Effects of Dietary Amaranth Grain Supplementation on Rumen Fermentation, Blood Parameters, and Productive Performance in Lactating Dairy Cows: Postprint

Authors: Sun Guoqing, Ma Jian, all text, Wang Yajing, Zhijun Cao, Li Shengli, remaining, male, Wang Yu, Lei Xiaoying, Ma Yabin

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with grain amaranth silage and hay on rumen fermentation, apparent nutrient digestibility, blood parameters, and production performance in lactating dairy cows. Forty-five healthy Holstein dairy cows with similar milk yield, parity, and days in milk were selected and randomly divided into 3 groups with 15 cows per group. The control group received roughage consisting of whole-plant corn silage, Chinese wildrye hay, and alfalfa hay. In experimental group I, grain amaranth silage replaced 30% of corn silage in the roughage, while in experimental group II, grain amaranth hay replaced 30% of alfalfa hay in the roughage. Nutritional levels were similar across the three diets. The experiment consisted of a 10-day preliminary period followed by a 60-day formal experimental period. The results showed: 1) Compared with the control group, experimental group I significantly increased the apparent digestibility of crude protein (CP), rumen fluid ammonia nitrogen (NH3-N) concentration, and blood total amino acid (T-AA) content ($P<0.05$), with no significant effects on apparent digestibility of other nutrients, blood parameters, or milk composition ($P>0.05$). 2) Compared with the control group, experimental group II had significantly higher blood T-AA content than the control group ($P<0.05$), with no significant effects on other blood parameters, rumen fermentation indices, apparent nutrient digestibility, or milk composition ($P>0.05$). In conclusion, dietary supplementation with appropriate amounts of grain amaranth does not affect the production performance of dairy cows while improving the economic efficiency of the farm.

Full Text

Effects of Dietary Supplementation of *Amaranthus hypochondriacus* L. on Ruminal Fermentation, Blood Parameters, and Performance of Lactating Dairy Cows

SUN Guoqing[1],[2], MA Jian[1]*, DU Wen[2], WANG Yajing[2], CAO Zhijun[2], LI Shengli[2], **YU Xiong[1], WANG Yu[3], LEI Xiaoying , MA Yabin **

(1. College of Animal Science, Xinjiang Agricultural University, Urumqi 830052, China; 2. College of Animal Science and Technology, China Agricultural University, Beijing 100193, China; 3. Zhongfu Huibang Animal Husbandry Co., Ltd., Beijing 102200, China; 4. Hebei Jinzhou Zhoujiazhuang Agriculture and Animal Husbandry Co., Ltd., Shijiazhuang 052262, China; 5. Hebei Provincial Livestock Breeding Station, Shijiazhuang 050061, China)

Abstract: This experiment was conducted to investigate the effects of dietary supplementation of *Amaranthus hypochondriacus* L. silage and hay on ruminal fermentation, nutrient apparent digestibility, blood parameters, and performance of lactating dairy cows. Forty-five healthy Holstein dairy cows with similar milk yield, parity, and days in milk were randomly assigned to three groups with 15 cows per group. The control group received a basal diet containing whole-plant corn silage, Chinese wildrye, and alfalfa hay as roughage sources. In trial group I, 30% of the corn silage was replaced with *Amaranthus hypochondriacus* L. silage, while in trial group II, 30% of the alfalfa hay was replaced with *Amaranthus hypochondriacus* L. hay. All three diets were formulated to have similar nutrient levels. The experiment consisted of a 10-day pre-trial period followed by a 60-day formal trial period. The results showed that: 1) Compared with the control group, trial group I exhibited significantly higher apparent digestibility of crude protein (CP), ruminal ammonia nitrogen (NH -N) concentration, and blood total amino acid (T-AA) content ($P<0.05$), with no significant effects on other nutrient digestibility metrics, blood parameters, or milk composition ($P>0.05$). 2) Compared with the control group, trial group II showed significantly higher blood T-AA content ($P<0.05$), with no significant differences in other blood parameters, ruminal fermentation indicators, nutrient apparent digestibility, or milk composition ($P>0.05$). In conclusion, dietary supplementation of *Amaranthus hypochondriacus* L. at appropriate levels does not adversely affect cow performance while improving farm economic efficiency.

