	Berseem	Rice straw	Basal ration
DM %	91.90	15.65	91.45	31.47
Composition of DM%				
OM	92.65	88.40	84.15	89.25
CP	16.25	15.35	2.55	13.15
CF	11.70	27.50	34.60	22.60
EE	3.15	2.70	1.15	2.57
NFE	61.55	42.85	45.85	50.93
Ash	7.35	11.60	15.85	10.75

* Concentrate feed mixture consisted of 32% undecorticated cotton seed cake, 24% wheat bran, 22% yellow corn, 12% rice bran, 5% line seed cake, 3% molasses, 1% limestone and 1% common salt.

Experimental design

A complete switch-back design was used with three treatments and three successive experimental periods, each period consisting of 28 days. The first 14 days of each period were considered a transition period followed by 14 days as the test period, as described by Lucas (1956). The cows were assigned randomly to the experimental rations.

Digestibility trails

A digestibility trail was carried out during the 1st period of the feeding trial using all experimental cows (4 in each group), to determine the digestibility coefficients and nutritive values of the experimental rations using acid insoluble ash (AIA) as a natural marker (Van Keulen and Young, 1977). Faeces samples were taken from the rectum of each cow twice daily at 12 h intervals during the collection period. Samples of tested feedstuffs were collected at the beginning, middle and end of the collection period. Representative samples of feedstuffs and faeces were chemically analyzed according to the methods of AOAC (1990). Digestibility coefficients were calculated from the equations given by Schneider and Flatt (1975).

$$\text{DM digestibility \%} = 100 - \left[100 \text{ x } \frac{\text{AIA\% in feed}}{\text{AIA\% in feces}}\right]$$

$$\text{Nutrient digestibility \%} = 100 - \left[100 \text{ x } \frac{\text{AIA\% in feed}}{\text{AIA\% in feces}}\right] \text{ x } \left[\frac{\text{Nutrient \% in feces}}{\text{Nutrient \% in feed}}\right]$$

Milk yield and samples

Cows were mechanically milked at 6 am and 5 pm, individual morning and evening milk yields were recorded daily for each cow and the 4% FCM for each cow was calculated from daily milk yield and the percentage of milk fat using the formula as given by Gains (1928): 4% FCM = [0.4x milk yield (kg)]+[15xfat yield (kg)].

Milk samples from consecutive evening and morning milkings were collected from each cow in the 4th week of each period and mixed in proportion to milk yield. Composite milk samples were analyzed for fat, protein, lactose, solids not fat (SNF), and total solids (TS) by Milko-Scan (model 133B), and ash by difference.

Rumen liquor samples

Rumen liquor samples were collected using a rubber stomach tube from the cows three times during the 4th week of each experimental period within 3 hours after offering the morning feed, and strained through four layers of cheesecloth. Ruminal pH value was determined directly using a digital pH- meter, while ammonia–nitrogen (NH_3-N) concentration was determined using magnesium oxide (MgO) as described by AOAC (1990), and total volatile fatty acid (TVFA) concentration was determined by a steam distillation method as described by Warner (1964).

Blood Samples

At the same time, blood samples were collected from all the cows three times during the experimental period from the jugular vein into dry, clean glass tubes using heparin as anticoagulant, and centrifuged for 15 minutes at 4,000 rpm to obtain plasma. Plasma levels of glucose, cholesterol, triglyceride, total protein, albumin, globulin (by difference), urea, AST and ALT were determined colorimetrically by spectrophotometer (Spectronic 21D, USA) using commercial kits manufactured by Diagnostic System Laboratories, Inc., USA.

Feed conversion

Feed conversion was determined as the amounts of DM, TDN and DCP required for producing 1 kg 4% FCM.

Economic efficiency

Economic efficiency was calculated as the ratio between the price of produced milk and the cost of feed consumed which were 2 LE/kg of 4% fat corrected milk produced, 1,800 LE/ton of concentrate feed mixture, 120 LE/ton of fresh berseem, 75 LE/ton of rice straw and 6,000 LE/ton of choline chloride, according to 2007 figures (LE= 0.13 £).

