# Effects of Moringa Stem and Leaf on Production Performance and Plasma Biochemical, Antioxidant, and Immune Indices in Dairy Cows: Postprint

**Authors:** Zhang Xingyi, Lin Cong, Li Yang, Wang Yizhen, Gao Hong, Zhang Guangning, Jiang Xin, Xu Hongjian, ZHANG Yonggen

**Date:** 2017-10-11T00:00:00+00:00

## Abstract

College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030

## Full Text

### Effects of Moringa oleifera Leaves and Peduncles on Production Performance, and Plasma Biochemical, Antioxidant and Immune Indexes in Dairy Cows

**ZHANG Xingyi, LIN Cong, LI Yang, WANG Yizhen, GAO Hong, ZHANG Guangning, JIANG Xin, XU Hongjian, ZHANG Yonggen***

*College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China*

**Corresponding author: Professor, E-mail: zhangyonggen@sina.com**

---

**Abstract**

This experiment aimed to investigate the effects of replacing 50% of alfalfa in the basal diet with Moringa oleifera leaves and peduncles on production performance and plasma biochemical, antioxidant, and immune indexes in lactating dairy cows. Eight healthy multiparous Holstein cows with similar body weight, parity, and milk production at 100–150 days postpartum were selected and divided into two groups of four cows each for a crossover trial. Cows in the experimental group (A) were fed a test diet in which Moringa oleifera leaves and peduncles replaced 50% of the alfalfa in the basal diet, while those in the control group (B) received the basal diet. The trial consisted of two periods, each lasting 18 days (15-day preliminary period followed by a 3-day sampling period). Blood and milk samples were collected, and dry matter intake (DMI) and milk yield were recorded. The results showed that: (1) Compared with group B, group A exhibited significantly increased DMI ($P<0.05$) and a tendency for higher milk yield ($0.05 \leq P<0.10$), along with significant improvements in milk protein percentage, milk protein yield, and total solids content, while milk somatic cell count showed a decreasing trend ($0.05 \leq P<0.10$); (2) Plasma cholesterol (CHOL) and triglyceride (TG) concentrations in group A were significantly lower than in group B ($P<0.05$), while alkaline phosphatase (ALP) activity and non-esterified fatty acid (NEFA) content tended to decrease ($0.05 \leq P<0.10$); (3) Group A significantly enhanced plasma total antioxidant capacity (T-AOC) and hydroxyl radical scavenging capacity ($P<0.05$), significantly reduced plasma malondialdehyde (MDA) content ($P<0.05$), and significantly increased plasma immunoglobulin G (IgG) concentration ($P<0.05$). Therefore, Moringa oleifera leaves and peduncles can enhance dairy cow production performance to a certain extent, prevent mastitis, improve plasma biochemical profiles, and elevate antioxidant capacity and immune function, making them a viable high-quality roughage for dairy production.

**Keywords:** Moringa oleifera leaves and peduncles; dairy cows; production performance; plasma biochemical index; antioxidant index; immune function

---

## Introduction

Roughage serves as the primary feed source for dairy cows and plays a crucial role in their nutritional system. However, with the development of China' s dairy industry and decreasing forage cultivation area, the shortage of high-quality roughage has become increasingly severe, prompting recent efforts to develop and utilize novel roughage resources. Moringa oleifera Lam., primarily cultivated in tropical and subtropical regions, is a functional plant with rich nutritional and medicinal value in its roots, bark, leaves, flowers, fruits, and seeds [1-3]. It is characterized by strong adaptability, rapid growth, high yield, and edibility. Moringa oleifera leaves (MOL) are rich in protein, minerals, vitamins, essential amino acids, and antioxidant components such as carotenoids and flavonoids, serving as natural antibacterial and antioxidant agents [4]. In contrast, Moringa oleifera peduncles (MOP) have lower nutritional value but higher physical fiber content and lower cost. Mixing leaves and peduncles in appropriate proportions as roughage for dairy production could help alleviate the shortage of high-quality roughage. Research has demonstrated that Moringa oleifera leaves exhibit strong antioxidant activity at both tender and mature stages, preventing oxidative damage to essential biomolecules [5]. The leaves also possess hypolipidemic, anti-atherosclerotic, immunity-enhancing, and anti-tumor properties [6-8]. Kholif et al. [9] reported that replacing sesame with Moringa oleifera leaves in lactating Nubian goats improved feed digestibility, milk yield, and rumen fermentation function. Alfalfa, known as the "king of forages," represents high-quality roughage, yet Moringa oleifera leaves have comparable nutritional levels with even higher protein content. If widely promoted and cultivated in China, Moringa oleifera could potentially surpass alfalfa in both nutritional value and cost-effectiveness given its high biomass production. Therefore, this study investigated the effects of replacing 50% of alfalfa with Moringa oleifera leaves and peduncles on dairy cow production performance and plasma biochemical, antioxidant, and immune indexes to provide a theoretical basis for the scientific and rational application of Moringa oleifera as roughage in dairy production.

