INFLUENCE OF FEEDING CORN IMPURITIES ON RUMEN BACTERIA AND FERMENTATION CHARACTERISTICS OF SHEEP

Thaer A. M. Al-Mamouri[1]
Researcher
[1]Mesopotamia General Seeds Company/ Ministry of Agriculture,
abothaeer06@gmail.com

J. A. Tawfeeq Al-Ani[2]
Prof.
[2]Dept. of Animal Prod., Coll. Agric. Engin. Sci., University of Baghdad
drjamalani@yahoo.com

ABSTRACT

This study was aimed to determine the effect of replacing treated and untreated corn impurities with urea instead of wheat bran on rumen bacteria and fermentation of Awassi lambs. Five levels of treated and untreated corn impurities as 44:0%, 32:11%, 20:22%, 10:32% and 0:39% bran:impurities in a 2×5 factorial experiment/ Completely Randomized Design using forty lambs with an initial weight of 27.45 ± 2.16kg and 4-5 months old. Individual feeding was used for 70 days and rumen fluid was sampled at 0, 3, 6h after morning feeding. Results showed an increasing ($p<0.05$) rumen pH at zero time especially corn impurities treated with urea and increasing ($p<0.05$) rumen ammonia at zero time and 3h after feeding with highly significant ($p<0.01$) increased at 6h, and superiority increased of volatile fatty acids (VFA's) for urea treated corn impurities ($P<0.05$) at zero time and after 3h of feeding, similar results for rumen bacterial count at zero time, 3h and 6h after morning feeding for two dilutions × 10^7 and × 10^9 Cfu/ml for impurities treated with urea. In conclusion, it is possible to use corn impurities instead of wheat bran, preferably treated with urea for positive increase rumen fermentation and total bacterial count in sheep.

Key words: By-products, ammonia, volatile fatty acids, corn impurities.
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تأثير تغذية شوائب الذرة الصفراء في بكتريا الكرش ومتغيرات التخمر في الأغنام

ثائر عبد علي منهل المعموري[1] **جمال عبدالرحمن توفيق العاني[2]**
باحث **أستاذ**
[1]شركة مابين النهرين العامة للبذور/ وزارة الزراعة **[2]قسم الإنتاج الحيواني/ كلية علوم الهندسة الزراعية/ جامعة بغداد**

المستخلص

أجريت هذه الدراسة لمعرفة تأثير احلال شوائب الذرة المعاملة وغير المعاملة باليوريا بدلاً من نخالة الحنطة في بكتريا وتخمرات الكرش في الحملان العواسي. تم احلال خمسة مستويات من شوائب الذرة: 44: 0٪ ، 32: 11٪ ، 20: 22٪ ، 10: 32٪ و 0: 39٪ نخالة: شوائب في تجربة عاملية 2×5/ تصميم عشوائي كامل، استعملت أربعين حمل عواسي بوزن ابتدائي 27.45 ± 2.16 كغم وعمر 4 – 5 أشهر. غذيت الحيوانات تغذية فردية لمدة 70 يومًا وأُخذت عينات من سائل الكرش عند 0، 3، 6 ساعة بعد التغذية الصباحية. أظهرت النتائج زيادة ($P<0.05$) الأس الهيدروجيني للكرش عند وقت السحب صفر وخاصة لمعاملات شوائب الذرة المعاملة باليوريا وزيادة ($P<0.05$) نتروجين أمونيا الكرش عند وقت السحب صفر وبعد 3 ساعات من التغذية وزيادة عالية المعنوية ($P<0.01$) بعد 6 ساعات من التغذية، وزيادة الأحماض الدهنية الطيارة (VFA) للشوائب المعاملة باليوريا ($P<0.05$) مقارنةً مع غير المعاملة عند الوقت صفر وبعد 3 ساعات من التغذية، نفس النتائج لأعداد بكتيريا الكرش عند الوقت صفر وبعد 3 ساعات و 6 ساعات من التغذية الصباحية بتخفيفين × 10^7 و × 10^9 (وحدة تكوين مستعمرة خلوية\ مل) لصالح الشوائب المعاملة باليوريا. نستنتج امكانية استخدام شوائب الذرة بدلا من نخالة الحنطة ويفضل معاملة شوائب الذرة باليوريا لزيادة تخمرات الكرش والعدد البكتيري الكلي لدى الاغنام.