Statistical analysis

All data were statistically analyzed according to Lucas (1956) using the fixed model, with RPC as fixed effect and cows as random effect. The data were also quadratically analyzed. Significant differences in the mean values among dietary treatments were analyzed by repeated measures and Duncan's tests. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Digestibility coefficients

The digestibility coefficients of DM, OM, CP, CF, EE and NFE increased significantly ($P<0.05$) with RPC supplementation (Table 2). However, these were similar for 15 and 30 g RPC supplementation. The digestibility coefficients of DM, OM, CP, CF, EE and NFE for 15 and 30 g RPC increased by 3.55 and 3.93, 3.63 and 4.02, 2.98 and 3.66, 4.59 and 5.04, 1.68 and 1.78, and 3.09 and 3.97%, respectively, compared with un-supplemented RPC. The quadratic analysis showed that RPC supplementation at 25 g had the highest digestibility coefficients of DM (R^2=0.76), OM (R^2=0.67), CP (R^2=0.60) and CF (R^2=0.77) being 67.96, 69.43, 68.93 and 67.30%, at 20 g the highest EE (R^2=0.32) digestibility (71.47%) and at 30 g the highest NFE (R^2=0.68) digestibility (71.24%). These results may be attributed to choline supplementation having increased protozoa populations in the rumen. These results are in agreement with those obtained by Sharma and Erdman (1988a) who supplemented Holstein cows with 10 and 20 g added choline/kg ration DM and reported that rumen apparent digestibility of DM, CP, NDF, and ADF was similar for the different choline levels.

Nutritive values

Results in Table 2 indicated that TDN and DCP values followed similar trends to the digestibility coefficients, which increased significantly ($P<0.05$) with RPC supplementation, but were similar for 15 and 30 g RPC supplementation. The TDN value for 15 and 30 g RPC increased by 3.11 and 3.75%, respectively, and the DCP value increased by 0.39 and 0.48%, respectively, compared with un-supplemented RPC. The quadratic analysis showed that RPC supplementation at 30 g produced the highest TDN (65.07% R^2=0.71) and DCP (9.65%, R^2=0.60). Choline is a quasi-vitamin that has a variety of functions in mammalian metabolism (Donkin, 2002; Pinotti *et al*., 2002).

Table 2: Digestibility coefficients and nutritive values by Friesian cows supplemented with rumen protected choline

Item	Choline chloride (g/head/day)			
	0	15	30	SEM
Digestibility coefficients %:				
DM	63.78^b	67.33^a	67.71^a	0.35
OM	65.05^b	68.68^a	69.07^a	0.37
CP	65.18^b	68.16^a	68.84^a	0.35
CF	61.82^b	66.41^a	66.86^a	0.44
EE	69.77^b	71.45^a	71.55^a	0.24
NFE	67.34^b	70.43^a	71.31^a	0.35
Nutritive values % (on DM basis):				
TDN	60.87^b	63.98^a	64.62^a	0.34
DCP	8.57^b	8.96^a	9.05^a	0.05

a, b and c: Values in the same row with different superscripts differ significantly at 5% level.

Feed intake

Rumen-protected choline supplementation did not significantly (P>0.05) affect the intake of concentrate feed mixture, berseem, rice straw and total DM (Table 3). However, the intake of TDN and DCP increased significantly (P<0.05) with RPC supplementation. The quadratic analysis showed that the levels of 25-30 g RPC had the highest intake of concentrate feed mixture (7.06 kg), 30 g RPC had the highest intake of fresh berseem, total feed, total DM (R^2=0.03), TDN (R^2=0.42) and DCP (R^2=0.33) being 41.67, 52.37, 16.32, 11.35 and 1.56 kg/day, respectively, and levels of 15-30 g RPC had the highest rice straw intake (3.54 kg/day). These results were attributed to increasing TDN and DCP values with rumen-protected choline supplementation (Table 2). These results are also in accordance with those obtained by Piepenbrink and Overton (2003) and Guretzky *et al.* (2006), who found that rumen-protected choline supplementation for cows did not affect dry matter intake either pre-partum or during the first 3 weeks post-partum.

