

## **EFFECT OF SUPPLEMENT GREEN TEA AND TETRACYCLINE ON NUTRIENT DIGESTIBILITY, SOME BLOOD METABOLITES AND GROWTH PERFORMANCE OF GROWING BUFFALO CALVES**

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### **SUMMARY**

The current study was conducted to investigate the effect of addition green tea and tetracycline hydrochloride or both of them to growing buffalo rations on digestibility, blood metabolites and animal performance. Twelve buffalo calves of 11-13 months of age weighing  $213 \pm 3.52$  kg were divided into 4 groups of 3 animals each. Dried green tea was added to calves in basic diet (control) at levels of 0.5 % (T1), 1 % tetracycline hydrochloride (T2), and 1 % dried green tea and 1% tetracycline hydrochloride (T3) for 4 month experimental period. All animals of these groups were fed 80% of their requirements as concentrate mixture while wheat straw and alfalfa hay was given. The quantity of concentrate mixture was adjusted every month according to change in body weight (NRC, 2001). The results indicated that dietary supplementation of green tea and tetracycline, or both of them did not significantly ( $p < 0.05$ ) affect on all nutrient digestibility, except EE digestibility was significantly ( $p < 0.05$ ) lower in group T3 than control. No statistically significant differences were observed among the feed supplemented groups for blood proteins, namely albumin and globulin. Buffalo calves fed rations supplemented with tetracycline in T2 or tetracycline and green tea in T3 decreased significantly ( $P < 0.05$ ) serum total cholesterol as compared with those fed control and green tea rations (163.80 and 166.99 vs. 196.17 and 203.31 respectively). Also, no significant differences were observed among all treatments for ALT and AST. However, it was noticed that buffalos fed supplemented tetracycline and green tea in T3 tended to be lower by 3.5 % and 7.3 % for ALT and AST respectively than control group. On the other hand, performance of growing calves in terms of average daily gain, total dry matter intake and feed conversion ratio did not affected by supplemented green tea and tetracycline or both of them to rations. From the results of this study it can be concluded that incorporating green tea at a rate of 0.5 % and tetracycline at a rate of 1 % is insufficient to improve digestibility and performances of growing buffalo, however mixed feed additive from green tea and tetracycline by rate 1% may be suitable for buffalo's calves, which slightly improvements were noticed.

**Keywords:** *growing buffalo calves, green tea, tetracycline, performance, blood metabolites, nutrient digestibility*

### **INTRODUCTION**

Many farms are using antibiotics as growth promoters for improving economic and effective animal production (Wierup, 2000). According to the National Office of Animal Health (NOAH, 2001), antibiotic growth promoters are used to "help growing animals digest their feed more efficiently, get maximum benefit from it and allow them to develop into strong and healthy individuals". Although the mechanism underpinning their action is unclear, it is believed that the antibiotics suppress sensitive populations of bacteria in the intestines. Tetracyclines including oxytetracycline, tetracycline, chlorotetracycline and doxycycline, are considered the main antibiotics used in livestock production. Tetracycline are licensed for use in a wide range of livestock and can be administered either supplemented to feed or by injection.

Antibiotic supplementation in animal feed can result in bacterial resistance in human pathogens through consumption of animal products. The European Union has banned the use of several antibiotics in animal feeds (Bowman, 1998). With the restriction or ban of dietary antimicrobial agents, we must explore new ways to improve and protect the health status of farm animals, to guarantee animal performance and to increase nutrient availability. So to produce food which is safe for human consumption, it is highly desirable to use medicinal plants or herbs and natural resources having natural

antibiotic properties. One of medicinal plants is green tea, (Yang *et al.*, 2003). Green tea can be used for animal feed additives to produce safe and hazard-free animal products. Medicinal plants as green tea are being suggested as effective alternatives to antibiotics (Fuller, 1989; Mohan *et al.*, 1996).

