

EFFECT OF FEEDING UREA ON DIGESTIBILITY, BEHAVIOUR, PLASMA UREA NITROGEN, AND GROWTH PERFORMANCE OF WEANED HOLSTEIN DAIRY CALVES

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ABSTRACT

The objective of the study was to evaluate the effect of urea addition in the diet of recently weaned dairy calves on their digestibility, behaviour, plasma urea nitrogen and growth performance. Sixteen (female=10, average body weight=63.01 kg; male=6, average body weight=66.47kg), 8-week-old Holstein-Friesian weaned calves were randomly assigned to treatment groups A and B (n=8/trt). The calves in group A served as control and in group B, they were fed a diet of 0.5% urea inclusion from 9-12 weeks of age and 1.0% urea from 13-16 weeks of age. All calves were housed and fed individually in cages. Feed intake and behavioural observations were recorded daily. Body measurements like heart girth, wither height, hip width, and hip height were measured at 8, 12, and 16 weeks of age. Blood samples were collected at 10, 12, and 14 weeks of age to measure Plasma Urea Nitrogen (PUN). All calves were weighed weekly to determine the average daily gain. The study found no negative effect of urea inclusion on calves' average daily gain, routine behavioral parameters, and growth performance. Similarly, urea inclusion did not negatively affect body weight, dry matter intake, or feed efficiency. However, it increased dry matter intake (DMI) digestibility in weaned dairy calves by 10.4%. The digestibility of non-fiber carbohydrates was 3.5% greater in calves fed urea-included diets. This study provides evidence that urea can be added to the ration of weaned dairy calves from the 8th week of age.

Keywords: Digestibility, Growth, Urea, Calves

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INTRODUCTION

Protein is the most expensive and critical macronutrient for the growth of weaned dairy calves. The crude protein (CP) requirement for weaned dairy calves (15-16% of the diet: DM basis) their diet is among the highest protein levels used in the life cycle of dairy cattle (Hill *et al.*, 2013). In the weaned calf diet, almost 100% of the protein comes from the preformed true protein sources (soyabean and canola), which makes their diet expensive. In adult dairy cattle ration, urea has been used for over 100 years to replace expensive protein sources (Kertz, 2010). Rumen microflora gives ruminants the unique ability to convert urea a non-protein nitrogen (NPN) source to good-quality animal protein products (milk and meat). Urea provides 6 times greater crude protein than soybean meal according to the NASEM (2021) model. However, in weaned calf ration, urea

inclusion is rarely considered, possibly due to the fear of urea toxicity.

Energy protein synchronization in rumen is a key concept in effectively utilizing urea and preventing its toxicity. Ruminal microbes in the presence of highly fermentable, non-fibrous carbohydrates (NFC) efficiently capture urea N to multiply in number and serve as high-quality endogenous protein for the host. Coincidentally, the quantity of NFCs used in the post-weaning calf ration (greater than 40%) is the highest in ruminant life (Dennis *et al.*, 2017; Dennis *et al.*, 2018; Hill *et al.*, 2013; Hu *et al.*, 2018), thereby providing an opportunity for the use of urea in their diet. Non-fibrous carbohydrates may allow rumen microbes of calves to capture urea nitrogen and synthesize high-quality endogenous protein, thereby preventing its toxicity risks.

Despite the favourable nutritional conditions in weaned calf ration, to the best of the authors' knowledge, urea has been rarely tested in the literature of

postweaning calves for the last thirty years. Therefore, the dairy industry has not adopted the idea of supplementing urea in weaned calf ration and the opportunity to decrease their rearing cost is not being utilized. This study aimed to evaluate the effect of feeding urea to weaned calves on their digestibility, plasma urea nitrogen, and growth performance. We hypothesize that in weaned calf ration, expensive true protein sources can be partially replaced with urea (NPN) without negatively affecting their digestibility, behaviour, plasma urea nitrogen, and growth performance.