الكلمات المفتاحية: مخلفات عرضية، امونيا، احماض دهنية طيارة، شوائب الذرة
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INTRODUCTION

Rumen is a dynamic system that contains a sufficient amount of microorganisms to ferment daily intake of feeds, otherwise problems will arise for the digestive system and subsequently for the host animal. Rumen fermentation is the release of different products after fermentation feeds by microorganisms in the rumen (19), where carbohydrates ferment to volatile fatty acids (VFA) or short-chain acids (SCFA) mainly propionate, acetate, butyrate and organic acids, produced by metabolism of acetogenic strains (12). Proteins ferment to ammonia, organic acids and free amino acids (24). Therefore, rumen is used to store feeds and simplify it by fermenting to its primary nutrients (20), which leads to change the pH of rumen fluid towards acidity and then returning it to moderation after absorbing or utilizing the fermentation products (26), and therefore the fermentation products in the rumen are ammonia nitrogen NH3-N and volatile fatty acids that responsible for the pH rumen environment. The end products of rumen fermentation of bulky feeds are directly related to their nutritional importance and determine the need to physically, chemically or biologically treatment, as the low content of crude protein means no ammonia nitrogen later and need to treat with urea or ammonia (24), while high lignin-cellulosic and hemicellulotice contents require treating with strong base like sodium hydroxide to break ligno-cellulosic linkage, or it may treated with weak base such as ammonia or urea, which require longer processing time with an increase in crude protein and cracking lignin bonds (14), or it may be treated biologically, such as treatment with fungi or bacteria, to break ligno-cellulosic bonds and increase nutrients. Corn impurities are cheap with high crude fiber and low crude protein content, so it is very preferable to treat with urea to increase crude protein and reduce the proportion of crude fibers to improve nutritional value. In ruminants, we can use urea with feeds directly 1-2%, or treated roughages to prepare 3.3% nitrogen (13) or treated grains (25). Adding urea directly requires great attention aimed to eliminate its negative aspects by providing 1-2%, gradually feeding, well mixing feed, frequency of feeding, giving compounds such as N-Carbamylglutamate (NCG) 0.4 – 0.6% as additives, providing clean water, avoid hot weather, having cement floors for the barn with good drainage, in the rumen, In ruminants, urea is commonly used in feeds to reduce costs (5), but it may cause problems for the fetus, because of urea hydrolysis rapidly to NH3-N by rumen microorganism's enzymes within 2h (15) and it is widely used as non-protein nitrogen (NPN) to provide ammonia that required for rumen microbial growth as true protein for the host animal or ruminants (4, 13). The high cost of feed ingrediants need to substitute with cheap sources or by-product. Treated roughage feeds with 1% urea led to improve digestibility, dry matter intake, feed efficiency and daily gain of growing camels (11) and could be use as an ingradient for completed feeds of ruminant. All sources of natural resources aren't sustainable, and the use of by-products saves us from pollution, achieves recycling, and preserves resources for the longest possible period, so, recycling of agricultural by-products like corn impurities achieves more feed for ruminants and decreases the cost of feeding. The main aim of substitution or feed additives is to increase the production and sustainability in addition to decrease pollution, Khalid and Al-Anbari (17) referred to enhance milk yield by adding 150 ml/ day glycerol to the rations of Holstein cows, while adding vitamin E enhanced growth (16), and adding fat-soluble vitamins pre- and post-mating of ewes led to improve the reproductive performance (1), and adding ajwain seeds to the rations enhance health and growth performance (10). According to the data of the Iraqi Ministry of Agriculture, impurities were 9557 tons from 2015 - 2020 and tended to increase every year with expansion of corn production, it's inexpensive and cost 30$ per ton, The benefit of ruminants in sustainable development, recycling and the use of agricultural and industrial by-products to produce meat and dairy has a promising future in the development and global trend to meet the requirements of population growth, reduce production costs and pollution. Nowadays, strategies for using residues in feeds have developed feeding systems to make more profit without affecting the animals, for

this reason, this study aimed to evaluate the effect of replacing treated and untreated corn impurities with urea instead of wheat bran on rumen fermentation and total bacterial count of Awassi lambs.