Green tea has over 200 bioactive compounds and contains over 300 different substances (Labdar, 2010). The chemical composition of tea is multifaceted, consisting of polyphenols (catechins and flavanoids), alkaloids (caffeine, theobromine, theophylline), volatile oils, polysaccharides, amino acids, lipids, vitamin C, minerals and other uncharacterised compounds (Karori *et al.*, 2007). The polyphenols compounds of green tea accounts for 30% to 36% weight of the water extractable materials in tea leaves and have been shown to improve body weight gain and feed efficiency in pigs (Hossain *et al.*, 2012), cattle (Sarker *et al.*, 2010) and broilers (Biswas and Wakita, 2001). These compounds also maintain micro flora balance and exhibit antimicrobial effects against pathogenic bacteria (Guray *et al.*, 2011; Hara-Kudo *et al.*, 2005). A decrease in the number of calf deaths was also observed, due to the effect of green tea in the prevention of digestive and respiratory organ diseases in calves while nursing (Ishihara *et al.*, 2001). Green tea catechins protect the brain, liver and kidney from lipid peroxidation injury (Lin *et al.*, 1998). Moreover Green tea polyphenoles protects against alcohol induced liver and serum lipid peroxidation (Skrzydłowska *et al.*, 2002).

Recently, green tea has been attracting much attention because of its wide medicinal functions such as reduction of plasma total cholesterol, cholesterol ester, very-low-density lipoprotein-cholesterol and the atherogenic index (Muramatsu *et al.*, 1986). Gad and Zaghoul (2013) found that green tea extract improve hepatic, renal and hematological parameters and serum lipid profile as well as the ultra-structure of the liver and kidney cells.

The objectives of this study were to investigate the effects of feeding green tea and tetracycline on growth performance, nutrient digestibility, and blood metabolites of growing buffalo calves.

## **MATERIALS AND METHODS**

### ***Animals, rations and management:***

The experiment was carried out at the research farm of Faculty of Agriculture, Al-Azhar University Assiut, Egypt. Twelve healthy buffalo calves of 11-12 months of age weighing  $213 \pm 3.52$  kg were divided into 4 groups of (3 animals each) according to their average live body weight. The source of green tea is china and the source of tetracycline dehydrate is Adwia company for Vet. Egypt. Supplements (green tea and tetracycline) were added to calves diet (concentrate mixture part) at levels of 0.5% green tea (T1), 1 % Tetracycline (T2), and 1 % Green tea and 1% Tetracycline (T3) for 4 month experimental period. The control diet consists of concentrate mixture, wheat straw and hay Alfalfa. The compositions of concentrate mixture were 50% yellow maize, 14% wheat bran, 15% linseed meal, 13.5% soybean meal, 5% molasses, 1.0 % calcium carbonate, 1.0% sodium chloride, and 0.5% minerals and vitamins mixture.

The animal's requirements for CP and TDN were calculated according to NRC (2001). The concentrates mixture level was 2 % while roughage level was 1% of body weight. All animals of all groups were fed 80% of their requirements as concentrate mixture while wheat straw and alfalfa hay was given ad libitum. The quantity of concentrate mixture was adjusted every month according to change in body weight (NRC, 2001). The animals were randomly allotted to experimental diets.

The ingredients and chemical composition of experimental diets are shown in Tables (1) and (2). Rations were offered twice a day and the feedorts were weighed daily through the experimental period and actual feed intake was calculated. Feed conversion ratio was calculated and expressed in terms of kg dry matter (DM per one-kg body weight gain. Diets were mixed daily and fed twice a day ad libitum. Calves were housed on concrete-floor in separate pens. They were fed individually in locally manufactured mangers. The calves were weighed initially and every month thereafter before morning feeding. Economic analysis of data was done using the technique of Perrin *et al.* (1979). Round the clock fresh and clean water was available to them. Calves were de-wormed at the start of the experiment. The study lasted for 6 months.

### ***Digestibility trials:***

Digestibility trials were conducted to determine the digestion coefficients and percentage nutrients digestibility for the four different experimental diets. Each digestibility trial was carried out using three

buffalos calves. Each trial lasted for 12-day, the first 5-day was considered as a preliminary period followed by a 7-day collection period.

