# Influence of TOMOKO® (Direct-Fed Microbials) on Productive Performance, Selected Rumen and Blood Constituents in Barky Finishing Lambs

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**Abstract:** A feeding trail of six weeks was conducted to investigate the influence of dietary supplementation of direct-fed microbials (TOMOKO®) on productive performance, selected rumen and blood constituents. Twenty four Barky finishing lambs of 30 kg average body weight were divided into 2 equal groups. Group one (G1) was fed on basal diet (60 % concentrate mixture and 40 % good quality hay) and used as a control. Group two (G2) was fed basal diet fortified with 500 grams of TOMOKO® per ton of feed. Body weight, average daily body weight gain (ADG), feed consumption and feed efficiency were recorded at 0, 21 and 42 days of experiment. Rumen juice samples; rumen pH, total protozoa count, total volatile fatty acid concentrations (TVFAs), ammonia nitrogen, urea and total protein were investigated, while serum concentrations of total protein, albumin, globulin and urea-nitrogen (BUN) were measured. Lambs fed TOMOKO® dietary supply showed significant ($p<0.05$) increased body weight and ADG. Significant differences ($p<0.05$) in rumen pH, total protozoa count, total volatile fatty acids, urea and total protein and serum total protein, globulin and urea nitrogen levels were recorded. Dietary supplementation of TOMOKO® to fattening lambs increased body weight gain, feed efficiency rate and improved the rumen fermentation pattern and health status.
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**Key words**: Fattening lambs, TOMOKO®, Rumen and serum constituents.

## 1. Introduction

Recent studies on ruminant nutrition have mostly focused on improving feed efficiency. A variety of feed additives have been developed to achieve this objective. Antimicrobial feed additives, which are widely used in the world have been prohibited or restricted in most countries due to the increased concern of causing resistance to antibiotics in bacterial pathogens. Therefore, the interest is directed to potential alternatives to antibiotic feed additives such as direct-fed microbials (DFM), which is composed of cultures of useful microorganisms (Alp and Kahraman, 1996; Elam *et al.,* 2003 and Krehbiel *et al.,* 2003). Tomoko is claimed to improve the productive performance of the broiler chicken (Saleh *et al.,* 2006). Addition of direct-fed microbials to the ration of sheep decreases numbers of harmful microorganisms in the intestines, improves fattening performance and feed conversion rate (Lema *et al.,* 2001). Feed evaluation for ruminant is often based on estimation of rumen digestibility, even though such measurements alone cannot predict how ruminants will utilize feed nutrients. It is essential to investigate the effect on rumen physical status, biochemical constituents (Zehra and Kiliç, 2009); and ciliates composition (Baraka, 2006). The examination of the rumen fluid has great importance in the investigation of microbial and biochemical alterations of reticulum and rumen (Barbosa, 2007).

The aim of this study was to investigate the influence of dietary fortification with Tomoko® on productive performance, selected rumen and serum constituents in fattening lambs.

## 2. Material and Methods

This feeding trial comprised a total of 24 Barky finishing male lambs belonging to a private sheep farm in Alexandria-Cairo desert road; of 30 kg average body weight. They were randomly divided into two equal groups of 12 lambs each. Group one (G1) was fed the basal diet containing 60 % concentrate mixture and 40 % good quality hay. Basal diet was formulated according to nutrient requirements of sheep (NRC, 1985) to meet the nutrient requirements of the finishing lambs (Table 1). Group two (G2) was fed the basal diet fortified with 500 grams of TOMOKO® per ton of feed. Tomoko is one of the biological feed additives and 100 % natural product of Koji, a Japanese fermentation process. Tomoko is composed of enzymes (acidic protease, ∞-amylase, phytase, glucoamylase and cellulase) and live fungal culture, *Aspergillus Awamori* cells. Lambs were allowed *ad libitum* consumption of fresh and clean water. Daily