MATERIALS AND METHODS

This study was conducted at Umar Dairy Farm (Sargodha, Punjab, Pakistan) from November 2022 to February 2023. Sixteen (female=10, male=6), 8-week-old Holstein-Friesian weaned calves were used in this study. All the experimental procedures were approved by the institutional guidelines of the ethical review committee (#337) of the University of Veterinary and Animal Sciences (UVAS), Lahore. All 16 calves (female=10, average body weight= 63.01 kg; male=6, average body weight=66.47 kg), were weaned at 8 weeks of age and then randomly assigned to one of two dietary treatment groups A and B (n=8 calves/group: 5 female, 3 male). The calves in group A served as control and were fed a diet without urea inclusion, while calves in group B were fed a diet of 0.5% urea inclusion from 9-12 weeks of age and 1.0% urea from 13-16 weeks of age (Table 1). Calves in both groups were offered iso-nitrogenous and iso-caloric diets. All calves were housed in individual cages. Each cage was 5 feet in length, 3.5 feet in width, and 4 feet in height. Cages were bedded with wood shavings and cleaned daily. All calves were fed for *ad libitum* (three times a day) and had free access to water from 8-16 weeks of age. Feed intake was recorded daily by subtracting refusal from the amount of feed offered. Heart girth, withers height, hip width, and hip height were measured at 8, 12, and 16 weeks of age (Mitchell *et al.*, 2021). Before and after the study, all calves were weighed for 2 consecutive days to get average daily gain for the whole study. Then, these calves were weighed weekly to determine the weekly average daily gain with an electronic scale (B-TEK | Sang D60 Calibration & Interfacing). Heart girth was measured using a measuring tape (Fiber tape GWF-1506) while wither height was determined using a long steel ruler. Body measurements and weighing of calves were done weekly to determine the average daily gain. Blood samples were collected from the jugular vein in a serum vacutainer at 10, 12, and 14 weeks, 4 h post morning feeding (Coverdale *et al.*, 2004) from all calves. All samples were immediately processed and refrigerated for analysis. By centrifugation, plasma was obtained and used for the analysis of plasma urea nitrogen (PUN) through the liquicolour kit method

(UREA/BUN-COLOR, BioSystems, S.A. Costa Brava 30, Barcelona, Spain) (Brambillasca *et al.*, 2010).

The activity and feeding behavior of each calf was recorded at 15 weeks of age through video camera for 12 hours from 8:00 am to 8:00 pm (Schirmann *et al.*, 2009). Readings were made from recorded videos after every 5 minutes by a single trained person. Behavioural observations were recorded for eating, standing ruminating, lying ruminating, standing and lying time (Mushtaq *et al.*, 2023). A total of 144 behavioural observations were made for the individual calf. Time spent on each activity (minutes/hour) was calculated by multiplying the number of respective behavioural observations with 5 and dividing the result by 12. Between 15-16 weeks of age, feed offered and feces excreted samples for each calf were sampled for digestibility evaluation using acid insoluble ash marker. Feces were collected thrice a day for 4 consecutive days. They were stored at -20°C until analyzed. Later, feed and fecal samples were composited. Samples were dried at 55°C for 72 hours for dry matter (DM) analysis and ground to 2 mm particle size using a Wiley mill (Model No. 2, Arthur H. Thomas Company; Philadelphia U.S.A.). Finely grounded feed samples were processed in the lab to determine DM, crude protein (CP) by the Kjeldal method, crude fat (CF) using the Soxhlet method and ash contents (A.). The NDF and ADF were carried out using the detergent method developed by Van Soest without alpha-amylase (Robertson and Van Soest, 1977).

Statistical Analysis: Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc.). For a change in body growth measurements, initial and final body weight (BW), ADG, feed: gain ratio, digestibility, plasma urea nitrogen and routine behaviour parameters treatment was tested as fixed effect. For all parameters repeated over time (body weight, average daily gain, structural measurements, and feed intake) repeated measures procedure was used. Goodness of fit criteria based on second-order Akaike information criterion values were used to select covariance structures for repeated measures procedure. The least-squares means are presented in tables and graphs. Significant differences were declared at $P \leq 0.05$. The effect of calf sex was not significant; therefore, it was not included in the model.