**Table (1): Ingredients composition (%) of concentrate mixture rations.**

Item	Control	T1	T2	T3
Concentrate mixture	71	70.5	70	70
Wheat bran	25	25	25	24
Green tea	-	0.5	-	1
Tetracycline	-	-	1	1
Limestone	2	2	2	2
Salt	1	1	1	1
Minerals Mixture	1	1	1	1
Total	100	100	100	100

*T1 Green tea      T2 Tetracycline      T3 Green tea + Tetracycline*

**Table (2): Chemical composition of experimental rations (%DM basis).**

Item	DM	OM	CP	CF	EE	NFE	Ash
Control	90.66	89.51	17.04	18.18	4.13	50.16	10.49
T <sub>1</sub>	90.52	89.63	16.92	17.02	3.94	51.75	10.37
T <sub>2</sub>	90.30	89.47	17.53	17.65	4.77	49.52	10.46
T <sub>3</sub>	90.53	89.43	16.94	16.83	4.37	51.29	10.57

*T1 Green tea      T2 Tetracycline      T3 Green tea + Tetracycline*

The rations were sampled daily during the trial and the samples were composited at the end of each trial, ground through 1 ml. screen, and stored for chemical analysis. Faeces were weighed just at the time of collection and 10% of the total amount were taken and dried at 60-70 °C for 24 h. The dried samples were mixed and ground for chemical analysis. The daily dried fecal samples from each animal were grounded through 1mm mill screen openings and were saved for chemical analysis.

***Chemical analysis and digestion coefficients measurements:***

The chemical analysis of feeds and faeces were carried out according to the procedures of Association of Official Analytical Chemists (AOAC, 1999) using duplicate samples. The apparent digestion coefficients of nutrients were calculated by expressing the difference between the content of nutrient in both consumed feed and faeces as a percentage of its intake.

***Blood sampling:***

Blood samples were collected from the jugular vein monthly after the morning feeding. Blood samples were immediately centrifuged at 3000 rpm for 20 min. and serum was stored at -20 °C until analysis. Serum total protein (TP g/dl), albumin (AL g/dl), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol were determined by spectrophotometer (Unico, USA) using commercial test kits (Spinreact, Spain). Serum globulin (GL g/dl) was obtained as the difference between the total protein and albumin concentration.

***Statistical analysis:***

Statistical analysis was carried out using general linear model (G.L.M) of SAS (2001) program, version 8.2. Differences between groups in nutrient digestibility, blood metabolites and performance were evaluated by one way ANOVA. The significance differences between treatments means were tested by Duncan Multiple Range Test (Steel and Torrie, 1980). The data were presented in mean ± S.E.M. Level of significance was set at P<0.05.

## RESULTS AND DISCUSSION

### *Nutrients digestibility:*

The average values of nutrients digestibility in the experimented diets are presented in (Table 3). In general, the digestibility of different nutrients did not differ significantly among all treatments except those of ether extract digestibility, it was significantly ( $P < 0.05$ ) lower by about 12.77 % for buffalo fed green tea and tetracycline T3 than that animal fed control rations. However, no significant differences were observed among buffaloes fed control, T1 and T2 rations for EE digestibility. Also, it was noticed that there are numerical differences in CF digestibility for calves feed green tea and calves fed green tea and tetracycline treatments, which was decreased by about 15% than that buffalo fed control group. The low digestibility for CF and EE may be due to tannins contents of green tea that are known to reduce ruminal turnover, as well as rate of digestion (Reed, 1995). Similar results were confirmed by Makoto *et al.*, (2004), who reported that whole-crop oat silage with the addition of green tea waste did not significantly affect on DM digestibility. Green tea containing high catechin may have an inhibitory effect on intestinal absorption of lipid (Ikeda *et al.*, 1992). This may be preventing an excessive accumulation of lipid in the liver and other tissues.