### 1.1 Experimental Materials and Design

The Moringa oleifera leaves and peduncles used in this experiment were provided by Beijing Meilink Biotechnology Co., Ltd. The variety was PKM1, a selected cultivar originating from Calcutta, India, characterized by rapid growth, multiple branches, and high seed yield. The mixing ratio of leaves to peduncles was 3:2, with nutrient composition shown in .

This crossover trial was conducted at Harbin Comprehensive Dairy Farm to eliminate individual cow differences and variations across different lactation stages. Eight healthy Chinese Holstein cows at 100–150 days postpartum, with similar parity, milk yield, and genetic background, were selected and divided into two groups of four cows each. Group A received the test diet with Moringa oleifera leaves and peduncles replacing 50% of alfalfa, while group B received the basal diet. The trial comprised two periods, each lasting 18 days (15-day preliminary period and 3-day sampling period). The experiment ran from December 27, 2015, to January 31, 2016, for a total of 36 days.

### 1.2 Feeding Management and Diet Composition

All cows were managed under identical conditions, fed three times daily at 05:00, 11:00, and 17:00, with each feeding lasting 2–3 hours. Water was available ad libitum, and diets were offered as total mixed rations (TMR). Diets were formulated according to the *Feeding Standard of Dairy Cows* (NY/T 34-2004) using CMP formulation software, consisting of concentrate, corn silage, and alfalfa hay. The concentrate contained corn, beet pulp, soybean meal, fat powder, and premix. Diet composition and nutrient levels are presented in .

### 1.3 Sample Collection and Processing

During the 3-day sampling period of each period, feed offered and refused was recorded daily to determine dry matter content and calculate dry matter intake (DMI). Milk yield was recorded and milk samples were collected at 06:00 and

18:00 each day (approximately 50 mL per sampling). The two samples were pooled according to the actual morning and afternoon milk yields for milk composition and somatic cell count analysis. The average milk yield during the sampling period represented the period' s milk production.

On the final day of each sampling period, blood samples were collected from the coccygeal vein before morning feeding using heparin sodium anticoagulant tubes. Samples were centrifuged at 3,500 rpm for 10 minutes, and plasma was aliquoted into five EP tubes and stored at -20°C for subsequent analysis of biochemical, antioxidant, and immune parameters.

**Milk composition** (fat, protein, lactose, total solids) was analyzed using a Foss MilkoScan FT+ milk composition analyzer. **Milk somatic cell count (SCC)** was determined using a DeLaval somatic cell counter.

**Plasma biochemical indices** including total protein (TP), albumin (ALB), globulin (GLB), cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), urea nitrogen (UN), glucose (GLU), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using an Italian fully automatic biochemical analyzer with reagent kits from Beijing Zhongsheng Beikong Biotechnology Co., Ltd. Non-esterified fatty acids (NEFA) and $\beta$-hydroxybutyric acid (BHBA) were measured using a Shimadzu UV-2401 spectrophotometer with reagent kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

**Plasma antioxidant indices** including catalase (CAT) activity, total superoxide dismutase (T-SOD) activity, total antioxidant capacity (T-AOC), hydroxyl radical scavenging capacity, and malondialdehyde (MDA) content were measured using reagent kits from Nanjing Jiancheng Bioengineering Institute.