RESULTS

In our study, there was no effect of urea inclusion on average daily gain ($P = 0.92$) and growth performances of calves. The initial and final body weight, body weight change, dry matter intake (DMI), and feed efficiency were not affected ($P \geq 0.37$) by the inclusion level of urea in the calves' diet. According to this urea experiment, there was no interaction ($P = 0.97$) between

the age of the calves and dietary treatments for average daily gain. Similarly, the dietary treatments did not affect ($P = 0.92$) average daily gain (Table 2). However, the average daily gain of calves was affected by age ($P \leq 0.01$) as shown in Figure 1. In this study, there was no interaction between the age and structural measurement treatments for heart girth, hip width, wither height, and hip height of weaned dairy calves. HG, HW, WH, and HH were not affected ($P \geq 0.27$) by urea inclusion in their diet. However, all structural measurements of calves were affected by age ($P \leq 0.01$) as shown in Figure 2. All measurements were increased normally with age.

There was an effect of urea inclusion level on dry matter intake digestibility ($P \leq 0.01$) of weaned dairy calves. DM digestibility increased by about 10.4% in calves fed a diet with urea inclusion. Digestibility of NDF, CP, and EE was remained unaffected ($P \geq 0.26$) by urea. However, the digestibility of non-fiber carbohydrates was 3.5 % greater in calves fed a diet with urea inclusion as shown in Table 3.

Table 1. Ingredients and nutrient composition.

Ingredients, % DM	A ¹		B ²	
	0% urea	0.5% urea	1% urea	
Wheat Straw	15.12	15.18	15.27	
Corn Grain	29.65	32.24	34.61	
Soybean meal	13.51	11.30	9.12	
Canola meal	13.31	11.14	8.99	
Barley Grain	11.28	11.33	11.92	
Molasses, cane	3.75	4.01	4.29	
Wheat bran	11.19	12.01	12.59	
Toxin Binder	0.33	0.33	0.33	
Mineral premix	0.82	0.82	0.82	
Sodium bicarbonate	0.65	0.66	0.66	
Calcium carbonate	0.49	0.49	0.48	
Urea	0	0.49	0.96	
Total	100	100	100	
Nutritional composition³, % DM				
CP	17.59	17.45	17.40	
Starch	30.5	32.5	34.5	
NDF	27.40	26.73	26.30	
NFC	44.75	46.28	47.5	
Ash	6.17	6.54	6.00	
M.E Mcal/Kg	2.7	2.7	2.7	

¹A= Treatment group A contain 0% of urea in diet as a control

²B= Treatment group B contain 0.5% of urea inclusion from 9-12 weeks of age and 1.0% from 13-16 weeks of age

³CP=Crude protein; DM= Dry matter; NDF= Neutral detergent fiber; NFC= Non fiber carbohydrates; ME= Metabolizable energy

In this study, weaned Holstein dairy calves did not exhibit ($P \geq 0.13$) any behavioural changes as a result of urea feeding. Eating (min/hr.), standing ruminating

(min/hr.), lying ruminating (min/hr.), total ruminating time (min/hr.), total standing time (min/hr.), and total lying time (min/hr.) were unaffected by the inclusion of urea in calves diet as shown in Table 4. There was also no impact ($P=0.997$) on the plasma urea nitrogen level as shown in Figure 3.

Table 2. Effect of urea inclusion on feed intake and growth performance of weaned dairy calves.

Parameters	Treatment ¹			P ² Value
	A	B	SEM	
Initial body weight, Kg	65.47	63.14	2.067	0.44
Final body weight, Kg	118.5	116.6	3.22	0.68
Body weight change, Kg	53.04	53.44	2.099	0.89
ADG ³ , Kg	0.947	0.954	0.0375	0.89
Total DMI, Kg	158.1	166.7	6.56	0.37
Daily DMI, Kg	2.823	2.977	0.1171	0.37
FE	0.339	0.324	0.0177	0.57

¹A = 0% urea in diet; B = 0.5% and 1% urea in diet, Treatment means expressed as least square means

²P value represents the main effects of dietary treatments

³ADG= Average daily gain; DMI= dry matter intake; FE= Feed efficiency

Table 3. Effect of urea inclusion in diet on nutrient digestibility of weaned dairy calves.