recording of feed consumption in each group was applied. While body weight, average daily body weight gain (ADG) and feed efficiency (FE) of each group were recorded. Health conditions of the lambs were closely monitored during the study. Collection of rumen and blood samples was carried out just before feeding. Rumen juice samples were collected from each lamb on days 0, 21 and 42 of experiment using a rubber stomach tube. Rumen pH (using SMP1 pH-meter) was examined immediately; then samples divided and stored for determination of total protozoa count according to the method described by Dehority (1984), generic protozoa composition according to Dehority (1993), ammonia concentration according to Zapletal (1967) and volatile fatty acids concentration according to Cottyn and Boucque (1968). Centrifuged strained rumen liquor supernatant was stored in deep freezer at -20°C until assayed. Blood samples were collected via jugular vein puncture before morning feeding on days 0, 21 and 42 of the experiment to separate serum and stored in deep freezer. Selected biochemical constituents were measured in rumen fluid (urea and total protein) and in serum (total protein, albumin and BUN) using Apel PD-303S spectrophotometer and specific diagnostic chemical kits. Obtained data were statistically analyzed using SPSS Statistical Computer Software, Copyright (c) SPSS Inc., 2007 version 16.0. Differences at $p<0.05$ were considered significant.

**Table 1: Composition and Chemical analysis of basal diet**

| **Ingredient composition** | | **As fed / Kg** | **DM / Kg** | **DM %** |
|---|---|---|---|---|
| Berseem hay | | 0.57 | 0.52 | 40.0 |
| Concentrate mixture (%): | | 0.87 | 0.78 | 60.0 |
| Yellow corn, ground | 61.30 | | | |
| Soya bean meal (44% CP) | 15.00 | | | |
| Wheat bran | 20.00 | | | |
| Common Salt | 1.00 | | | |
| Sodium bicarbonate | 0.90 | | | |
| Sheep Premix* | 0.30 | | | |
| Limestone, ground | 1.50 | | | |
| **Total** | | **1.44** | **1.30** | **100** |
| **Chemical analysis of basal diet** | | | | |
| CP % | 15.60 | | | |
| TDN % | 73.00 | | | |
| Ca % | 1.00 | | | |
| P % | 0.45 | | | |

* Composition of sheep premix: vitamin A 12 000 000 IU; vitamin $D_3$ 3 000 000 IU; vitamin E 30 g; Mn 50 g; Fe 50 g; Zn 50 g; Cu 5 g; I 0.85g; Co 0.15g; Se 0.15 g.

## 3. Results and Discussion

The effect of dietary supplementation of Tomoko® on body weight and average daily weight gain (ADG) of Barky male lambs was shown in Table (2). The results revealed that significant increase ($p<0.05$) in body weights of lambs in G2 than that of control at day 42 of the experiment. Moreover, ADG of lambs fed rations supplemented with DFM (TOMOKO®) was significantly ($p<0.05$) higher than control for the period of day 22-42. Overall ADG of lambs in G2 were found significantly higher ($p<0.05$) than that of control. While the data of feed consumption of the lambs and feed efficiency is provided in the Table (3) and results indicated that there were no significant changes in the feed consumption and feed efficiency of lambs during different periods between G1 and G2. The results of the present study were consistent with other published articles indicating that addition of direct-fed microbial to the ration of sheep resulted in increased body weight and ADG, (Lubbadeh *et al.,* 1999; Lema *et al.,* 2001 and Chiofalo *et al.,* 2004). Henderson *et al.* (1986) found that feeding a sheep with silage inoculated through bacterial cultures resulted in an increased consumption of feed dry matter and increased ADG compared to the control. In a similar study done by Emanuelle *et al.* (1992), feeding the lambs with inoculum-added dry forage improved the feed consumption, body weight gain, and feed conversion rate of the animals. Krehbiel *et al.* (2003) reported that data, which were on the effects of direct-feed microbials added to the feed, might be due to differences in cultures used as direct-feed microbial as well as variations in breed, age, gender and environmental conditions. Lee *et al.* (2000) stated that the effect of DFM originating from an anaerobic fungal culture with superior fibrolytic activity into the rumen can improve nutrient utilization in sheep.

**Table 2: Body weight and average daily body weight gain of fattening lambs fed DFM (TOMOKO®) ***

| | Group 1 | Group 2 |
|---|---|---|
| **Body weight / Kg** | | |
| Initial | 30.30[a] | 30.70[a] |
| Day 21 | 34.40[a] | 34.90[a] |
| Day 42 | 40.35[b] | 41.86[a] |
| **Average daily weight gain/g** | | |
| Day 0- 21 | 195.24[a] | 200.00[a] |
| Day 22- 42 | 283.33[b] | 331.43[a] |
| Day 0- 42 day | 239.29[b] | 265.71[a] |

*Means in rows with different superscript letter are statistically different at $p<0.05$ .