MATERIALS AND METHODS

Chemical treatment and experimental feeds

Chemical treatment of corn impurities with urea (ureated corn impurities UCI) was carried out by adding urea to prepare 3.3% nitrogen (7.17% urea as dry basis) at air temperature (about 30 °C) and a humidity 60% (adding 60L water per 100 kg dry matter corn impurities (added 50L water for 90% dry matter of impurities which equivalent 60% humidity) for 30 days of incubation period (13) as follows: estimate required urea to achieve 3.3% nitrogen (7.17 kg urea/ 100 Kg DM of impurities); estimate the amount of required water to achieve 60% of humidity as DM basis of impurities, then, dissolve urea with water to prepare urea solution, put the impurities on clean nylon and spray prepared urea solution with manual mixing of impurities until homogeneity, then wrapped tightly to ensure the ammonia gas liberated from the decomposed urea does not come out. After 30 days, open the nylon with continuous mixing for drying and volatilization of the remaining ammonia gases. After drying, it was sampled for analysis (Table 1) as AOAC (6) and collected in bags until they were used in experiment. Ingredients of concentrated feeds included barley, wheat bran, soybean meal, mineral and vitamins, wheat bran were replaced by treated and untreated corn impurities with urea as 44:0%, 32:11%, 20:22%, 10:32% and 0:39%, all mixed to produce ten experimental treatments then sampled for analysis (Table 1) as AOAC (6), Concentrate was fed at 3% of body weight as DM basis at 8.0am, while alfalfa hay was provided *ad libitum*. pH value of feeds was measured by weight 1g of feed then added 10ml of distilled water. After 10 minutes, filtering the sample with a cheesecloth, and measure it with a portable pH meter from HANNA Instrument (20).

Experimental animals and management

Forty Awassi male lambs aged 4 -5 months with initial weight 27.45 ± 2.16 kg randomly distributed to ten treatments to replace treated (ureated) and untreated corn impurities with urea instead of wheat bran at levels of 0, 11, 22, 30 and 39(%). Individual feeding was conducted for 70 days of experiment preceded by 14 days as adaptation period. All animals were provided clean water, vaccines and kept continuous veterinary supervision. Concentrate feed was given at 3% of live body weight as DM basis, while alfalfa hay offered ad-libitum with remaining.

Rumen fermentation

Rumen fermentation contain pH, ammonia nitrogen, and volatile fatty acids (VFA) or short chain fatty acids (SCFA). Rumen fluid was collected using oral stomach tube at different times after feeding as 0, 3, 6 h. pH values were directly measured with portable pH meter (HANNA Instrument), then, some of rumen fluid used to determine bacterial count by kept at 4ºC and 0.1N HCl was added to another part of fluid and kept in deep freeze to determine the total volatile fatty acids (TVFA) and ammonia nitrogen cementations (NH3-N). Ammonia nitrogen was measured according to AOAC (6) as follows: after thawing frozen rumen fluid, 5ml was taken into a Kjeldahl digestion tube, added 0.5g MgO and 0.5 ml $CaCl_2$ 25% and 10 ml of distilled water, then measured with Kjeldahl apparatus. Ammonia was received with 5 ml of receiving solution, 2% boric acid and drops of the mixture (methyl red 0.099g and bromocresol green 0.066g dissolved in 100 ml ethyl alcohol), then titrated with 0.05 HCL to calculate ammonia concentration according to the equation:

Ammonia%(mg/100ml) = 14.008 × 0.05 × (titration volume of HCL for sample – titration volume for blank) × 100/5ml

Rumen volatile fatty acids were measured after thawing frozen rumen fluid by taking 5ml rumen fluid into Kjeldahl digestion tube, added 1ml orthophosphoric acid, The tube was washed with a little distilled water, receiving flask contain drops of phenol dye (50ml of absolute ethanol + 1g of phenolphthalein + 50ml of distilled water + drops of NaOH 0.05M), collect 50ml, then titrate with sodium hydroxide 0.1M (27), total volatile fatty acids concentration calculated as following:

Total volatile fatty acids (mmol/100ml) = 0.1M × (titration volume of NaOH sample – titration volume for blank) × 100/5ml.