Digestibility parameter (%) ³	Treatment ¹			
	A	B	SEM	P ² Value
DM	66.92	73.86	1.034	≤ 0.01
NDF	52.83	54.87	4.944	0.56
CP	62.71	64.45	1.046	0.26
NFC	93.42	96.69	1.023	0.04
EE	71.60	71.39	0.754	0.65

¹A = 0% urea in diet; B = 0.5% and 1% urea in diet

²P value represents the main effects of dietary treatments

³CP= crude protein, EE= ether extract, NDF= neutral detergent fiber, DM= dry matter, NFC= non-fiber carbohydrates.

Table 4. Effect of urea inclusion in the diet on behavior parameters of weaned dairy calves.

Behavior Parameters, min/hr	Treatment ¹			
	A	B	SEM ³	P ² value
Eating	11.0	13.3	1.01	0.13
Standing Ruminating	0.78	0.63	0.37	0.72
Lying Ruminating	6.57	7.32	0.68	0.37
Total Ruminating Time	7.37	7.96	1.03	0.61
Total Standing Time	29.7	32.0	1.44	0.28
Total Lying Time	30.4	28.0	1.44	0.26

¹A = 0% urea in diet; B = 0.5% and 1% urea in diet

²P value represents the main effects of dietary treatments

³SEM = Standard Error of Means

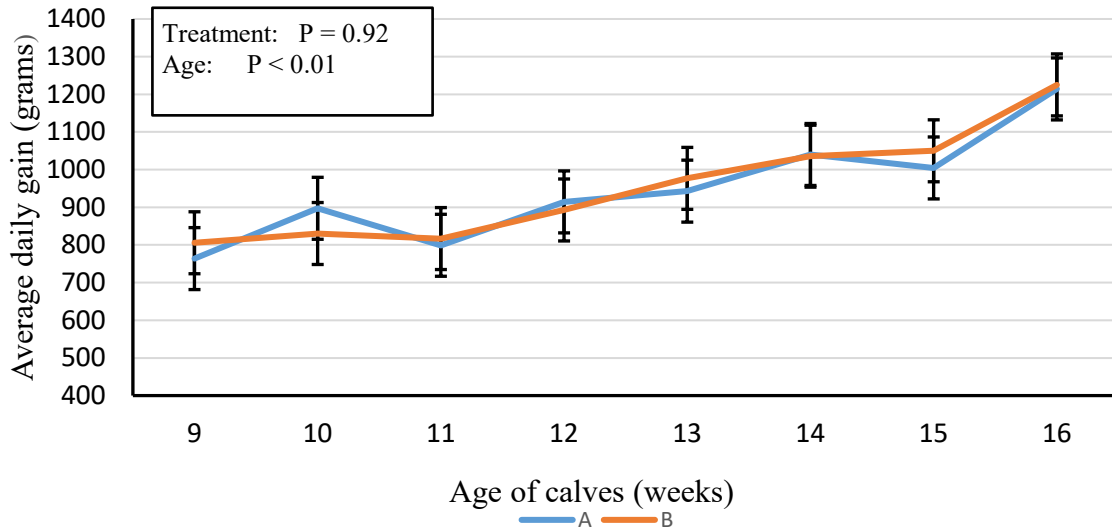


Figure 1. Effect of urea inclusion in the diet on average daily gain of weaned dairy calves