Keywords: *Amaranthus hypochondriacus* L.; Holstein dairy cows; ruminal fermentation; blood parameters; performance

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Amaranthus hypochondriacus L., an annual herbaceous plant belonging to the family Amaranthaceae, originates from Central and South America and Southeast Asia. Commonly known as protein grass or thousand-ear grain, it is characterized by high yield, superior quality, strong stress resistance, and rapid growth, making it a dual-purpose crop for both grain and forage. In northern China, the fresh yield of *Amaranthus hypochondriacus* harvested at the seed stage can reach 130 t/ha, substantially higher than the 60 t/ha typical for corn silage. The hay yield can achieve 20 t/ha, far exceeding the 7.5 t/ha of alfalfa hay. The plant has demonstrated excellent performance across many regions in China, with timely harvest of stems and leaves meeting the standards for protein feed. Fresh stems and leaves can be processed into green fodder, silage, or leaf meal for feeding pigs, cattle, rabbits, and chickens with good results. After seed harvest, the remaining mature green stalks and rachis also serve as quality livestock feed, suitable for silage or powder production, and represent superior feed for herbivorous livestock and poultry, offering better nutrition and palatability than corn stalks.

With the continuous development of large-scale dairy farming in China and strict quality control of raw milk, farms have become increasingly dependent on high-quality roughage. As a novel forage crop, *Amaranthus hypochondriacus* offers advantages of high yield and nutritional value. Its inclusion in dairy diets represents a new feeding strategy that may reduce costs and improve efficiency. Currently, whole-plant corn silage is the primary silage source for dairy cows in China; however, its protein content is relatively low compared to high-quality silages such as *Amaranthus hypochondriacus*. Rezaei et al. [1] reported that replacing corn silage with different proportions of *Amaranthus* silage did not affect dairy cow performance and could be applied as a quality feed ingredient in dairy diets. Alfalfa hay is the world' s most important legume forage, rich in protein, vitamins, and minerals, and represents one of the best roughages for dairy cows. Liu et al. [2] found that replacing alfalfa hay with certain amounts of raw and processed willow bark did not significantly affect milk yield and reduced production costs. However, research on *Amaranthus hypochondriacus* as a roughage for dairy cows remains scarce in China. Therefore, this study aimed to investigate the effects of dietary supplementation of *Amaranthus hypochondriacus* silage and hay on ruminal fermentation, nutrient apparent digestibility, blood parameters, and performance of dairy cows, and to explore its impact on farm economic efficiency, thereby providing a scientific basis for the utilization and promotion of *Amaranthus hypochondriacus* in dairy production.

1.1 Experimental Materials

Amaranthus hypochondriacus L. silage was prepared by harvesting fresh whole plants at the seed stage, chopping to approximately 3 cm length, baling, and sealing in silage bags. *Amaranthus hypochondriacus* L. hay was prepared by harvesting whole plants at the seed stage, chopping to approximately 3 cm length, and machine-drying. Whole-plant corn silage was prepared from corn harvested at the two-thirds milk line stage, chopped to approximately 1.5 cm length, and ensiled in a silage bunker. Alfalfa hay was provided by the experimental farm and purchased from Huanghua City, Hebei Province.

1.2 Experimental Design and Animal Management

Forty-five Holstein dairy cows with similar days in milk, milk yield, parity, and milk composition were randomly divided into three groups of 15 cows each. The control group received a basal diet without *Amaranthus hypochondriacus*, trial group I had 30% of whole-plant corn silage replaced with *Amaranthus hypochondriacus* silage, and trial group II had 30% of alfalfa hay replaced with *Amaranthus hypochondriacus* hay. Cows were housed in free-stall barns and fed total mixed rations ad libitum with free access to water. Milking was performed twice daily at 06:00 and 18:00. All groups were managed under identical feeding, management, and environmental conditions throughout the 70-day experimental period, which included a 10-day pre-trial period and a 60-day formal trial period.