**Table (3): Effect of different supplements on nutrient apparent digestibility (%) and feeding value of different rations.**

Items	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P
DM	82.90 ± 0.39	82.43 ± 0.63	82.74 ± 0.34	82.60 ± 0.42	0.900
OM	64.66 ± 0.44	64.42 ± 1.34	64.31 ± 0.63	63.84 ± 0.83	0.925
CP	75.34 ± 1.58	74.65 ± 1.65	75.30 ± 0.65	75.93 ± 2.12	0.952
CF	54.51 ± 3.44	47.52 ± 4.24	51.16 ± 1.92	47.33 ± 2.93	0.402
EE	83.13 <sup>a</sup> ± 3.69	77.18 <sup>ab</sup> ± 1.87	76.08 <sup>ab</sup> ± 2.63	72.51 <sup>b</sup> ± 2.75	0.013
NFE	62.35 ± 0.75	64.84 ± 0.80	63.08 ± 1.55	63.71 ± 1.42	0.534
TDN, %	61.80 ± 0.33	61.14 ± 1.06	61.67 ± 0.92	60.70 ± 1.23	0.835
SV					
Theoretical	61.08 ± 0.15	60.74 ± 1.06	61.19 ± 0.88	60.29 ± 1.18	0.894
SV true	44.73 ± 1.18	46.48 ± 0.59	45.84 ± 1.32	46.08 ± 0.34	0.621

Means within row bearing different superscripts differ significantly ( $p < 0.05$ ).

T1 Green tea      T2 Tetracycline      T3 Green tea + Tetracycline

The feeding value in terms of total digestible nutrients (TDN), starch value (SV) were not significant affected ( $P < 0.05$ ) for all rations.

### *Blood metabolites:*

The data of serum parameters are summarized in (Table 4). No statistically significant differences were observed among the feed supplemented groups for blood proteins, namely albumin and globulin. The values of albumin and globulin are similar to those of other early stage ruminants such as buffalo calves (Kumar and Dass, 2006), lambs (Harton, 1992) and dairy calves (Belibasakis and Tsirgogianni, 1996). The values of albumin/globulin ratio in the present study were higher ( $P < 0.05$ ) for buffaloes fed diet supplemented with green tea and tetracycline at rate of 1% (T3) than those fed diet with green tea at rate of 0.5% (T1). These results are in agreement with those of Gad and Zaghoul (2013) they reported that treatment of aged rats with green tea extract caused a significant increase in levels of total protein, albumin, globulin, albumin/ globulin (A/G) ratio. Buffaloes fed rations supplemented with tetracycline in T2 or tetracycline and green tea in T3 decreased cholesterol significantly ( $P < 0.05$ ) as compared with those fed control and green tea diets (163.80 and 166.99 vs. 196.17 and 203.31 respectively). On the other hand, supplemented green tea to ration T1 did not decrease cholesterol in serum and this may attributed to the low percentage of green tea supplemented to the ration (0.5%). However, the cholesterol value was lower in T3, this may be due to the higher level of green tea in the ration (1%) that decrease absorbed cholesterol in the intestine. Hence, the decrease in total lipid and cholesterol may be due to the effect of green tea by-products on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase that is required for cholesterol synthesis in the liver (Ariana *et al.*, 2011). Marcel and cho (2004) reported that green tea reduced cholesterol and lipids absorption in the gastrointestinal tract. Ahmed *et al.* (2014) reported that

increase green tea by-products levels (0%, 0.5%, 1.0% or 2.0%) in goats rations decreased linearly plasma glucose and cholesterol ( $p < 0.01$ ). Also, Han et al, (1999) and Juhel *et al.* (2000) found that green tea inhibit digestive lipases. Similarly, Md and Masaak (2001) found that levels of liver cholesterol, liver fat and blood serum cholesterol were significantly reduced ( $P < 0.05$ ) by green tea supplement to broilers rations. Yang *et al.* (2003) reported that addition of green tea by-product to chickens diets tended to decrease blood cholesterol and cholesterol content in chicken meat as compared with control, but a significant difference was not observed ( $p > 0.05$ ).