**Plasma immune indices** including immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) were determined by immunoturbidimetry using the automatic biochemical analyzer with reagent kits from Nanjing Jiancheng Bioengineering Institute.

### 1.4 Data Processing and Analysis

All data were initially processed using Excel software and subjected to statistical analysis using the MIXED procedure of SAS 9.4 software. Duncan' s multiple range test was used for inter-group comparisons. $P<0.01$ indicated extremely significant difference, $P<0.05$ indicated significant difference, and $0.05 \leq P<0.10$ indicated a tendency.

### 2.1 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Dairy Cow Production Performance

As shown in , group A exhibited significantly higher DMI and energy-corrected milk yield compared with group B ($P<0.05$). Milk yield in group A averaged 2.09 kg/d higher than group B, showing an increasing trend ($0.05 \leq P<0.10$). Milk protein yield, milk protein percentage, and total solids content were significantly higher in group A ($P<0.05$), while milk fat percentage, milk fat yield, lactose percentage, and lactose yield showed no significant changes ($P>0.05$). Milk somatic cell count in group A tended to be lower than in group B ($0.05\leq P<0.10$).

### 2.2 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Plasma Biochemical Indices

As shown in , compared with group B, group A had significantly lower plasma CHOL and TG concentrations ($P<0.05$), while ALP activity and NEFA content showed decreasing tendencies ($0.05\leq P<0.10$). No significant differences were observed between groups for other parameters ($P>0.05$).

### 2.3 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Plasma Antioxidant Indices

As shown in , group A supplemented with Moringa oleifera leaves and peduncles significantly increased plasma T-AOC and hydroxyl radical scavenging capacity ($P<0.05$) and significantly decreased plasma MDA content ($P<0.05$) compared with group B.

### 2.4 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Plasma Immune Indices

As shown in , group A significantly increased plasma IgG concentration compared with group B ($P<0.05$), while IgA and IgM concentrations remained unchanged ($P>0.05$).

### 3.1 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Dairy Cow Production Performance

Dry matter intake effectively reflects dairy cow production performance and health status [10]. Previous studies have demonstrated that Moringa oleifera leaves can significantly increase DMI [11] and milk yield [12] in dairy cows. In this experiment, group A showed significantly higher DMI and energy-corrected milk yield, with milk yield tending to increase compared with group B. This may be attributed to the improved palatability of the diet containing Moringa oleifera and its higher rumen-undegradable protein content [13]. Literature has confirmed that Moringa oleifera leaves contain high levels of rumen-undegradable protein, which promotes increased DMI in dairy cows [14-15]. DMI is a critical factor affecting milk yield; under similar dietary composition and nutrient levels, higher DMI generally leads to greater milk production [16], consistent with our findings. Group A exhibited significantly higher milk protein percentage, milk protein yield, and total solids content, while lactose and fat percentages remained unchanged. Basitan et al. [17] confirmed that supplementing dairy cow diets with Moringa oleifera significantly increased milk yield. Sánchez et al. [18] also reported that increased milk yield was associated with higher milk fat and protein production. Improved rumen fermentation function can enhance milk protein, fat, and lactose yields. Khalel et al. [19] demonstrated that Moringa oleifera leaves improved rumen fermentation, significantly increasing milk yield and milk composition including total solids, fat, and protein percentages, but not lactose percentage, similar to our results. Milk somatic cell count serves as an indicator of udder health, showing a significant negative correlation with milk yield [20-21]. In this study, group A tended to have lower somatic cell counts than group B, with values below $20\times10^{4}$ cells/mL (within normal range), whereas group B approached $50\times10^{4}$ cells/mL, indicating risk of subclinical mastitis [22]. The reduction in somatic cell count may be due to the antibacterial and anti-inflammatory properties of Moringa oleifera leaves; studies have shown that methanol extracts significantly inhibit Staphylococcus aureus and Escherichia coli growth with inhibition rates of 90.34% and 37.21%, respectively [4], with S. aureus being a primary mastitis pathogen. In summary, diets containing Moringa oleifera leaves and peduncles can improve dairy cow production performance, significantly reduce milk somatic cell count, prevent subclinical mastitis, enhance udder health, and improve milk quality.