Rumen liquor parameters

The pH, TVFA and NH_3-N concentrations of rumen liquor was not significantly (P>0.05) affected by RPC supplementation (Table 4). The quadratic analysis showed that RPC supplementation had little effect on ruminal pH (R^2 = 0.07), TVFA (R^2 = 0.08) and NH_3-N (R^2 = 0.08). These results may be attributed to the fact that protected choline was not degraded in rumen. These values are within the normal range as obtained by Van Soest (1983), who stated that the optimum pH value for growth of cellulytic microorganisms was 6.7 with a range under normal conditions of about ±0.5 pH. Russell and Dombrowski (1980) reported that ruminal VFA production was closely related to ruminal pH, which can be considered an important regulator of microbial yield. Also, depression of both ruminal pH value and NH_3-N concentration with the rise in VFA concentration may be attributed to the proper functioning of rumen microorganisms, which utilize ammonia-N to synthesize microbial protein as reported by Breves and Schroder (1991). Sharma and Erdman (1988b) found that rumen protected choline does not appear to have any effect on pH, TVFA and NH_3-N concentrations in rumen liquor of Holstein cows.

Blood plasma constituents

Results presented in Table 4 showed that RPC supplementation led to a significant decrease (P<0.05) in the concentrations of plasma cholesterol and triglycerides. However, the differences in plasma glucose, total protein, albumin, globulin and urea-N concentrations and the activities of AST and ALT among the different experimental groups were not significant (P>0.05). The quadratic analysis showed that RPC supplementation at 30 g yielded the highest plasma concentrations of glucose (71.78 mg/dl, R^2=0.30), total protein (7.67 g/dl, R^2=0.11), albumin (3.22 g/dl, R^2=0.10) and globulin (4.33 g/dl, R^2=0.12) and the lowest concentrations of cholesterol (131.96 mg/dl, R^2=0.96), triglycerides (mg/dl, R^2=0.79), urea-N (38.79 mg/dl, R^2=0.10) and AST (39.11 μ/l, R^2=0.12), while 20 g RPC produced the highest ALT (26.01 μ/l, R^2=0.05). These results are in agreement with the findings of Zahra *et a*l. (2006) and Guretzky et al. (2006), which showed that Holstein cows receiving rumen-protected choline supplementation had significantly lower serum cholesterol concentration and greater serum triglycerides compared with cows that did not receive RPC. In comparison, Bindel *et al*. (2005), Zahra et al. (2006), Toghdory et al. (2007) and Ambrosio *et al*. (2007) reported that blood metabolites such as glucose, total protein, albumin, globulin and urea-N concentrations and the activity of AST and ALT in cows and goats were not affected by choline supplementation.

Table 3: Average daily feed intake by Friesian cows supplemented with rumen protected choline

Item	Choline chloride (g/head/day)			
	0	15	30	SEM
Average daily feed intake (as fed)		kg/head/day		
Concentrate feed mixture	7.00	7.04	7.05	0.03
Fresh berseem	41.10	41.32	41.40	0.16
Rice straw	3.52	3.54	3.54	0.01
Total intake	51.62	51.90	51.99	0.19
Average daily feed intake (on DM basis)		kg/head/day		
Total DM	16.08	16.17	16.20	0.05
TDN	9.79^b	10.35^a	10.47^a	0.24
DCP	1.38^b	1.45^a	1.47^a	0.03

a and b: Values and means in the same row with different superscripts differ significantly at 5% level.

Table 4: Rumen activity and blood plasma constituents of Friesian cows supplemented with rumen protected choline

Item	Choline chloride (g/head/day)			
	0	15	30	SEM
Rumen activity				
pH value	6.74	6.72	6.67	0.02
TVFAs (meq / 100 ml)	14.83	14.96	15.03	0.16
NH_3-N (mg / 100 ml)	16.91	16.75	16.63	0.07
Blood plasma constituents				
Glucose (mg / dl)	69.26	71.36	69.64	1.01
Cholesterol (mg / dl)	160.91^a	140.41^b	132.16^c	4.35
Triglycerides (mg / dl)	112.97^a	108.24^{ab}	104.09^b	1.61
Total protein (g / dl)	7.56	7.37	7.51	0.09
Albumin (g / dl)	3.07	3.21	3.26	0.06
Globulin (g / dl)	4.49	4.16	4.25	0.08
Urea-N (mg / dl)	39.51	39.10	38.60	0.71
AST (units / l)	40.40	39.65	38.70	0.56
ALT (units / l)	26.43	25.96	25.79	1.15

a and b: Values and means in the same row with different superscripts differ significantly at 5% level.

