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Effect of Rumen-Protected Choline Supplementation on Growth Performance of Fattening Calves

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Abstract: This study aimed to evaluate the influence of choline chloride supplementation on the growth performance of feedlot calves. Thirty-two crossbred calves with a mean initial body weight of 305.71±4.09 Kg were divided into three groups of 10, 11 and 11 animals, randomly. The animals were distributed to one of three dietary treatments, control, choline15 and choline 30 (the rations supplemented with 0, 15 and 30 g choline chloride, respectively). The animals were fed on concentrate feed at a rate of about 2.15% of their live body weight and rice straw open access. Supplementing animal ration with choline chloride did not significantly affect all nutrients' digestibility and feeding value. The animals supplemented with 30 g choline chloride recorded significantly higher (P=0.037) total serum protein concentration compared to the other groups. The group received a ration supplemented with 30 g of choline chloride and recorded significantly higher final weight, total gain, and mean daily gain. Data showed significant improvement in TDN, CP and DCP conversion for the animal received ration supplemented with 30 g choline chloride compared to the control group. In conclusion, supplementing fattening calves' ration with rumen-protected choline could improve average daily gain and feed conversion.

1 Introduction

Choline is considered an essential nutrient for animals; it is characterized by its high requirements and thus, in some classifications, it is not considered as a vitamin (NRC 2021). Choline is a source of phosphatidylcholine and acetylcholine, and it is considered a methyl group donor through its metabolism to betaine. Methyl groups are essential for many metabolic reactions like methionine recycling and fat mobilization (Tawny and Heather 2017). The rumen non-protected choline degraded rapidly in the animal rumen (Jayaprakash et al 2016). Lagace (2015) reported that phosphatidylcholines are the most plentiful phospholipids compounds in animal cells, and they are incorporated in the metabolism of lipids (absorption & transport) in addition to anabolism of lipoproteins compounds; while Kuo and Ehrlich (2015) reported that neurotransmitter compound (acetylcholine) plays an important role in the contraction of the muscle and activity of the brain. Choline allowances in farm animals have not been well determined as a result of several reasons (Kawas et al 2020). 1) Feedstuff materials contain a different ratio of natural choline that display a variety of bioavailability, and 2) the choline in feeds interacts with other nutrients involved in trans-methylation reactions (methionine, folic acid, and Britain cobalamin) (Kawas et al 2020). 3) The combination of fat, proteins, and carbohydrates in the ration at different levels of animal age, energy consumption, and weight gain is affecting choline lipotropic action, thus, the requirement of this nutrient. 4) Choline (non-protected choline) in feed naturally is rapidly degraded by rumen flora, and the groups of methyl are metabolic to ultimately methane and trimethylamine (Jayaprakash et al 2016). For these reasons, feedstuffs rich in choline can only slightly contribute to the duodenal choline supply, so, choline supply must be provided in the protected form in ruminant (Kawas et al 2020). Despite the factors affecting determining choline requirements in ruminant animals, growing evidence points to, the production of ruminant animals may be enhanced with choline addition (NRC 2021). Feeding animals with ration supplemented with rumen-protected choline led to a decrease in the accumulation of fats in the liver and increased milk production in cows (Humer et al 2019), increase average daily gain, and improved carcass characteristics in beef cattle (Habeeb et al 2017), also, improve growth rate, decrease fat percentage in tissues and enhance meat quality in feedlot lambs (Huawei et al 2015). Also, high carbohydrate (rapidly fermented) feedlot ratio leads to a decrease in rumen pH and affects the balance of the microorganisms in the rumen, consequently, affecting the degradability of choline in the rumen (Francisco et al 2019).

2 Materials and Methods

The present study was carried out at Abdel Rahman Amin Farms for Livestock Production, Al-Faiyum Governorate, Egypt. In cooperation with Labs of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University, Egypt.

2.1 Rumen-protected choline (RPC) (choline chloride)

Choline chloride is an organic compound with the formula [(CH3)3NCH2CH2OH]Cl. It is functional, containing both quaternary ammonium salt and alcohol.

2.2 Animals and Treatments

Thirty- two male crossbred calves with an average initial body weight of (305.71 ± 4.09 Kg) were randomly assigned into three groups (10 animals for one group and 11 for each of both other groups), to receive one of the following experimental rations. The first group served as a control (no choline chloride supplementation); the second group (low level of choline chloride supplementation) was supplemented with 15g choline chloride/head/day and the third group (high level of choline chloride supplementation) was supplemented with 30g choline chloride/head/day. The animals were fed about 2.15 % of their live body weight concentrate feed mixture and rice straw *ad. lib*. The concentrate feed mixture (55 % yellow corn, 10.5 % soybean meal 10.5% wheat bran 10% corn gluten feed 6% sunflower seed meal 5% black seed meal and 0.4% Di-calcium phosphate, 1% salt, 0.3% minerals and vitamins mixture and 1.3% buffering agent (**Table 1)**. Minerals and vitamins mixture composition: Mg: 100, Mn: 125.8, Zn: 41.7, Fe: 166.7, Cu: 32, P: 100 (g/kg DM); I: 810, Se: 480, Co: 200 (mg/kg DM); vitamin A: 20000000 (IU/kg DM), D: 2000000 and E: 10000 (IU/kg DM). The diet was balanced for minerals and vitamins and formulated to cover the nutrient requirements of calves according to NRC (2000). The diets were offered daily in two parts; at 8.00 a.m. and 16.00 p,m, and the animals had free excess to clean fresh water.