### 3.2 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Plasma Biochemical Indices

Plasma biochemical indices effectively reflect nutritional status and aid in early diagnosis and prevention of metabolic diseases. In this study, all plasma biochemical parameters in both groups remained within normal reference ranges [23]. No significant differences were observed between groups for TP, ALB, GLB, HDL, LDL, UN, GLU, or ALT activity. CHOL and TG are important indicators of blood lipid levels and lipid metabolism; abnormal lipid metabolism in ruminants can lead to metabolic diseases such as ketosis. The significant reduction in CHOL and TG in group A may be attributed to functional components in Moringa oleifera leaves that lower blood pressure and cholesterol [24]. Ghasi et al. [25] found that aqueous extracts of Moringa oleifera leaves alleviated elevated plasma CHOL in high-fat-fed Wistar rats. Jain et al. [26] also confirmed that methanol extracts reduced total cholesterol and TG in high-fat-fed mice, consistent with our findings. BHBA serves as an effective indicator for predicting ketosis; no significant difference between groups indicated no ketone body elevation. NEFA is an important indicator of negative energy balance; when cows experience energy deficit, body fat mobilization increases, raising plasma NEFA levels [27]. The tendency for lower NEFA in group A suggests that the diet met energy requirements and reduced fat mobilization. Improved dietary energy utilization is associated with synchronized energy and nitrogen release [28], indicating that the Moringa oleifera diet achieved better synchronization, enhancing energy utilization and reducing NEFA production. ALT and ALP are important aminotransferases reflecting liver function; their activities remain relatively stable but fluctuate with liver damage. In this study, no significant differences in ALT or ALP activities were observed, consistent with Allam et al. [29] who fed broiler chickens Moringa oleifera leaf extract.

### 3.3 Effects of Moringa oleifera Leaves and Peduncles Replacing 50% Alfalfa on Plasma Antioxidant and Immune Indices

Cellular vitality and function depend partly on balanced redox status. Excessive reactive oxygen species (ROS) and free radicals disrupt this balance, causing oxidative damage to macromolecules (DNA, proteins, lipids), leading to cellular dysfunction and apoptosis [30]. Cells activate endogenous protective enzymes such as CAT and SOD to combat oxidative stress. T-AOC reflects comprehensive antioxidant capacity of enzymatic and non-enzymatic systems, while MDA, as an end product of lipid peroxidation, indicates the degree of lipid peroxidation, cellular damage, and free radical metabolism [31]. Our results showed that group A significantly increased plasma T-AOC and hydroxyl radical scavenging capacity while decreasing MDA content. This may be due to antioxidants in Moringa oleifera leaves that reduce lipid peroxidation damage and inhibit ROS and free radical formation [29,32]. Studies have identified active components in Moringa oleifera leaf extracts—including phenolics, anthocyanins, thiocarbamates, and glycosides—that scavenge free radicals, activate antioxidant enzymes, and inhibit oxidases [33]. Therefore, feeding Moringa oleifera can enhance antioxidant capacity in dairy cows. Immunoglobulins are crucial immune-active substances participating in humoral immunity, binding to foreign antigens such as bacteria and viruses to facilitate their elimination [34]. Reports indicate that Moringa oleifera contains lectins that enhance human immunity [35]. Our findings demonstrate that Moringa oleifera leaves and peduncles significantly increased plasma IgG concentration, which possesses antibacterial, antiviral, and antitoxin activities, suggesting that Moringa oleifera can improve immune function in dairy cows.

In conclusion, Moringa oleifera leaves and peduncles can enhance dairy cow production performance, prevent mastitis, improve plasma biochemical profiles, and elevate antioxidant capacity and immune function, making them a viable high-quality roughage for dairy production.