Milk yield

Rumen-protected choline supplementation resulted in a significant increase ($P<0.05$) in actual milk and 4% FCM yield (Table 5). The actual milk yield increased by 1.32 and 2.24 kg/head/day or 8.75 and 14.85% for 15 and 30 g RPC, respectively, compared with the un-supplemented RPC treatment. The corresponding values of 4% FCM were 1.55 and 2.60 kg/head/day or 10.86 and 18.31%, respectively. The quadratic analysis showed that RPC supplementation at 30 g produced the highest actual milk ($R^2=0.58$) and 4% FCM ($R^2=0.53$) yields of 17.35 and 16.92 kg/day, respectively. The increase in milk yield with RPC supplementation may be due to one or more of the following reasons: i) higher nutrient digestibility (Table 2) and TVFA concentration and lower NH_3-N concentration in the rumen (Table 4) of animals supplemented with RPC; ii) RPC supplementation increases milk yield of high producing dairy cows during the first 60 days of lactation by improving lipid export from liver metabolism. iii) RPC feeding contributes to the prevention of metabolic disorders and economic losses related to abnormal lipid metabolism, therefore RPC supplementation is recommended for all high-yielding dairy herds during the peri-parturient period, especially when the body condition of the cows is too high. Feeding RPC increases choline concentration and milk yield, which indicates a better choline supply in supplemented cows. The large milk yield response is a consequence of the metabolic changes caused by RPC supplementation. These results are in agreement with those obtained by Pinotti *et al*. (2000), who found that cows consuming 20 g/day RPC produced 3.5 kg/day more milk than the controls. *Zahra et al.* (2006) reported that Holstein cows received 56 g/day of RPC produced on average 1.2 kg/day more milk yield through 60 days compared with those not receiving RPC supplementation. Erdman and Sharma (1991) fed Holstein cows 0, 0.078, 0.156, and 0.234% RPC and found that added choline resulted in a linear increase in 3.5% FCM and feeding 0.156 and 0.234% choline increased FCM by 2.4 and 1.7 kg/day, respectively, over the control. Guretzky *et al*. (2006) fed Holstein and Jersey cows diets which were top dressed once daily with 60 g of a RPC product and found that daily yield of 3.5% FCM increased by 2 kg/day compared with the control group.

Milk composition

Only the contents of fat and total solids (TS) increased significantly ($P<0.05$) with RPC supplementation (Table 5). However, the yield of all milk constituents except ash increased significantly ($P<0.05$) with RPC supplementation as a result of increasing milk yield. The quadratic analysis showed that RPC supplementation at 30 g produced the highest contents of fat ($R^2=0.34$), protein ($R^2=0.04$), lactose ($R^2=0.02$), SNF ($R^2=0.02$) and TS ($R^2=0.09$) being 3.89, 3.17, 4.44, 8.29 and 12.14%, respectively and the highest yields of fat ($R^2=0.51$), protein ($R^2=0.40$), lactose ($R^2=0.38$), SNF ($R^2=0.39$) and TS ($R^2=0.43$) being 0.65, 0.55, 0.78, 1.45 and 2.10 kg/day, respectively. These results agree with those obtained by Piepenbrink and Overton (2003), and Guretzky *et al*. (2006) who showed that cows fed RPC had increased milk fat, total solids contents and yield. Many researchers reported that RPC supplementation have no significant effect on the contents of protein, lactose, SNF and ash, but their yields increased ($P<0.05$) significantly (Sharma and Erdman, 1988a,b; Erdman and Sharma, 1991; Zahra *et al*., 2006; Ambrosio *et al*., 2007).