1.3 Experimental Diets

Three experimental diets were formulated according to the principle of equal energy and equal nitrogen, with variations based on different silage and hay additions. The composition and nutrient levels of the experimental diets are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (dry matter basis), %

Note: 1) Each kilogram of premix contained: VA 320,000 IU, VD 74,000 IU, VE 3,000 IU, D-biotin 3,000 mg, niacinamide 2,000 mg, -carotene 120 mg, Cu 680 mg, Mn 1,800 mg, Zn 3,000 mg, Se 20 mg, I 40 mg, Co 24 mg. 2) Net energy for lactation (NEL) is a calculated value, while other nutrient levels are measured values.

1.4 Sample Collection and Analysis

1.4.1 Dry Matter Intake (DMI) Determination and Diet Sample Collection and Analysis During the formal trial period, feed intake was measured continuously for 3 days every 15 days, recording daily feed offered and refusals for each cow. Diet and refusal samples were collected daily to determine dry matter (DM) content for calculation of group DMI. Feed samples were collected every 15 days using the quartering method to obtain diet, refusal, and individual feed ingredient samples, which were oven-dried at 65°C to produce air-dried samples, ground, and stored for subsequent analysis. Routine nutrient composition analysis was conducted according to the methods described by Zhang Liying [3].

1.4.2 Rumen Fluid Collection and Analysis During the final 2 days of the formal trial period, rumen fluid (100 mL) was collected from 5 cows per group at 0 h (pre-feeding) and at 2, 4, 6, and 8 h post-feeding. After filtration through four layers of cheesecloth, pH was immediately measured using a pH meter. The fluid was then centrifuged at 3,000 rpm for 15 min, and the supernatant was used to determine ammonia nitrogen (NH -N) concentration by colorimetry [4] on a UV spectrophotometer. Additional filtered rumen fluid was stored at -20°C for subsequent determination of volatile fatty acid (VFA) concentrations by gas chromatography [5].

1.4.3 Fecal Sample Collection and Analysis During the final 3 days of the trial period, fecal samples were collected continuously via rectal sampling (300–500 g per collection). Feces collected over 3 days were mixed, weighed, and a 1% subsample was taken. To prevent ammonia nitrogen loss, 10% tartaric acid was added to each 100 g of fresh feces. Samples were oven-dried at 65°C, rehydrated, ground, and stored for routine nutrient analysis [3]. Acid-insoluble ash (AIA) in feces and feed was used as an indigestible marker to calculate apparent digestibility according to the method described by Zhong et al. [6]:

Apparent digestibility (%) = [1 - (Ad × Nf)/(Af × Nd)] × 100

Where Ad and Af represent AIA content (g/kg) in diet and feces, respectively; Nd and Nf represent the corresponding nutrient content (g/kg) in diet and feces, respectively.

1.4.4 Serum Collection and Analysis Five cows were randomly selected from each group for blood collection (10 mL via tail vein) using vacuum tubes at 0, 15, 30, 45, and 60 days of the formal trial period, 2 h post-feeding. Blood samples were allowed to clot for 60 min, then centrifuged at 3,200 rpm for 15 min to separate serum, which was aliquoted into four 1.5 mL tubes and stored at -20°C for analysis. Blood parameters measured included: total cholesterol (TCHO), triglycerides (TG), glucose (GLU), urea nitrogen (UN), -hydroxybutyric acid (BHBA), total protein (TP), albumin (ALB), non-esterified fatty acids (NEFA), total amino acids (T-AA), insulin (INS), insulin-like growth factor 1 (IGF-1), prolactin (RPL), growth hormone (GH), and glucocorticoid (COR).

1.4.5 Milk Sample Collection and Analysis Daily milk yield was recorded throughout the formal trial period, and milk samples were collected every 15 days. Morning and evening milk samples were mixed in a 6:4 ratio, and 40 mL of the mixed sample was preserved with potassium dichromate for milk composition analysis, including milk protein percentage, milk fat percentage, lactose percentage, somatic cell count, and milk urea nitrogen content.

1.4.6 Feed Efficiency Calculation Feed efficiency was calculated as: Feed efficiency = Milk yield / DMI.

1.5 Statistical Analysis

Experimental data were organized using Excel 2007 and analyzed using SPSS 19.0 software for one-way ANOVA. Duncan' s multiple range test was used for post-hoc comparisons. Differences were considered significant at $P<0.05$. Results are expressed as mean ± standard deviation.