## References

[1] ANWAR F, LATIF S, ASHRAF M, et al. Moringa oleifera: a food plant with multiple medicinal uses[J]. Phytotherapy Research, 2007, 21(1): 17-25. [2] RAMACHANDRAN C, PETER K V, GOPALAKRISHNAN P K. Drumstick (Moringa oleifera): a multipurpose Indian vegetable[J]. Economic Botany, 1980, 34(3): 276-283. [3] KUMAR P S, MISHRA D, GHOSH G, et al. Medicinal uses and pharmacological properties of Moringa oleifera[J]. International Journal of Phytomedicine, 2010, 2(3): 210-216. [4] KUMAR V, PANDEY N, MOHAN N, et al. Antibacterial & antioxidant activity of different extract of Moringa oleifera leaves-an in-vitro study[J]. International Journal of Pharmaceutical Sciences Review and Research, 2011, 12(1): 89-94. [5] SREELATHA S, PADMA P R. Antioxidant activity and total phenolic content of Moringa oleifera leaves stages of maturity[J]. Plant Foods for Human Nutrition, 2009, 64(4): 303-311. [6] UDUPA S L, UDUPA A L, KULKARNI D R. A comparative study on the

effect of some indigenous drugs on normal and steroid-depressed healing[J]. Fitoterapia, 1998, 69(6): 507-511. [7] PRAKASH A O, PATHAK S, SHUKLA S, et al. Pre and post-implantation changes in the uteus of rats: response to Moringa oleifera lam. extract[J]. Ancient Science of Life, 1988, 8(1): 49-54. [8] FAIZI S, SIDDIQUI B S, SALEEM R, et al. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from Moringa oleifera[J]. Phytochemistry, 1995, 38(4): 957-963. [9] KHOLIF A E, MORSY T A, GOUDA G A, et al. Effect of feeding diets with processed Moringa oleifera meal as protein source in lactating Anglo-Nubian goats[J]. Animal Feed Science and Technology, 2016, 217: 45-55. [10] ZHENG J S, XIA C, ZHANG H Y, et al. Effects of rumen-protected choline on production performance and energy metabolism of periparturient dairy cows[J]. Journal of China Agricultural University, 2012, 17(3): 114-120. [11] MENDIETA-ARAICA B, SPÖRNDLY E, REYES-SÁNCHEZ N, et al. Feeding Moringa oleifera fresh or ensiled to dairy cows-effects on milk yield and milk flavor[J]. Tropical Animal Health and Production, 2011, 43(5): 1039-1047. [12] SÁNCHEZ N R, SPÖRNDLY E, LEDIN I. Effect of feeding different levels of foliage of Moringa oleifera to creole dairy cows on intake, digestibility, milk production and composition[J]. Livestock Science, 2006, 101(1/2/3): 24-31. [13] KHOLIF A E, GOUDA G A, MORSY T A, et al. Moringa oleifera leaf meal as a protein source in lactating goat' s diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile[J]. Small Ruminant Research, 2015, 129: 129-137. [14] M' HAMED D, FAVERDIN P, VERITÉ R. Effects of the level and source of dietary protein on intake and milk yield in dairy cows[J]. Annales De Zootechnie, 2001, 50(3): 205-211. [15] KHOLIF A E, KHATTAB H M, EL-SHEWY A A, et al. Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with Pleurotus ostreatus[J]. Asian-Australasian journal of animal sciences, 2014, 27(3): 357-364. [16] LIU Y N, SHI Y H, YAN X B, et al. Effects of replacing part of concentrate with alfalfa hay on production performance and economic benefits of dairy cows[J]. Acta Prataculturae Sinica, 2013, 22(6): 190-197. [17] BASITAN I S, JARCIA E G. Yield, quality and feed cost efficiency of milk produced by Anglo-Nubian goats fed different mixtures of Napier (Pennisetum purpureum) grass and malunggay (Moringa oleifera)[J]. Philippine Journal of Veterinary and Animal Sciences, 2013, 39(2): 193-200. [18] SÁNCHEZ N R, LEDIN S, LEDIN I. Biomass production and chemical composition of Moringa