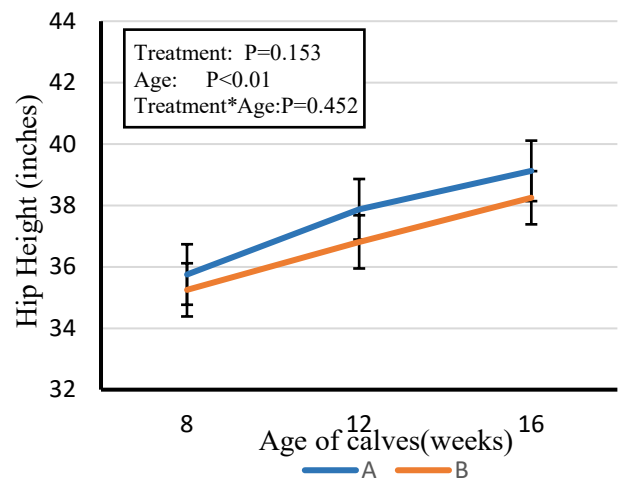
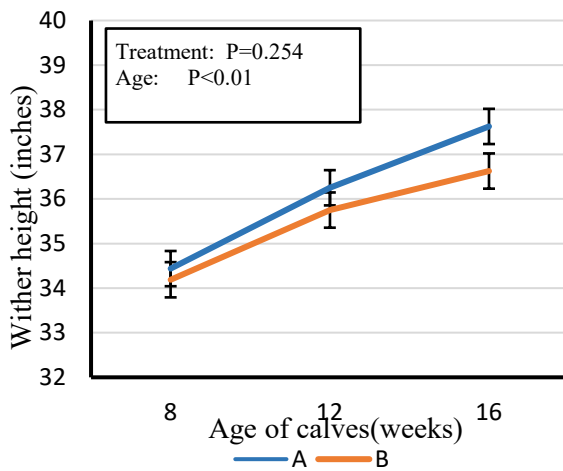
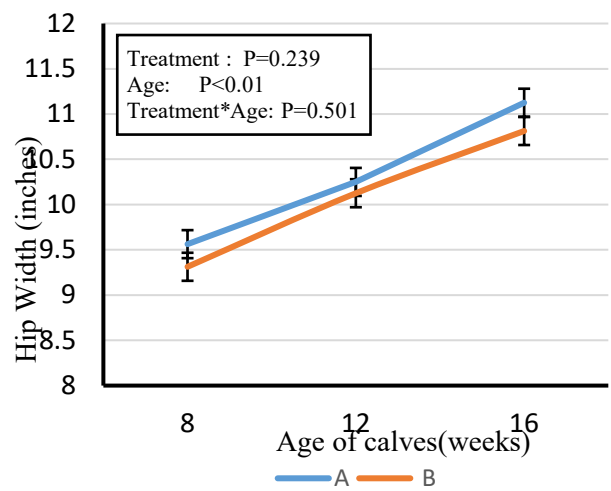
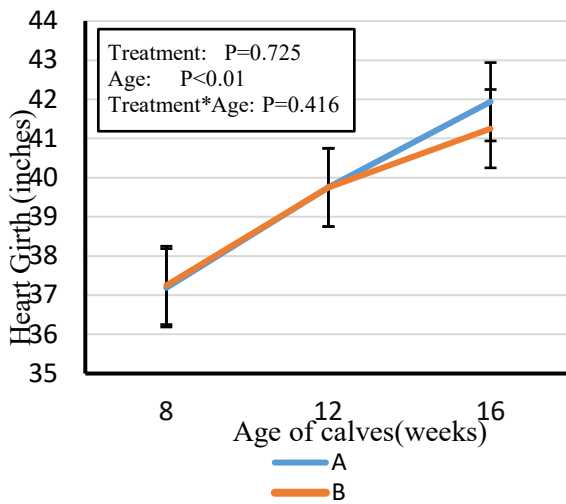


Figure 2. Effect of urea inclusion in the diet on structural measurements of weaned dairy calves