2.1 Nutritional Composition of Amaranthus Silage and Hay

Nutritional composition analysis of different roughages prior to feeding is presented in Table 2 . The CP content of *Amaranthus hypochondriacus* silage was 18.00% higher than that of whole-plant corn silage, with higher neutral detergent insoluble crude protein (NDICP), NDF, and ADF contents, but lower DM content. The NDICP content of *Amaranthus hypochondriacus* hay was higher than that of alfalfa hay, while its CP content was lower.

Table 2 Nutrient composition of *Amaranthus hypochondriacus* silage, whole corn silage, *Amaranthus hypochondriacus* hay, and alfalfa hay (dry matter basis)

2.2 Effects of Dietary Amaranthus Supplementation on Ruminal Fermentation

As shown in Table 3 , ruminal pH did not differ significantly among groups (P>0.05). Ruminal NH -N concentration in trial group I was significantly higher than in the control group (P<0.05), but did not differ from trial group II (P>0.05). No significant differences were observed among the three groups in total VFA, acetate, propionate, butyrate, valerate, isobutyrate, or isovalerate concentrations (P>0.05).

Table 3 Effects of different roughage combinations on ruminal fermentation in dairy cows

Note: In the same row, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), while different letters indicate significant difference (P<0.05). The same applies below.

2.3 Effects of Dietary Amaranthus Supplementation on Nutrient Apparent Digestibility

As shown in Table 4 , apparent digestibility of CP in trial group I was significantly higher than in trial group II and the control group (P<0.05). No significant differences were observed among groups in apparent digestibility of DM, NDF, or ADF (P>0.05), although numerically, trial group I showed higher values for all indices.

Table 4 Effects of different roughage combinations on apparent digestibility of nutrients (dry matter basis)

2.4 Effects of Dietary Amaranthus Supplementation on Blood Parameters

As shown in Table 5 , blood T-AA content in both trial groups I and II was significantly higher than in the control group (P<0.05), while other blood parameters were not affected by dietary composition (P>0.05). Blood RPL and GH concentrations were numerically higher in trial group II, blood UN and

COR concentrations were numerically higher in trial group I, and blood TP and NEFA concentrations were numerically higher in the control group, but none of these differences reached statistical significance (P>0.05).

Table 5 Effects of different roughage combinations on blood parameters of dairy cows

2.5 Effects of Dietary Amaranthus Supplementation on Performance

As shown in Table 6 , no significant differences were observed among the three groups in DMI, milk yield, milk urea nitrogen content, or feed efficiency (P>0.05). Milk somatic cell count was numerically lower in trial group I than in the other two groups, but the difference was not significant (P>0.05). No significant differences were found among groups in other milk composition parameters (P>0.05).

Table 6 Effects of different roughage combinations on DMI, feed efficiency, milk yield, and milk composition of dairy cows

2.6 Effects of Dietary Amaranthus Supplementation on Farm Economic Efficiency

The annual fresh yield of *Amaranthus hypochondriacus* at the seed stage can reach 130 t/ha, with hay yield of 24 t/ha, substantially higher than the yields of corn stalks, alfalfa, and Chinese wildrye. As shown in Table 7 , trial groups I and II generated additional profits of 2.70 and 2.31 yuan per cow per day, respectively, demonstrating that incorporating *Amaranthus hypochondriacus* into dairy cow roughage can improve farm economic efficiency.

Table 7 Effects of different roughage combinations on economic effectiveness of farms (yuan)

3.1 Effects of Dietary Amaranthus Supplementation on Ruminal Fermentation

Ruminal pH results from the combined effects of VFA interaction with salivary buffers, VFA absorption by ruminal epithelium, and passage of digesta out of the rumen, with normal ruminal pH ranging from 6 to 7 [7]. In this experiment, ruminal pH remained within the normal range across all three dietary treatments with no significant differences, which is conducive to maintaining the activity of fiber-degrading bacteria. Mertens [8] reported that dietary particle size is negatively correlated with ruminal pH. In this study, the higher NDF content of *Amaranthus hypochondriacus* hay compared to alfalfa hay may be related to this relationship.