Rumen bacteria count

One ml of sample was taken and added 9ml of physiological saline solution, and the dilutions were completed in test tubes containing 9 ml of dilution solution to reach the dilution that gives appropriate numbers between 25-300 colonies of microorganisms. Then, 1ml of appropriate dilution for each sample was transferred to empty Petri dishes using a sterile pipette, then the culture media was poured out Chocolate Agar or Nutrient agar. After mixing the medium well into the dishes, it was placed inside the anaerobic containers in an inverted form with several anaerobic conditions (Gas pack) to make the conditions anaerobic and then the dishes were incubated at 37 °C for 48 hours. After development on each of the media, the number of colonies was calculated for each of them, as Roberts and Greenwood (21).

Statistical analysis: All results were statistically analyzed using factorial experiment/ Completely Randomized Design (CRD) 2 × 5, One-way ANOVA analysis and statistical program (22) was performed. Duncan's multiple range test was used to determine the significant differences among treatments (9). The statistical model was:

$$Yijk = \mu + Ai + + Bj + AB(ij) + eijk$$

Table 1. Ingredient and chemical composition of concentrated feeds, alfalfa hay, corn impurities and ureated corn impurities on DM basis (%)

	Corn impurities (%)					Ureated corn impurities (%)					alfalfa		
Ingredients	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**hay**	**CI**	**UCI**
Barley	**46**	**46**	**46**	**46**	**46**	**46**	**46**	**46**	**46**	**46**			
Wheat bran (WB)	**44**	**32**	**20**	**10**	**0**	**44**	**32**	**20**	**10**	**0**			
Corn impurities (CI)	**0**	**11**	**22**	**30**	**39**	**0**	**11**	**22**	**30**	**39**			
Soya bean meal (SBM)	**8**	**9**	**10**	**12**	**13**	**8**	**9**	**10**	**12**	**13**			
Minerals & Vitamins	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**			
Dry matter (DM) %	**91.29**	**90.51**	**91.46**	**91.69**	**91.57**	**91.29**	**89.62**	**81.67**	**78.81**	**71.42**	**87.37**	**93.46**	**61.85**
Organic matter (OM) %	**93.53**	**93.4**	**92.55**	**91.36**	**90.12**	**93.53**	**93.37**	**92.13**	**91.53**	**90.71**	**91.20**	**87.97**	**87.75**
Crude protein (CP) %	**15.96**	**15.30**	**14.91**	**14.05**	**13.64**	**15.96**	**16.17**	**17.00**	**17.38**	**17.52**	**16.32**	**7.36**	**16.77**
Ether extract (EE) %	**3.92**	**2.98**	**3.18**	**2.80**	**3.06**	**3.92**	**3.73**	**3.81**	**3.60**	**3.50**	**1.47**	**3.06**	**1.54**
Crude fiber (CF) %	**12.20**	**12.66**	**12.89**	**13.16**	**14.12**	**12.20**	**12.44**	**12.45**	**12.68**	**13.35**	**24.33**	**19.95**	**17.09**
Inorganic matter (ash) %	**6.47**	**6.6**	**7.45**	**8.64**	**9.88**	**6.47**	**6.63**	**7.87**	**8.47**	**9.29**	**8.80**	**12.03**	**12.25**
Nitrogen free extract (NFE)	**61.45**	**62.46**	**61.57**	**61.35**	**59.30**	**61.45**	**61.03**	**58.87**	**57.87**	**56.34**	**49.08**	**57.60**	**52.35**
***Me**	**12.35**	**12.13**	**12.05**	**11.82**	**11.60**	**12.35**	**12.26**	**12.08**	**11.74**	**11.74**	**10.51**	**10.89**	**8.80**
pH value	**6.25**	**6.20**	**6.00**	**6.03**	**6.00**	**6.25**	**6.40**	**6.31**	**7.80**	**7.85**	**6.37**	**6.11**	**8.18**