Table 1. The diet ingredients chemical composition (%)

Item	Concentrate	Rice straw
Dry matter	91.64	94.16
Constituents, % on DM basis		
OM	92.65	82.87
CP	16.21	4.22
CF	7.19	38.94
EE	7.31	1.22
NFE	61.94	38.49
NFC	54.73	4.99
Ash	7.35	17.13
NDF	18.40	72.45
ADF	7.19	44.18

2.3 Nutrients digestibility

In the last week of the fattening trial, five animals were chosen randomly, from each group and subjected to the grab sample method to determine nutrient digestibility, in which the internal marker method was applied according to Hansen et al (2017) using acid insoluble ash (AIA) as an internal marker.

2.4 Sampling procedure

Samples of concentrate and rice straw were taken twice a week during offering the ration to animals, each ingredient was mixed and a representative sample was stored until subsequent analysis.

Blood samples were withdrawn from the jugular vein after 3 hrs. of morning feeding of 10 animals for each group. Immediately, the serum samples were extracted by centrifuging the sample at 2000 g for 20 min, then serum samples were transferred to clean dried Eoendorf then, stored in a deep freezer at -20ºC for specific subsequent chemical analysis.

2.5 Chemical analysis

Ration ingredients, as well as feces samples, were subjected to analysis for DM, ash, Crude fiber (CF); Crude protein (CP), and ether extract (EE) contents according to AOAC (2019). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were analyzed sequentially by AOAC (2019) using the Ankom200 Fibre Analyzer. NDF and ADF are expressed inclusive of residual ash.

Non-fiber carbohydrate (NFC) =
(100− (%NDF+%CP + %fat + %ash) (NRC 2021).

2.6 Statistical analysis

All data were statistically analyzed according to the statistical analysis system User's Guide, (SAS 2012). Separation among means was carried out by using the Duncan Multiple test (Duncan 1955). The data of blood serum parameters and nutrients digestibility were analyzed using a one-way model

$$Yi = \mu + Ti + Ei$$

Where
Yi = is the effect of the observation
μ = is the overall mean

while growth performance data were analyzed using a one-way model using the initial weight as a covariate.

$$Yij = \mu + ti + b\ (Xij + \bar{x}i) + eij$$

Yij = is the j^{th} observation under i^{th} treatment;
μ = overall mean
ti = the fixed effect of the i^{th} treatment (i=1,2,..,i);
Xij = is the i^{th} observation of the covariate under the i^{th} treatment;
$\bar{x}$i = is the i^{th} treatment mean, and
eij = is the associated on observed error term

3 Results and Discussion

3.1 Nutrients digestibility

Supplementation of fattening calve ration with choline chloride had no significant effect on nutrients (DM, OM, CP, CF, EE, NFE, NDF, ADF and NFC) digestibility (**Table 2)**. Furthermore, the data indicated a slight increase in feeding values as DCP and TDN ($P \geq 0.3$) for the animals fed ration supplemented with 30 g choline chloride compared to the control group and the group fed ration supplemented with 15 g choline chloride (**Table 2)**.

The non-significant ($P > 0.3$) results observed for nutrient digestibility and feeding values are due to the that all animals received the same ratio and approximately very close quantity, which resulted in the same pattern in fermentation and degradation, parallel to that choline chloride is rumen-protected choline (RPC) form, which not degraded in the rumen consequently not affected on rumen flora and its metabolism consequently did not affect rumen fermentation and degradation.

The present results agree with those obtained by Bakr and Mohamed (2020) who supplemented Holstein cow's ration with 40 and 50 g choline chloride /kg DM and reported that all nutrient digestibility was not significantly affected by the different choline chloride levels compared to the control ration. Contrary, Supriyati et al (2016) found that all studied nutrient digestibility increased significantly for the animals that received ration supplemented with choline chloride during the late pregnancy and the lactation periods. However, the results were similar for 15 and 30 g RPC supplementation, and the authors referred the results to that rumen-protected choline enhances ruminal growth of the protozoal population.

3.2 Blood serum parameters

Fattening calve ration supplementation with 30 g choline chloride resulted in a significant increase ($p=0.037$) in total serum protein concentration compared to the control group and the group supplemented with 15 g choline chloride (**Table 3)**. In this connection, Xia et al (2018) reported that total plasma protein concentration is positively correlated with the nutritional animal status of the animal, especially with dietary protein level.