Ruminal NH -N is an intermediate product of feed protein and non-protein nitrogen degradation and microbial protein synthesis, and its concentration reflects the balance between protein degradation and synthesis, being influenced primarily by dietary protein degradation, ruminal wall absorption, microbial utilization, and digesta outflow rate [7]. The significantly higher NH -N concentration in trial group I compared to the control group may be attributed to the higher NDF content of *Amaranthus hypochondriacus* silage compared to whole-plant corn silage, which increased retention time in the rumen through enhanced rumination, leading to more complete digestion and consequently higher NH -N concentration. Additionally, as dietary particle size decreases, binding with ruminal microbes increases, which tends to reduce ruminal NH -N concentration. In this experiment, the chopping length of *Amaranthus hypochondriacus* silage was greater than that of whole-plant corn silage, consistent with the findings of Wu et al. [9].

Volatile fatty acids in the rumen are intermediate metabolites in energy utilization for ruminants and serve as substrates for milk fat and body fat synthesis, with acetate, propionate, and butyrate accounting for approximately 95% of total VFA [7]. Beauchemin et al. [10-11] found that VFA production was not affected by dietary NDF content, which aligns with the results of this study. No significant effects of dietary composition were observed on acetate, propionate, butyrate, isobutyrate, or isovalerate concentrations. Numerous studies have investigated the effects of dietary composition on ruminal VFA and acetate/propionate ratio, with inconsistent results. Li [12] reported that when wet corn fiber accounted for 15% of total dietary fiber, ruminal propionate concentration increased significantly while acetate concentration and acetate/propionate ratio decreased significantly. Yang et al. [13] found that when concentrate proportion reached 65% of the diet, the acetate/propionate ratio was significantly higher than in other treatments. Beauchemin et al. [10-11] demonstrated that as dietary physically effective neutral detergent fiber (peNDF) content decreased, ruminal propionate concentration increased, butyrate concentration decreased, and acetate/propionate ratio decreased. In this study, the acetate/propionate ratio was higher in both trial groups than in the control group, likely due to the higher NDF content of *Amaranthus hypochondriacus* enhancing fiber-degrading bacterial activity.

3.2 Effects of Dietary Amaranthus Supplementation on Nutrient Apparent Digestibility

Brunette et al. [14] suggested that replacing corn silage with pearl millet silage could improve apparent digestibility of NDF and CP, attributing this to higher quality and more digestible CP. In this study, the higher apparent digestibility of CP in trial group I compared to the control and trial group II may be due to the higher content of true protein in *Amaranthus hypochondriacus* silage, which is more readily digested by the rumen. Zhou et al. [15] demonstrated that dietary NDF content affects apparent digestibility of NDF and ADF, with trends consistent with the results of this study. Xia et al. [16] reported that different roughages can create associative effects due to variations in fiber and protein content, thereby improving apparent digestibility. In this experiment, the inclusion of *Amaranthus hypochondriacus* increased roughage utilization and improved apparent digestibility. Li et al. [17] showed that increasing the proportion of alfalfa hay in the diet elevated NDF apparent digestibility, similar to the findings of this study.

3.3 Effects of Dietary Amaranthus Supplementation on Blood Parameters

Blood T-AA typically promotes milk protein synthesis and is influenced by dietary NDICP content [18]. In this study, blood T-AA concentration was significantly higher in both trial groups than in the control group, yet milk protein percentage was only numerically higher in the trial groups without significant differences. This may be due to limitations imposed by blood GLU concentration and acetate content, which increased the energy required for amino acid conversion to urea, thereby reducing amino acid utilization efficiency.