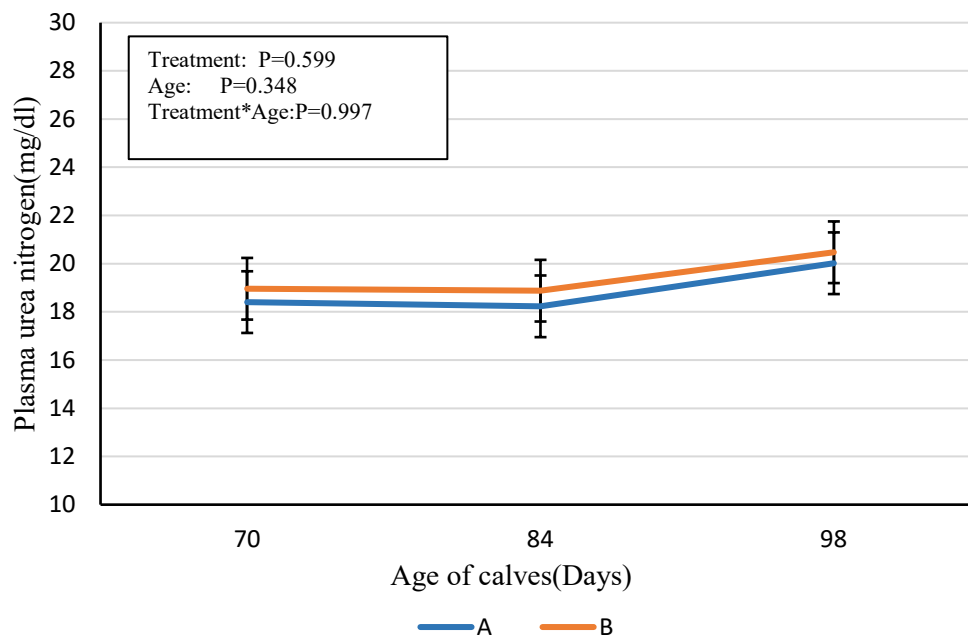


Figure 3.Effect of urea inclusion in the diet on plasma urea nitrogen of weaned dairy calves

DISCUSSION

Despite the evidence from old studies that young calves less than 3 months of age can tolerate and adapt to urea inclusion in calf starter, the idea of supplementing calf starter with urea has not been adopted. In last 30 years, little work was available on urea treatment in dairy calves in post weaning period, especially in terms of digestibility and growth performance. This study provides vital information to fill knowledge gaps about the effect of urea feeding on digestibility, behavior, plasma urea nitrogen, and growth performance of recently weaned calves about 8 to 16 weeks of age. The main objective of this study was to evaluate the effect of feeding urea along with high starch in calf ration on digestibility, plasma urea nitrogen, and growth performance of post-weaning calves. The diets used in the study were isonitrogenous and isocaloric. There were minor differences in NFC% across diets (less than 2% units). Possibly, these differences were not enough to confound the results, because NFC% across all diets were high (more than 44%). Differences in NFC% appeared to keep the diets isocaloric with the same ingredients. In this study, effects were observed due to urea supplementation and not due to increasing NSC levels in urea-supplemented diets.

In current study, there was no negative effect of urea inclusion on feed intake and growth performance of weaned dairy calves. Similar to our study, Wallenius and Murdock (1977) discovered no differences in feed intake and average daily gain of calves fed 1.1% urea replacing

9.3% soybean meal. Dry matter intake and weight gain has been reported to decrease upon urea inclusion in calf ration, when its inclusion has exceeded 2.0%. In our study dry matter intake did not decrease, as maximum urea inclusion was 1.0%. Higher urea levels in diet can cause death Kertz *et al.* (1977); Wilson *et al.* (1975) but in our study, no such effects were observed. In the most recent trial, 0.9% urea inclusion in calf ration (replacing 5-7% SBM in 16% CP calf starter) did not affect feed intake or average daily (Abdelgadir *et al.*, 1996). Some studies found that the substitution of soybean meal for urea in calf starters can decrease the average daily gain of calves by 70 grams in the first 12 weeks of age. In their experiment, 1.9% urea replaced 15% soybean in 18% crude protein calf starter. The urea inclusion recommendation for dairy cows is 1% and can be safely applied to the ration of dairy calves (Miron *et al.*, 1968; Winter, 1973).

The increase in dry matter digestibility was observed in our study upon urea inclusion in the diet of calves. Digestibility of NDF, CP, and EE was not affected by urea inclusion in their diet. However, the digestibility of NFC was 3.5 % greater in calves fed a diet with urea inclusion. Starch and dry matter digestibility increase linearly upon increasing starch inclusion from 26-44% in 8 to 16-week-old dairy calves (Dennis *et al.*, 2018). When starch concentration is increased in the diet it increases the dry matter digestibility (Chishti *et al.*, 2021; Hu *et al.*, 2018). The urea inclusion perhaps increased rumen degradable protein, which may have led to increased DM digestibility as suggested by numerical increase in NDF and CP digestibility. Rumen degradable

protein requirements are rarely considered for weaned dairy calves.

Animal behavior mainly lying behavior is a main indicator of its comfort and interaction with the environment (Bonk *et al.*, 2013). In the current study, there was no difference in all behavior parameters indicating similar social interaction for calves from both treatment groups. To conclude, in the current study, we found no negative effect of urea feeding on plasma urea nitrogen, behavior, growth, and structural development of calves. So, urea feeding can be safely adopted in weaned Holstein dairy calves.

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