oleifera under different management regimes in Nicaragua[J]. Agroforestry Systems, 2006, 66(3): 231-242. [19] KHALEL M S, SHWERAB A M, HASSAN A A, et al. Nutritional evaluation of Moringa oleifera fodder in comparison with Trifolium alexandrinum (berseem) and impact of feeding on lactation performance of cows[J]. Life Science Journal, 2014, 11(10): 1040-1054. [20] SALITANATI, AHMET MAIMATI, ZHAN X M. Study on the relationship between mastitis and somatic cell count in dairy cows[J]. Herbivorous Livestock, 2012(3): 81-83. [21] MAO Y J, YANG Z P, WANG X L, et al. Study on correlation between somatic cell count, udder traits and milk performance of Holstein cows in southern China[J]. China Dairy Cattle, 2002(2): 12-13. [22] RUEGG P L, DOHOO I R. A benefit to cost analysis of the effect of premilking teat hygiene on somatic cell count and intramammary infections in a commercial dairy herd[J]. The Canadian Veterinary Journal La Revue Vétérinaire Canadienne, 1997, 38(10): 632-636. [23] LI X P, TAO Y, ZHANG X E, et al. Establishment of normal reference ranges for blood biochemical indices of dairy cows in Shihezi, Xinjiang[J]. China Dairy Cattle, 2011(18): 47-50. [24] ZHANG J. Tissue Culture and Active Component Analysis of Moringa oleifera[D]. Master' s Thesis. Fuzhou: Fujian Agriculture and Forestry University, 2013. [25] GHASI S, NWOBODO E, OFILI J O. Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera high-fat fed wistar rats[J]. Journal Ethnopharmacology, 2000, 69(1): 21-25. [26] JAIN P G, PATIL S D, HASWANI N G, et al. Hypolipidemic activity of Moringa oleifera Lam., Moringaceae, on high fat diet induced hyperlipidemia in albino rats[J]. Revista Brasileira De Farmacognosia, 2010, 20(6): 969-973. [27] LEBLANC S J. Monitoring programs for transition dairy cows[C]//Proceedings of the 26th World Biuatrics Congress. Nice, France: World Biuatrics Congress, 2006: 460-472. [28] RICHARDSON J M, WILKINSON R G, SINCLAIR L A. Synchrony of nutrient supply to the rumen and dietary energy source and their effects on the growth and metabolism of lambs[J]. Journal of Animal Science, 2003, 81(5): 1332-1347. [29] ALLAM H, ABDELAZEM A M, SALAH H, et al. Some hemato-biochemical, bacteriological and pathological effects of Moringa oleifera leaf extract in broiler chickens[J]. International Journal of Basic and Applied Sciences, 2016, 5(2): 99-104. [30] MBIKAY M. Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review[J]. Frontiers in Pharmacology, 2012, 3: 24. [31] ZHU H J. Effects of Different Selenium Sources and Levels on Growth Performance and Blood Antioxidant Indices in Broiler Chickens[D]. Master's Thesis. Changsha: Hunan Agricultural University, 2006. [32] OGBE A O, AFFIKU J P. Proximate study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health[J]. Journal of Microbiology Biotechnology and Food Sciences, 2011, 1(3): 296-308. [33] LUQMAN S, SRIVASTAVA S, KUMAR R, et al. Experimental assessment of Moringa oleifera leaf and fruit for its antistress, antioxidant, and scavenging potential using in vitro and assays[J]. Evidence-Based Complementary Alternative Medicine, 2011, 2012: 519084. [34] LIU A G, YANG G Y, LIU X L, et al. Study on extraction of immunoglobulin from bovine serum[J]. Food Science, 2007, 28(9): 261-265. [35] GUO L Q, FENG F Z, WU L, et al. Study on medicinal value and application of Moringa oleifera[J]. Tropical Agricultural Science, 2015, 35(6): 11-15.

*Note: Figure translations are in progress. See original paper for figures.*