***Metabolic energy (MJ/kg DM) = 0.012 × crude protein + 0.031 × ether extract + 0.005 × crude fiber + 0.014 × nitrogen free extract… (18). CI= Corn impurities; UCI= Ureated corn impurities; T1= WB 44% and CI 0; T2 = WB 32% and CI 11%; T3 = WB 20% and CI 22%; T4 = WB 10% and CI 30%; T5 = WB 0 and CI 39%; T6= WB 44% and UCI 0; T7 = WB 32% and UCI 11%; T8 = WB 20% and UCI 22%; T9 = WB 10% and UCI 30%; T10 = WB 0 and UCI 39%.**

RESULTS AND DISCUSSION

Rumen fermentation: The effect of replacing treated and untreated corn impurities with urea on rumen pH indicated to increase ($P<0.05$) the values of rumen pH for treatments with treated corn impurities for zero time which means before morning feeding or after 24h (Table 2), and significant increase after 6h from morning feeding. There were no significant differences after 3h of feeding. the increased of rumen pH value due to degradation of urea by bacterial ureases and produced more ammonia to blood streem then recycled from liver to rumen as endogenous sources of urea with saliva, that leads to significant increase of rumen pH (28) and increasing levels of non protin nitrogen (NPN) with daily feeding reduces the cost feeding and profuce more N excretion (13). The effect of replacing treated and untreated corn impurities

with urea on rumen ammonia nitrogen (mg/100 ml) (Table 3) referred to increase ammonia nitrogen (P<0.05) for replacing 39% treated corn impurities with urea or T10 for all collected times (0, 3h and 6h) after feeding. Higher fermentation mean an effective fermentation medium with good conditions for microbial growth that leads to an increase feed intake, daily gain, feed efficiency, rumen flow rate and indicates a good quality feed (26). Crude fiber or structural carbohydrate is very important for the physiology ruminants digestive system, so, corn impurities or other by-products could be great especially with urea treatment (3). Babale et al. (7) found better rumen characteristics, pH, ammonia-N and volatile fatty acids when replaced corn bran with corn cobs. Carro et al. (8) noticed higher pH value with increasing roughages to concentrate 80:20%, fermentation products represent a kind of waste of energy and protein intake, especially when eating more than animal requirement, Agle et al. (2) referred to cumulative ammonia emissions from manure were lower for low and medium crude protein diets compared to the high crude protein diet and demonstrated that low rumen degradable protein diets will produce manure with lower ammonia-emitting potential without affecting performance.

Table 2. Effect of replacing treated and untreated corn impurities with urea on rumen pH (mean ± SE)

Tret.	pH/ zero time	pH/ After 3h	pH/ After 6h
T1	6.96±0.05bc	6.29±0.08	5.68±0.18ba
T2	7.18±0.19bac	6.29±0.19	6.25±0.27a
T3	6.83±0.19c	6.09±0.19	6.28±0.09a
T4	7.16±0.01bac	5.81±0.18	6.29±0.10a
T5	7.09±0.04bac	6.36±0.03	6.06±0.08a
T6	6.96±0.05bc	6.29±0.08	5.68±0.18ba
T7	7.11±0.17bac	6.11±0.16	6.33±0.16a
T8	7.13±0.06bac	5.87±0.22	5.92±0.23ba
T9	7.29±0.10ba	5.73±0.18	5.34±0.03b
T10	7.39±0.02a	5.52±0.53	6.34±0.23a
Sign.	*	NS	*

Different litters in same column means significant differences; NS= non-significant differences; * Significant differences at level 0.05; T1= WB 44% and CI 0; T2 = WB 32% and CI 11%; T3 = WB 20% and CI 22%; T4 = WB 10% and CI 30%; T5 = WB 0 and CI 39%; T6= WB 44% and UCI 0; T7 = WB 32% and UCI 11%; T8 = WB 20% and UCI 22%; T9 = WB 10% and UCI 30%; T10 = WB 0 and UCI 39%.