**Table 3: Feed consumption and feed efficiency of fattening lambs fed DFM (TOMOKO®)**

| | Group 1 | Group 2 |
|---|---|---|
| **Feed consumption (gram/day)** | | |
| Day 0 -21 | 1300 | 1305 |
| Day 22 – 42 | 1440 | 1442 |
| Day 0 – 42 | 1380 | 1387 |
| **Feed efficiency (Gain: Feed)** | | |
| Day 0- 21 | 0.15 | 0.15 |
| Day 22-42 | 0.20 | 0.23 |
| Day 0- 42 | 0.17 | 0.19 |

Rumen constituents (Table 4) showed that pH in both groups significantly changed ($p<0.05$) at day 21 and day 42. By the end of the experiment a mild decrease was recorded in G2, but generally within the normal range of rumen pH in sheep. These changes can be referred to the addition of TOMOKO®, which was in agreement with that recorded by Gomez-Alarcon *et al.* 1990, Machmüller *et al,.* 2003, Khaled et al. 2005,Trabalza-Marinucci *et al.*, 2008 and Akbar *et al.*, 2009. The control of rumen pH normally occurs as an interaction between increasing rumen input of bases and buffers ($HCO3^-$ from diet and saliva), food yields bases or buffers (ammonia from degraded protein, non-protein nitrogen food), absorption of volatile fatty acids (VFAs) and removing non-ionized VFAs in exchange with $HCO3^-$ (Maloiy 1972, Farid *et al,.* 1979, Rubsamen and Engelhardt, 1979 and Baraka, 2001).

**Table 4: Rumen pH and ciliates composition (Means ± SE) of fattening lambs fed DFM (TOMOKO®) ***

| Parameters | Sampling time (day) | Group 1 | Group 2 |
|---|---|---|---|
| pH | 0 | $6.06 \pm 0.06^{a}$ | $5.34 \pm 0.08^{c}$ |
| | 21 | $6.00 \pm 0.16^{ab}$ | $6.17 \pm 0.04^{a}$ |
| | 42 | $6.09 \pm 0.03^{a}$ | $5.71 \pm 0.16^{b}$ |
| Total protozoa ($\times 10^4$/ml) | 0 | $28.13 \pm 4.75^{bcd}$ | $53.75 \pm 3.77^{a}$ |
| | 21 | $19.00 \pm 2.78^{d}$ | $49.38 \pm 7.96^{ab}$ |
| | 42 | $15.75 \pm 2.18^{d}$ | $37.50 \pm 5.91^{bc}$ |
| Entodinium (%) | 0 | 65.33 ± 1.89 | 76.95 ± 8.05 |
| | 21 | 86.00 ± 3.81 | 82.47 ± 11.11 |
| | 42 | 74.85 ± 5.24 | 84.73 ± 4.09 |
| Diplodinium (%) | 0 | 0.00±0.00 | 0.00±0.00 |
| | 21 | 0.00±0.00 | 0.00±0.00 |
| | 42 | 0.00±0.00 | 0.60±0.60 |
| Epidinium (%) | 0 | 20.10 ± 1.61 | 22.72 ± 7.88 |
| | 21 | 13.15 ± 3.49 | 17.53 ± 11.11 |
| | 42 | 18.53 ± 8.56 | 19.18 ± 5.75 |
| Holotricha (%) | 0 | $13.83 \pm 1.94^{a}$ | $0.28 \pm 0.28^{c}$ |
| | 21 | $0.80 \pm 0.80^{c}$ | $0.00 \pm 0.00^{c}$ |
| | 42 | $5.88 \pm 3.40^{b}$ | $0.00 \pm 0.00^{c}$ |
| Ophryoscolex (%) | 0 | $0.80\pm0.46^{a}$ | $0.00\pm0.00^{b}$ |
| | 21 | $0.00\pm0.00^{b}$ | $0.00\pm0.00^{b}$ |
| | 42 | $0.00\pm0.00^{b}$ | $0.00\pm0.00^{b}$ |

*Means in rows with different superscript letter are statistically different at $p<0.05$.

Stabilization of rumen pH was the reason for the improved microbial growth. Enzymes present in the TOMOKO® (Acidic protease, α-amylase, Phytase, Glucoamylase and Cellulase) that are responsible for the activity of live fungal culture that composed of *Asperagillus awamori* cells in the rumen and capable for digestion of plant cell wall material (Williams 1989 and Williams *et al.,* 1991). Different species in the genus *Aspergillus* may give effects comparable to *A. oryzae*. *Asperagillus niger* was at least as effective as *A.oryzae* in enhancing nutrient digestion in cows (Campos *et al.,* 1990).