Table 2. Effect of fattening calves ration supplementation with choline chloride on nutrient digestibility (%) and feeding values (%)

Item	Control	Choline 15	Choline 30	SE	P value
	Nutrients digestibility				
DM	68.81	68.13	71.53	2.42	0.505
OM	71.89	72.09	74.85	2.32	0.512
CP	68.67	62.05	70.03	3.69	0.314
CF	50.79	55.38	56.16	4.13	0.546
EE	88.45	88.23	89.98	1.04	0.404
NFE	75.17	75.87	78.25	2.21	0.492
NDF	46.05	51.65	53.68	4.17	0.351
ADF	35.15	43.74	45.29	5.35	0.319
NFC	85.56	85.27	87.39	2.13	0.681
	Feeding Value				
DCP	7.43	6.64	7.49	0.39	0.300
TDN	72.30	72.25	74.89	2.17	0.530

Table 3. Effect of fattening calve ration supplementation with choline chloride on some blood serum parametres

	Control	Choline 15	Choline 30	SE	P value
TPC, g/dL	6.12 ^{b}	6.44 ^{ab}	6.58 ^{a}	0.079	0.037
Albumin, g/dL	3.52	3.24	3.20	0.066	0.093
Urea, mg/dL	35.60	36.20	35.50	0.433	0.802
ALT, u/L	35.80	35.60	35.17	0.474	0.867
AST, u/L	36.00	36.60	37.17	0.397	0.509
Creatinine, mg/dL	1.08	1.06	1.12	0.023	0.638

Different letters in the same row refer to significant differences (P< 0.05)

The study conducted by Habeeb et al (2017) supplemented buck ration with RPC and found that there is a significantly higher total serum protein concentration of animals that received RPC compared to the non-supplemented group.

The majority of blood metabolites and liver function were within the normal range, with no differences among the experimental treatments in albumin, urea and creatinine concentration as well as ALT and AST activity. Furthermore, Habeeb et al (2017) found no significant effect for RPC supplementation on albumin, urea and creatinine concentration as well as no significant effect on ALT and AST activity.

3.3 Growth performance

Although the animals received a ration supplemented with 30 g choline chloride recorded significantly higher final weight and total gain. The animal-fed ration supplemented with 15 g choline chloride did not record any significant differences in the final weight, total gain, and ADG compared with the control group. Moreover, no significant differences were recorded between animals that received a ration supplemented with 15 g and those that received 30 g of choline chloride (**Table 4)**.

The same trend was obtained by Jaeger et al (2009) who fed beef cows on a ration supplemented with RPC and recorded no significant effect on body weight change and ADG during the experimental period. Moreover, Hajilou et al (2014) fed young Holstein calves on ration supplemented with RPC and found that initial, final BW and ADG were not significantly affected by treatments.

On the other hand, Habeeb et al (2017) found that RPC supplementation to the Zaraibi buck's diet during summer led to a significant increase in final weight compared to the non-supplemented animal, consequently, an increase in bucks' total gain by 3.1 kg as well as average daily gain increased by 29.8%.

The authors attributed the increase in bucks' daily gain to the significant increases in feed intake as well as improvements in nutrient digestibility and nutritive values for animals who received rations supplemented with rumen-protected choline. Furthermore, Huawei et al (2015) reported that feeding lambs on diets containing choline chloride led to an increase ADG in finishing lambs.

3.4 Feed conversion

The data showed numerical improvement for DM conversion (p=0.061) for the group that received supplemented ration with 15 and 30 g choline chloride compared to the control (**Table 5)**. On the other hand, data showed significant improvements in feed conversion as TDN, CP and DCP conversion (P=0.021, 0.050, and 0.008, respectively) for the animal received ration supplemented with 30 g choline chloride in comparison with the control group while no significant differences were noted between the animal received ration supplemented with 15 g and those received 30 g choline chloride. Furthermore, no significant differences were recorded between the control treatment and those who received supplemented ration with 15 g choline chloride.

Same as the results obtained by Huawei et al (2015), which recorded improved feed efficiency in lamb fed diet supplemented with RPC, but responses depended on RPC level.

Table 4. Effect of fattening calve ration supplementation with choline chloride on growth performance

	Control	Choline 15	Choline 30	SE	P value
Initial weight, kg	304.61	304.61	304.61	0.00	-
Final weight, kg	429.68^{b}	443.09^{ab}	462.37^{a}	9.46	0.041
total gain, kg	125.07^{b}	134.47^{ab}	157.75^{a}	9.46	0.041
Average daily gain, kg	0.94b	1.04ab	1.19a	0.07	0.043

Different letters in the same row refer to significant difference (P< 0.05)

Table 5. Effect of fattening calve ration supplementation with choline chloride on feed conversion

	Control	Choline 15	Choline 30	SE	P value
DM	10.52	8.93	8.23	0.71	0.061
TDN	8.86^{a}	6.60^{b}	6.94^{b}	0.58	0.021
CP	1250^{a}	1052.38^{ab}	969.68^{b}	83.81	0.050
DCP	875.84^{a}	611.17^{b}	678.10^{b}	57.09	0.008

Different letters in the same row refer to significant difference (P< 0.05)

4 Conclusion

It could be concluded that supplementing fattening calves ration with 30 g choline chloride may be led to an increase in average daily gain and improve feed conversion. In addition supplementation with 30 g choline chloride has no adverse effect on nutrient digestibility and feeding value as well as the majority of blood metabolites kidney function and liver function.

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