Table 3. Effect of replacing treated and untreated corn impurities with urea on rumen ammonia nitrogen concentrations (mg/100 ml) (mean ± SE)

Tret.	NH3-N (mg/100ml) zero time	NH3-N (mg/100ml) After 3h	NH3-N (mg/100ml) After 6h
T1	20.75±0.53ba	25.99±0.59bdc	15.66±0.36bc
T2	18.31±0.25b	19.81±0.78d	13.94±0.75c
T3	19.30±0.62b	21.80±1.4d	14.21±0.64c
T4	19.38±0.70b	29.68±4.80bac	17.99±0.92ba
T5	18.63±1.11b	20.35±0.58d	14.52±0.58c
T6	20.75±0.53ba	25.99±0.59bdc	15.66±0.36bc
T7	18.76±1.42b	21.85±1.79d	14.70±1.11c
T8	18.72±0.74b	23.31±0.99dc	14.30±1.10c
T9	21.15±0.86ba	32.07±1.41ba	15.72±0.36bc
T10	22.71±0.56a	32.61±2.21a	19.58±0.32a
Sign	*	*	**

Different litters in same column means significant differences; * Significant differences at level 0.05; ** Significant differences at level 0.01; T1= WB 44% and CI 0; T2 = WB 32% and CI 11%; T3 = WB 20% and CI 22%; T4 = WB 10% and CI 30%; T5 = WB 0 and CI 39%; T6= WB 44% and UCI 0; T7 = WB 32% and UCI 11%; T8 = WB 20% and UCI 22%; T9 = WB 10% and UCI 30%; T10 = WB 0 and UCI 39%.

Similar results for replacing treated and untreated corn impurities with urea on rumen volatile fatty acids (VFA). There were significantly increasing (P<0.05) for feeding 30%

treated corn impurities or T9 in comparison with untreated corn impurities at zero time and after 3h of feeding (Table 4). Short chain fatty acids (SCFA) or VFA's produced in the rumen through ruminal fermentation by microorganisms, and high concentrations of rumen VFA's indicated to increase feed digestibility and more source of energy for microbial organisms and host animal, while the success of synchronization between ammonia nitrogen and VEA's meaning more feed efficient and utilization (24). Babale et al. (7) tried to reduce feeding cost and production by replacing corn bran with corn cobs and replaced up to 40 % without negative effects with increasing mean production of VFA's with increasing maize cobs, while the increasing of rumen VFA's from fermented carbohydrate lead to decrease rumen pH value later due to the acidity of VFA's, that reason prevents animals to consumed highly concentrated or highly fermented roughages (8).

Rumen bacteria count

The results of replacing treated and untreated corn impurities with urea on rumen bacterial count or rumen microorganisms that live in anaerobic environment (Table 5) referred to high significance (P<0.01) in total bacteria count for corn impurities as compared with wheat bran with superiority for UCI or T9 at zero time $\times 10^7$ and $\times 10^9$ Cfu/ml and significant increasing after 3h for $\times 10^7$ Cfu/ml (P<0.05) and after 6h for $\times 10^9$ Cfu/ml, while there were higher increasing after 6h for $\times 10^7$ Cfu/ml (P<0.01) especially when treating corn impurities with urea (T9) compared to other treatments. Babale et al. (7) replaced corn cobs with corn bran and found increases in total bacteria count with 50% corn cobs relative to a better rumen ecosystem. Hassan and Tawffek (14) stated that decreasing phenolic compounds leads to a better rumen ecosystem and increases rumen bacteria count. So, ruminants as an herbivorous, depend on plants with structural carbohydrates that are degraded by rumen bacteria. After feeding, the number of rumen bacteria increased as a result of the increase in available nutrients, and increasing total bacteria counts leads to improving digestibility and feed efficiency, with availability of rumen suitable conditions, especially pH value, synchronization between ammonia nitrogen and volatile fatty acids as a source of energy (24). We found an increase in total bacteria count, especially with corn impurities, which is appropriate to moderate pH values and the rise of ammonia and volatile fatty acids productions, there were different kinds of cellulolytic bacteria, Zhang et al. (30) observed high rumen pH values when feeding straw and increasing of Oscillibacter cellulolytic bacteria. The importance of adaptation period is to create microbial population suitable for fermentation and utilization from the feed, Wu et al. (29) noticed change of rumen microbial populations with different feeds and environmental influences, like increasing dilution and flow rate of rumen liquor when feeding hydroponic fodder (26). Sha et al. (23) found similar rumen microbial community with no differ between two aged cattle 2 and 3 years when examined the functional characteristics and species composition of rumen microorganisms.