Triglycerides are formed by the condensation of three fatty acids with glycerol' s three hydroxyl groups, exported from the liver as lipoproteins, and can serve as precursors for milk fat synthesis or be absorbed by tissues. Albumin can re-esterify NEFA into TG. Blood TP and ALP concentrations decrease when liver function is impaired. Blood NEFA, primarily derived from mobilization and lipolysis of TG in adipose tissue, serves as a key indicator of energy balance in dairy cows [19]. BHBA is an intermediate metabolite of NEFA esterification and can be used as an early diagnostic indicator for ketosis [20]. Shi et al. [21] reported that blood BHBA concentration increased linearly with decreasing dietary non-fibrous carbohydrate content. In this study, the numerically higher blood BHBA concentration in trial group II may be attributed to slightly higher dietary NDF content. Urea nitrogen is the primary end product of protein metabolism and a key indicator of kidney function. In ruminants, GLU is primarily synthesized from propionate absorbed from the rumen via hepatic gluconeogenesis [22], representing the dynamic balance of glucose absorption, transport, and energy metabolism, and is positively correlated with INS concentration. Similar conclusions were reached in this study, though the numerically lower ruminal propionate concentration in trial group II may have been influenced by feeding time. No significant differences were observed among groups in blood TG, NEFA, BHBA, or GLU concentrations, as the three diets were formulated to be isoenergetic and isonitrogenous. The lack of significant differences in TP, ALP, and UN concentrations indicates that dietary *Amaranthus hypochondriacus* supplementation did not adversely affect liver or kidney function.

Prolactin (PRL) and glucocorticoids (COR) are primary hormones that stimulate mammary cell differentiation, initiation, and maintenance of lactation. Insulin-like growth factor 1 (IGF-1) is a polypeptide that promotes growth and cell differentiation, as well as protein synthesis and lipolysis, and acts synergistically with GH. Wen et al. [23] reported that blood GH concentration is affected by dietary nutrient composition, with dietary arginine and leucine potentially promoting GH secretion. In this study, no significant differences in blood GH concentration were observed among groups, though trial group II was numerically higher, possibly due to the rich amino acid content of *Amaranthus hypochondriacus* hay, which requires further investigation. Glucocorticoids are steroid hormones influenced by dietary composition and environment. Han et al. [24] demonstrated that blood COR concentration is affected by lactation stage and decreases linearly. Lepage et al. [25] suggested that blood COR concentration is influenced by dietary tryptophan content. In this study, with similar days in milk across groups, blood COR concentration was numerically higher in both trial groups than in the control group, but the difference was not significant.

3.4 Effects of Dietary Amaranthus Supplementation on Performance of Lactating Cows

Due to the higher NDF and ADF content of *Amaranthus hypochondriacus* hay compared to alfalfa hay, its use in dairy diets might be expected to reduce DMI [26]. However, this study showed numerically higher DMI in trial groups, with similar findings reported by Ma et al. [27]. This may be because the acid detergent insoluble crude protein (ADICP) in *Amaranthus hypochondriacus* participates in Maillard reactions, creating a special aroma that enhances palatability [28]. In this study, the ADICP content of both *Amaranthus hypochondriacus* hay and silage was slightly higher than that of alfalfa hay and corn silage, which may have promoted feed intake. Staples [19] reported a high correlation between milk yield and feed efficiency. In this study, numerically higher milk yield in trial groups was linearly correlated with DMI and feed efficiency across groups.

Milk protein is synthesized by mammary cells primarily from amino acids, glucose, and acetate, with blood amino acids mainly derived from small peptides produced by rumen-undegraded protein (RUP) digestion in the small intestine [18]. Wright [29] reported that increasing RUP supply could elevate blood amino acid concentration. NDICP, which degrades slowly in the rumen but can be digested in the intestine and absorbed as peptides and amino acids, is a major component of RUP [30]. In this study, the numerically higher milk protein percentage in trial groups may be related to the higher NDICP content of *Amaranthus hypochondriacus* silage and hay compared to corn silage and alfalfa hay. Milk urea nitrogen concentration reflects dietary protein level and energy-nitrogen balance, with normal values ranging from 10–14 mg/dL in healthy herds [31]. Due to the isoenergetic and isonitrogenous nature of the experimental diets, milk urea nitrogen concentrations were approximately 10.50 mg/dL across all groups, within the normal range. Somatic cell counts in this study ranged from 7×10 to 12×10 cells/mL across groups, well below both the European Union standard (40×10 cells/mL) and the current U.S. standard (75×10 cells/mL).

Conclusions: 1. Dietary supplementation of *Amaranthus hypochondriacus* silage significantly increased ruminal NH -N concentration, blood T-AA content, and apparent digestibility of CP. 2. Dietary supplementation of *Amaranthus hypochondriacus* hay significantly increased blood T-AA content.

In summary, incorporating *Amaranthus hypochondriacus* into dairy cow diets can improve farm economic efficiency without adversely affecting cow health or performance.

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