Table 5: Average daily milk yield and composition of Friesian cows supplemented with rumen protected choline

Item	Choline chloride (g/head/day)			
	0	15	30	SEM
Actual milk yield (kg/day)	15.22^b	16.54^a	17.46^a	0.27
4% FCM (kg/day)	14.22^b	15.77^a	16.82^a	0.29
Milk constituents content %				
Fat	3.56^b	3.69^a	3.75^a	0.02
Protein	3.12	3.15	3.16	0.01
Lactose	4.39	4.39	4.43	0.01
SNF	8.21	8.25	8.30	0.02
TS	11.78^b	11.94^a	12.05^a	0.03
Ash	0.71	0.71	0.71	0.003
Milk constituents yield (kg/day)				
Fat	0.54^b	0.61^a	0.66^a	0.01
Protein	0.47^b	0.52^a	0.55^a	0.01
Lactose	0.67^b	0.73^a	0.77^a	0.01
SNF	1.25^b	1.36^a	1.45^a	0.02
TS	1.79^b	1.97^a	2.11^a	0.03
Ash	0.11	0.12	0.12	0.002

a, b: Values and means in the same row with different superscripts differ significantly at 5% level.

Feed conversion

RPC supplementation improved feed conversion expressed as the quantities of DM, TDN and DCP required to produce one kg 4% FCM (Table 6). The quantities of DM, TDN and DCP per kg 4% FCM decreased significantly (P<0.05) with RPC supplementation and cows supplemented with 30 g RPC showed the lowest quantities (R^2=0.49, 0.24 and 0.24, respectively). The quadratic analysis showed that RPC supplementation at 30 g produced the lowest DM, TDN and DCP per kg 4% FCM (0.87, 0.61 kg and 87.21 g, respectively). These results can be attributed to similar feed intake (Table 3) and increasing 4% FCM yield with RPC supplementation (Table 5).

Economic efficiency

Results in Table 6 revealed that in spite of the average daily feed cost increasing significantly (P<0.05), average daily feed cost per kg 4% FCM decreased significantly (P<0.05) with increasing level of RPC supplementation. The average income from daily milk yield increased by 3.11 and 5.20 LE and net revenue increased by 2.92 and 4.88 LE for 15 and 30 g RPC, respectively, compared with the un-supplemented treatment (P<0.05). The quadratic analysis showed that RPC supplementation at 30 g had the highest daily feed cost (R^2=0.22), income from 4% FCM yield (R^2=0.53) and income improvement of 4% FCM yield (R^2=0.97) being 19.63, 33.87, 5.43 LE and 18.27%, respectively and the lowest feed cost/kg 4% FCM (R^2=0.46) being 1.06 LE. These results can be attributed to increasing 4% FCM yield with RPC supplementation (Table 5).

Table 6: Feed conversion and economic efficiency of Friesian cows supplemented with rumen protected choline

Item	Choline chloride (g/head/day) 0	15	30	SEM
Feed conversion				
DM kg / kg 4% FCM	1.14^{a}	1.03^{b}	0.97^{b}	0.02
TDN kg / kg 4% FCM	0.69^{a}	0.66^{ab}	0.62^{b}	0.01
DCP g / kg 4% FCM	97.05^{a}	91.95^{ab}	87.40^{b}	2.15
Economic efficiency				
Daily feed cost (LE)	19.18	19.37	19.50	0.04
Feed cost (LE)/ kg 4% FCM	1.36^{a}	1.24^{b}	1.17^{b}	0.02
Income of milk yield (LE)	28.44^{b}	31.55^{a}	33.64^{a}	0.57
Income improvement (LE)	0.00^{c}	3.11^{b}	5.20^{a}	0.65
Income improvement %	100.00^{c}	110.94^{b}	118.28^{a}	2.54

a, b and c: Values and means in the same row with different superscripts differ significantly at 5% level.

CONCLUSION

From the results of the present study, it could be concluded that rumen protected choline (RPC) supplementation at 30 g/head/day to lactating Friesian cows improved nutrient digestibility, milk yield and composition, feed conversion and economic efficiency.

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