Table 4. Effect of replacing treated and untreated corn impurities with urea on rumen volatile fatty acids concentrations (Mmol/100ml) (mean ± SE)

Tret.	VFA (Mmol/100ml) zero time	VFA (Mmol/100ml) After 3h	VFA (Mmol/100ml) After 6h
T1	**3.40±0.33bac**	**5.47±0.24a**	**3.53±0.40**
T2	**2.67±0.22bac**	**4.03±0.24bc**	**3.10±0.56**
T3	**2.43±0.09bc**	**3.23±0.09c**	**3.03±0.45**
T4	**3.27±0.41bac**	**4.77±0.42ba**	**4.27±0.19**
T5	**2.37±0.18c**	**3.7±0.32bc**	**2.9±0.25**
T6	**3.40±0.33bac**	**5.47±0.24a**	**3.53±0.40**
T7	**2.73±0.20bac**	**4.63±0.29ba**	**3.03±0.27**
T8	**3.30±0.44bac**	**4.60±0.84ba**	**3.73±0.33**
T9	**3.87±0.38a**	**5.00±0.25ba**	**3.87±0.70**
T10	**3.73±0.60ba**	**5.83±0.57a**	**3.97±0.27**
Sign.	*	*	NS

Different litters in same column means significant differences; NS= non-significant differences; * Significant differences at level 0.05; T1= WB 44% and CI 0; T2 = WB 32% and CI 11%; T3 = WB 20% and CI 22%; T4 = WB 10% and CI 30%; T5 = WB 0 and CI 39%; T6= WB 44% and UCI 0; T7 = WB 32% and UCI 11%; T8 = WB 20% and UCI 22%; T9 = WB 10% and UCI 30%; T10 = WB 0 and UCI 39%.

Table 5. Effect of replacing treated and untreated corn impurities with urea on rumen bacterial count (Cfu/ml) (mean ± SE)

Tret.	Bact. Count, zero time × 10^7 Cfu/ml	Bact. Count, zero time × 10^9 Cfu/ml	Bact. Count, After 3h × 10^7 Cfu/ml	Bact. Count, After 3h × 10^9 Cfu/ml	Bact. Count, After 6h × 10^7 Cfu/ml	Bact. Count, After 6h × 10^9 Cfu/ml
T1	163±13.51ed	130±11.69c	170±18.26b	157±20.08	159±17.89d	136±17.53c
T2	147±20.83e	129±17.33c	207±12.72ba	179±12.77	209±45.35bdc	179±43.30bc
T3	199±19.88bcd	154±26.03c	195±35.41ba	153±46.28	209±16.75bdc	172±21.94c
T4	241±13.38ba	211±13.48bc	215±48.46ba	179±45.02	278±15.28ba	252±16.46a
T5	225±7.51bc	209±5.20ba	235±5.20ba	216±6.93	245±1.76bac	227±2.89ba
T6	163±13.51ed	130±11.69c	170±18.26b	157±20.08	159±17.89d	136±17.53c
T7	243±9.96ba	225±10.48a	254±10.26ba	231±11.10	276±13.38ba	264±13.86a
T8	192±6.93ecd	174±4.16bc	208±47.39ba	181±46.91	229±17.33bdc	215±16.91ba
T9	274±5.03a	254±6.43a	276±11.02a	248±12.39	290±4.06a	269±2.40a
T10	163±12.77ed	139±12.67c	170±12.70b	153±12.35	190±6.23dc	175±8.19bc
Sign.	**	**	*	NS	**	*

Different litters in same column means significant differences; NS= non-significant differences; * Significant differences at level 0.05; ** Significant differences at level 0.01; T1= WB 44% and CI 0; T2 = WB 32% and CI 11%; T3 = WB 20% and CI 22%; T4 = WB 10% and CI 30%; T5 = WB 0 and CI 39%; T6= WB 44% and UCI 0; T7 = WB 32% and UCI 11%; T8 = WB 20% and UCI 22%; T9 = WB 10% and UCI 30%; T10 = WB 0 and UCI 39%.

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