Rumen total protozoa count in both groups showed gradual parallel significant reduction ($p<0.05$) during the experiment and a significant change ($p<0.05$) at forty two day in both groups was obvious. These changes can be referred to the compensation of protozoa count to pH and metabolic products of digestion. Total count obtained in control group was in agreement with that recorded by Machmüller *et al.,* 2003 and Sărăndan *et al.,* 2009; while higher numbers than that of G1 and G2 were recorded by Crha *et al.,* 1991 and Akbar *et al.,* 2009.

Generic composition of protozoa showed that *Entodinium* spp. percentage was within the normal level in both groups during the experiment and in agreement with levels recorded by Varadyova *et al.,* 2007, while Sărăndan *et al.,* 2009 reported higher levels. *Diplodinium* spp. was only recorded in forty two day in G2. *Holotricha* spp. showed significant changes ($p<0.05$) along the experiment in both groups. This can be explained on the basis of as *Entodinium* spp. is responsible for utilization of formed lactic acid in the rumen (Baraka, 2006) and fungal cultures improve the use of lactate by the rumen organism, *Selenomonas ruminantium* by providing a source of dicarboxcylic acids (e.g., malic acid) and other growth factors (Martin and Streeter, 1995).

The recent researchers have shown that numbers of some protozoa genera (e. g., *Entodinium* spp.) remain high in cattle fed wheat (Kreikemeier *et al.,* 1990) and barely (Hristov *et al.,* 2001), *Entodinium* spp. may reduce the risk of clinical and sub-clinical acidosis through their ability to utilize lactate (Newbold *et al.,* 1987) and to moderate the rate of starch digestion by ingestion of starch granules (Williams and Coleman, 1992).

Rumen volatile fatty acids (Table 5) showed a significant decrease ($p<0.05$) in twenty one day followed by a significant increase ($p<0.05$) in last samples in both groups. The fluctuation in rumen volatile fatty acids can be explained depending on that, the quantity of volatile fatty acids absorption depends on the pH of the fore-stomach fluid when, the pH decreases below the physiological limit the rate of absorption increases (Dycker *et al.,* 1994). The recent papers recorded lower levels of VFA as mentioned by Machmüller *et al.,* 2003, Carro *et al.,* 2005, Akbar *et al.,* 2009 and Carro *et al.,* 2009.

**Table 5: Selected rumen constituents (Means ± SE) of fattening lambs fed DFM (TOMOKO®) ***

| Parameters | Sampling time (day) | Group 1 | Group 2 |
|---|---|---|---|
| Total VFAs (mmol/L) | 0 | $158.13 \pm 9.09^{b}$ | $161.0 \pm 11.20^{b}$ |
| | 21 | $113.25 \pm 10.54^{b}$ | $128.9 \pm 21.89^{b}$ |
| | 42 | $209.00 \pm 14.77^{a}$ | $210.00 \pm 19.55^{a}$ |
| Ammonia-N (mmol/L) | 0 | $347.86 \pm 3.16$ | $358.36 \pm 9.94$ |
| | 21 | $366.38 \pm 2.73$ | $356.86 \pm 7.93$ |
| | 42 | $339.86 \pm 9.27$ | $342.90 \pm 7.53$ |
| Urea (mmol/L) | 0 | $648.79 \pm 77.03^{b}$ | $728.41 \pm 120.6^{b}$ |
| | 21 | $523.80 \pm 78.31^{bc}$ | $315.70 \pm 25.72^{c}$ |
| | 42 | $817.59 \pm 150.52^{a}$ | $759.4 \pm 125.03^{b}$ |
| Total protein (g/L) | 0 | $12.28 \pm 0.95^{bc}$ | $18.78 \pm 0.97^{a}$ |
| | 21 | $10.55 \pm 0.52^{c}$ | $14.21 \pm 1.43^{b}$ |
| | 42 | $12.45 \pm 0.62^{bc}$ | $14.88 \pm 1.54^{b}$ |

*Means in rows with different superscript letter are statistically different at $p<0.05$.