**Table (4): Effect of different supplements on blood metabolites.**

Item	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P
Total protein (g/dl)	6.92±0.10	7.02± 0.11	6.80± 0.23	6.60± 0.21	0.389
Albumin (g/dl)	4.12±0.07	4.14± 0.02	4.08± 0.04	4.17± 0.01	0.595
Globulin (g/ dl)	2.79± 0.08	2.88± 0.10	2.71± 0.24	2.42± 0.21	0.299
Al/Glo ratio	1.49 <sup>ab</sup> ± 0.07	1.45 <sup>b</sup> ± 0.04	1.59 <sup>ab</sup> ± 0.13	1.84 <sup>a</sup> ± 0.17	0.014
ALT (U/l)	14.77± 0.87	14.22± 0.87	13.66± 0.83	14.22± 0.87	0.844
AST (U/l)	16.33± 0.78	15.00± 1.00	14.33 ± 0.72	15.22± 1.70	0.653
Cholesterol mg/dl	196.17 <sup>a</sup> ± 6.21	203.31 <sup>a</sup> ± 10.49	163.80 <sup>b</sup> ± 3.96	166.99 <sup>b</sup> ± 4.87	0.0003

Means within row bearing different superscripts differ significantly ( $p < 0.05$ ).

T1 Green tea T2 Tetracycline T3 Green tea + Tetracycline

No significant differences were observed among all treatments for ALT and AST. However, it was noticed that buffalos fed supplemented tetracycline and green tea in T<sub>3</sub> tended to be lower by 3.5 % and 7.3 % for ALT and AST respectively than control group. Similar findings were reported by Gad and Zaghloul (2013) who supplemented green tea extracts to aged rats decreased significantly serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and a highly significant decrease in levels of total lipids, total cholesterol and triglycerides compared to control rats.

**Calves performance:**

Results presented in (Table 5) shows that the differences among all groups in body weight, daily gain were not significant ( $P > 0.05$ ). However, daily gain tended to be higher in calves fed ration supplemented with green tea than other treatments. The results indicated that there were no significant differences in

**Table (5): Performance of buffalo calves fed experimental diets.**

Item*	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P
Feeding period(day)	120	120	120	120	-
Initial weight (kg)	211.67±9.28	214.00 ± 14.47	213.33 ± 19.75	213.67 ± 18.89	0.999
Final weight (kg)	313.33 ± 6.89	322.00 ± 28.93	314.33 ± 24.13	315.67 ± 35.89	0.995
BW gain (kg)	101.67 ± 7.69	108.00 ± 14.57	101.00 ± 10.39	102.00 ± 17.04	0.997
Daily gain (kg)	0.847± 0.064	0.900 ± 0.121	0.841 ± 0.086	0.850 ± 0.141	0.977
Feed Intake (FI, kg/day)					
DMI of concentrate	3.78± 0.148	3.73± 0.143	3.76± 0.144	3.77± 0.148	0.996
DMI of wheat straw	1.73 <sup>ab</sup> ± 0.007	1.71 <sup>b</sup> ± 0.007	1.72 <sup>ab</sup> ± 0.008	1.75 <sup>a</sup> ± 0.007	0.058
DMI of hay alfalfa	1.29± 0.282	1.29± 0.282	1.29± 0.282	1.29± 0.282	1.000
Total DM intake	6.81± 0.160	6.74± 0.166	6.78± 0.164	6.81± 0.161	0.991
Feed conversion ratio kg DMI/kg gain)	8.04 ± 0.189	7.50 ± 0.185	8.04 ± 0.192	8.01± 0.190	0.118
feed cost/day E.P.*	11.64 ± 0.27	11.47 ± 0.28	11.46 ± 0.28	11.44 ± 0.27	0.953
Feed cost/kg WG**	13.75 ± 0.324	12.82 ± 0.316	13.75 ± 0.328	13.70 ± 0.325	0.118

Means within row bearing different superscripts differ significantly ( $p < 0.05$ ).

T1 Green tea T2 Tetracycline T3 Green tea + Tetracycline

\* E.P. =Egyptian pound \*\* WG=Weight gain

total DM intakes among all treatments (6.81, 6.74, 6.78 and 6.81) for control, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. On the other hand, buffalos fed ration content green tea and tetracycline was significant higher for dry matter intake of wheat straw than fed green tea (1.75 vs. 1.71). Supplemented green tea or tetracycline to rations has no effect on feed conversion ratio of growing buffalos (Table 5). Although no significant differences were observed among all groups for feed conversion ratio, numerical differences were found between T<sub>1</sub> and control (0.73 unit DM /unit gain). Results presented in (Table 5) showed that the differences in feed cost/day and feed cost /kg were not significant (P<0.05). These results agreement with Jerome *et al.* (2002) who reported that neither drug nor dose significantly were in affected the rate of feed intake, average daily gain and feed conversion ratio for pigs fed concentrate mixture with different level of tetracycline.

## CONCLUSION

Based on the findings among the feed additive supplementation, mixed feed additive from green tea and tetracycline may be suitable for buffalo's calves. However, supplemented green tea at rate of 0.5% and tetracycline at rate of 1 % were insufficient to make effect on digestibility or performance of growing buffalos. Further studies are required to apply higher levels of green tea or use green tea extracts and by-products on rumen fermentation, performance and meat quality for growing buffalos.

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### إضافة الشاي الأخضر والتتراسيكلين على هضم المكونات الغذائية وخصائص الدم وإداء النمو لعجول الجاموس النامية

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أجريت هذه الدراسة لبحث تأثير إضافة الشاي الأخضر أو التتراسيكلين أو كليهما لعلائق عجول الجاموس على القيمة الهضمية وخصائص الدم وإداء النمو في عجول الجاموس النامية. تم تقسيم 12 من الحيوانات الي اربع مجاميع بكل مجموعة ثلاثة حيوانات متوسط وزنها  $213 \pm 3.52$ . غذيت المجموعة الاولى علي العليقة المقارنة بدون اضافات وغذيت المجموعة الثانية (1م) علي العليقة المقارنة مضافا اليها 0.5% من الشاي الاخضر بينما غذيت المجموعة الثالثة (2م) علي العليقة المقارنة مضافا اليها 1% من التتراسيكلين في حين غذيت المجموعة الرابعة (3م) علي العليقة المقارنة مضافا اليها كل من الشاي الاخضر والتتراسيكلين بمعدل 1% وكانت مدة الفترة التجريبية (4 شهور). تم تغطية 80% من احتياجات الحيوانات من المواد المركزة بينما أعطى تبن القمح ودريس البرسيم لتكملة الاحتياجات. علما بان كمية المواد المركزة يتم تغييرها شهريا تبعاً للزيادة الشهرية وذلك طبقاً لـ (NRC, 2001). أظهرت النتائج أن إضافة الشاي الاخضر أو التتراسيكلين أو كليهما لم يكن لة تأثير معنوي على هضم المكونات الغذائية لعلائق عجول الجاموس النامية ويستثنى من ذلك هضم الدهن حيث كان منخفضاً معنوياً في مجموعة عجول الجاموس المغذاة على كل من الشاي الاخضر والتتراسيكلين مقارنة بالعليقة الكنترول.

لم تسجل اى اختلافات معنوية عند تغذية العجول الجاموس على العلائق المختلفة في كل من البروتين الكلى والاليومين والجلوبولين بسيرم الدم. إضافة التتراسيكلين م2 أو الشاي الأخضر مع التتراسيكلين م3 ادى الى انخفاض مستوى الكوليسترول الكلى في سيرم العجول النامية مقارنة بالعليقة المحتوية على الشاي الاخضر أو العليقة المقارنة. أيضاً لم تشاهد اى اختلافات معنوية في كل المجاميع بين كل من ALT, AST ولكن وجد ان عجول الجاموس المغذاة على العليقة م3 أظهرت انخفاض في كل ALT, AST بمقدار 3.5 و 7.3% على التوالي مقارنة بالعليقة المقارنة. أداء النمو للعجول النامية معبرا عنة بالغذاء الكلى المأكول اليومي أو معدل النمو اليومي أو الكفاءة التحولية للغذاء الى نمو لم يتأثر معنوياً بإضافة كل من الشاي الأخضر أو التتراسيكلين أو كليهما الى العلائق. وقد خلصت الدراسة الي ان إضافة الشاي الاخضر بمعدل 0.5% والتتراسيكلين بمعدل 1% لم يكن كافي لتحسين القيمة الهضمية وأداء العجول النامية، ولكن عندما أضيف الاثنين معا بمعدل 1% كان مناسباً لإضافة في علائق العجول حيث لوحظ تحسن في القيمة الهضمية وصفات الدم وأداء العجول النامية.