Rumen ammonia showed no significant changes, while marked decreases in G1 at day forty two were obvious, these levels were higher than that mentioned by Machmüller *et al.,* 2003, Carro *et al.,* 2005, Akbar *et al.,* 2009 and Carro *et al.,* 2009; That can be explained by that level of rumen ammonia is derived from the degradation of protein or non-protein nitrogen feed to ammonia and urea. Rumen urea varied significantly at day twenty one ($p<0.05$) and day forty two and by the end of experiment increased significantly ($p<0.05$) in both groups. Ammonia is absorbed to the liver for urea formation and the urea to blood for formation of the blood urea nitrogen level. Both of urea and ammonia in the rumen are used by the rumen bacteria for synthesis of bacterial protein, bicarbonates in the rumen are needed for the formation of carbon skeleton to enhance the change of simple nitrogenous

compounds into more complex molecules of bacterial protein (Payne and Payne, 1987). The rumen urea concentration depends on the rate of production and absorption of ammonia nitrogen, as well as on the rate of detoxification of ammonia into urea in the liver (Visek, 1972).

Rumen protein in G1 decreased significantly ($p<0.05$) at day twenty one and return to normal level at day forty two, while in G2 decreased significantly ($p<0.05$) in days 21 and 42 consequently; which confirms the increase in absorption from rumen (Abdel-Rahman and Khaled, 2004).

Serum constituents (Table 6) revealed that total protein level in control group showed significant descending at day twenty one ($p<0.05$) and ascending at day forty two ($p<0.05$); while by the end of experiment a significant increase ($p<0.05$) at forty two day in control in comparison with that of G2, that was a reflection to the increase in protein level produced and absorbed from rumen. The levels recorded by Borjesson *et al.,* 2000, Whittaker *et al.,* 2000, Mostaghni *et al.,* 2005, Mousa, 2008, Trablaza-Marinucci *et al.,* 2008 and Afaf *et al.,* 2009 were within the mean of both groups, but lesser than that gained at the end of experiment in G1. Serum albumin showed a non significant fluctuation while the changes in calculated globulin showed the opposite manner of increases and decreases parallel to that of total protein in both groups with significant increase ($p<0.05$) of globulin in G2 at day twenty one and decrease ($p<0.05$) in control group at day twenty one.

**Table 6: Selected blood constituents (Means ± SE) of fattening lambs fed DFM (TOMOKO®) ***

| Parameters | Sampling time (day) | Group 1 | Group 2 |
|---|---|---|---|
| Serum Protein (g/L) | 0 | $79.11 \pm 2.73^{ab}$ | $55.44 \pm 5.49^{c}$ |
| | 21 | $68.39 \pm 5.64^{abc}$ | $77.14 \pm 6.88^{ab}$ |
| | 42 | $85.43 \pm 4.40^{a}$ | $66.59 \pm 6.90^{bc}$ |
| Albumin (g/L) | 0 | $31.92 \pm 1.48$ | $36.36 \pm 1.88$ |
| | 21 | $39.03 \pm 1.93$ | $33.50 \pm 5.07$ |
| | 42 | $35.75 \pm 0.88$ | $31.87 \pm 0.69$ |
| Globulin (g/L) | 0 | $47.19 \pm 1.50^{ab}$ | $19.07 \pm 7.20^{c}$ |
| | 21 | $29.37 \pm 5.38^{bc}$ | $43.65 \pm 6.37^{ab}$ |
| | 42 | $49.63 \pm 5.26^{a}$ | $34.7 \pm 6.46^{abc}$ |
| BUN (mmol/L) | 0 | $19.37 \pm 2.59^{a}$ | $6.64 \pm 1.46^{b}$ |
| | 21 | $21.48 \pm 2.15^{a}$ | $8.47 \pm 1.14^{b}$ |
| | 42 | $18.46 \pm 3.44^{a}$ | $8.82 \pm 0.91^{b}$ |

*Means in rows with different superscript letter are statistically different at $p<0.05$.

As blood urea nitrogen level depends on ammonia absorbed to the liver (Payne and Payne, 1987); it can explain the significant increase ($p<0.05$) in twenty one and forty two days in G2.

It can be concluded that in Fattening lambs that fed TOMOKO® supplemented ration, there were significant increase ($p<0.05$) in lamb body weight and ADG, significant decrease ($p<0.05$) in rumen pH, total protozoa count and total protein; while significant increases ($p<0.05$) in total volatile fatty acids and urea were recorded. Serum total protein, globulin and urea nitrogen were significantly increased ($p<0.05$).

Dietary supplementation of TOMOKO® to fattening lambs increased body weight gain, feed efficiency rate and improved the rumen fermentation pattern and health status.

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